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## Antimicrobial resistance in wildlife - potential for dissemination

**Opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee  
for Food and Environment**

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Antimicrobial resistance in wildlife - potential for dissemination

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Norwegian Scientific Committee for Food and Environment (VKM)

Po 4404 Nydalen

N – 0403 Oslo

Norway

Phone: +47 21 62 28 00

Email: [ykm@ykm.no](mailto:ykm@ykm.no)

[ykm.no](http://ykm.no)

[ykm.no/english](http://ykm.no/english)

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# **Antimicrobial resistance in wildlife - potential for dissemination**

## **Preparation of the opinion**

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to answer the request from the Norwegian Environment Agency. The project group consisted of two VKM members from the Panel on Genetically Modified Organisms (GMO) and one from the Panel on Microbial Ecology, one external expert, and a project leader from the VKM secretariat. One VKM member from the the Panel on Microbial Ecology and external referee reviewed and commented upon the manuscript. The VKM Panel on Microbial Ecology evaluated and approved the final opinion drafted by the project group.

## **Authors of the opinion**

Members of the project group that contributed to the drafting of the opinion (in alphabetical order after chair of the project group):

Kaare Magne Nielsen (chair), member of Panel on GMO-VKM. Affiliation: 1) VKM and 2) OsloMet – Oslo Metropolitan University,

Tor GjØen, Affiliation: 1) VKM and 2) University of Oslo,

Nana Asare, Affiliation: VKM-Secretariat, Affiliation: VKM,

Bjørn-Tore Lunestad (external expert), Affiliation: Institute of Marine Research, Bergen,

Bjørnar Ytrehus, Affiliation: 1) VKM and 2) Norwegian Institute for Nature Research,

Siamak Yazdankhah, member of project group and project leader, VKM-Secretariat, Affiliation: VKM,

Members of the Panel on Microbial Ecology that contributed to the assessment and approval of the opinion (in alphabetical order before chair/vice-chair of the Panel/Committee):

Jacques Godfroid, Affiliation: 1) VKM and 2) University of Tromsø,

Anders Jelmert, Affiliation: 1) VKM and 2) Institute of Marine Research,

Jörn Klein, Affiliation: 1) VKM and 2) University College of Southeast Norway,

Arinze Okoli, Affiliation: 1) VKM and 2) Centre for Biosafety, GenØk,

Arne Tronsmo, Affiliation: 1) VKM and 2) Norwegian University of Life Sciences.

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## **Competence of VKM experts**

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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**Key words:** Antimicrobial resistance, wildlife, VKM, Norwegian Scientific Committee for Food and Environment, Norwegian Environment Agency

# Summary

Antimicrobial resistance (AMR) is recognised as one of the greatest public health concerns of our time. The development of AMR occurs in nature as a defence by microbes against naturally occurring antimicrobials. However, the selective pressure, generated by the use of antimicrobial agents in human and veterinary medicine, livestock and plant production as well as aquaculture practices, is the major driving force leading to the increased emergence and spread of resistance in bacteria. The sharing of common habitats and water resources could result in transfer of antimicrobial-resistant bacteria (ARB) between wildlife, food-producing animals and humans. Bacterial populations with various transferable AMR traits are reported in wildlife, and wildlife is thus a well-established source of AMR bacteria entering the food chain both in meat and in foods of plant origin. The relative importance of such reservoirs and transfer routes of AMR in comparison to other sources leading to AMR development in pathogenic bacteria remains unclear.

The Norwegian Environment Agency (Miljødirektoratet) asked the Norwegian Scientific Committee for Food and Environment (VKM) for an assessment regarding the role of wildlife in dissemination of AMR. The Norwegian Environment Agency would like VKM to give an opinion on wildlife and AMR:

- Identification of transferable AMR bacteria in wildlife (terrestrial and aquatic animals).
- Methods used for sampling and analysis of data in reported studies.
- AMR in bacteria in wildlife, according to their habitat (close to urban areas, rural areas, marine or freshwater environment or migratory).
- Possible routes of antimicrobial residues to induce ARB in the environment.
- Transfer of AMR bacteria between wildlife and other hosts, possible routes of dissemination of ARB to wildlife from domestic animals and vice versa, exchange routes of AMR between human and wildlife.

VKM appointed a working group, consisting of one member of the Panel on Microbial Ecology, one member of the Panel on Genetically Modified Organisms, one external member, and VKM staff to prepare a draft Opinion. The Panel on Microbial Ecology has reviewed and revised the draft prepared by the working group and approved the Opinion document.

In this opinion, we summarise the majority of research conducted on AMR in wildlife, identify knowledge gaps and areas of uncertainty, and explore the interfaces between wildlife, domestic animals, and humans in the context of resistance emergence, persistence, and transmission.

## **Methodology**

This assessment is based on internationally published data identified using specific terms and defined inclusion and exclusion criteria in the following databases: PubMed, EmBase,



ScienceDirect, and Web of Science. Information from Norway is based on internationally published articles and data retrieved from the NORM/NORM-VET database.

## Results

We have considered over 230 peer reviewed studies identified in the different databases searched. These studies revealed variation in the choice of experimental designs, sampling strategies, and methods for isolation and characterisation of AMR in wildlife. In most cases, resistant bacterial isolates were first characterised by cultivation-based methods. In some cases, genetic analysis based on known resistance determinants was also performed. The large majority of studies describe point prevalences of AMR in a small wildlife population. The published studies rarely investigate development of resistance over time or space. Direct comparisons between studies are difficult due to limited methodological coherence between studies, time of sampling, and the non-uniform methodological approaches and reporting. The degree of anthropogenic exposure is rarely addressed. These shortcomings represent an obstacle for using these data to infer routes of transmission between habitats, wildlife, domesticated animals and humans. Standardization and larger collaborative studies are needed to explore the epidemiological aspects of AMR in wildlife.

The studies assessed in this report were first sorted according to animal group (e.g., amphibians and reptiles, birds, fish and other water-living animals, and terrestrial mammals) and further evaluated based on the following criteria: number of studies, timespan of publications, geographical areas where the studies were performed, and resistance types with focus on specific resistant bacterial species.

Most studies focused on bacterial species common in the gastrointestinal tract of humans and domestic animals. Two bacterial groups were investigated in 60 % of the studies: *E. coli* and *Enterococcus* spp. These and many of the other bacterial species examined in the studies considered are known to establish in both humans and animals, including wildlife.

A number of interfaces between animal species where transmission of AMR is physically possible between wildlife, humans and domestic animal have been identified: a) Humans and domestic animals can be in direct contact or in close proximity with wildlife; b) Water is a major transmission medium for AMR (sewage, rivers, irrigation water, lakes and sea water); c) Soil communities, possibly due to the production of naturally-occurring antibiotics by some soil bacteria and fungi. The main source of AMR in agricultural soil is the use of irrigation water, direct faeces or urine deposition (e.g., in pastures), manure use, or effluent flows. The latter also include feces and urine from domestic animals treated with antimicrobials.

Birds, particularly migratory birds, is considered to have the highest dispersal capacity for AMR bacteria due to their biannual migration patterns between countries and even continents. Omnivorous species often feed on anthropogenic waste and live near human habitations and farms. Such species (e.g. rodents) are often ubiquitous and can thereby act as a major link between wildlife, domestic animals, and humans. Furthermore, small

anthrophilic prey species, such as rodents, is also a bridge between human/domestic animals and their predators including birds, of which some have seasonal migration patterns.

Few of the examined studies have explored how AMR patterns in wildlife change over time. Most studies represent snapshots of limited scale and duration. Thus, the tempospatial dynamics of resistance traits within and between wildlife populations remain most often unknown.

Taken together, the examined studies suggest a tendency of wildlife populations living in close proximity to humans can have higher levels of AMR than those populations with minimal contact with humans or anthropogenic antimicrobial sources. This observation suggest directionality. However, robust observations of directionality has not been observed, and the dynamics of the interactions between bacterial populations in wildlife, domesticated animals and humans is currently best described as complex and multidimensional.

### **Uncertainties**

A range of uncertainties to our understanding of the probability of development and dissemination of AMR from wildlife have been identified. Many of these are due to data gaps, lack of a determination of relevant time scales and a lack of a quantitative approach and broader theoretical framework that can guide the experimental design. Although many point prevalence studies are available, few have been designed to establish directionality of resistance transfer.

### **Conclusion**

In a One Health context, AMR represents a complex ecological problem affected by a multitude of factors including type and level of selective pressure, mechanisms of transmission and persistence, and routes of dispersal.

More than 230 studies were considered in this Opinion, we nevertheless remain unable to establish clear links and causality between AMR in wildlife, domesticated animals and humans. This is due to a number of limitations. Most studies focus on a few bacterial species in one wildlife animal species in a defined geographical area over a short time period. In a some cases, the study areas were geographically very large (an administrative region or a state) and included various habitats, but with small sample sizes and unclear level and type of anthropogenic exposure. Few of the studies related their outcome to the use of antimicrobial agents in the same area or to the occurrence of AMR in domestic animals or humans in the same region.

Direct comparisons between experimental studies are also difficult due to non-uniform methodology and reporting. A wide range of sampling strategies and methods for isolation and characterisation of antimicrobial resistance have been used. In most cases, resistant isolates were first characterised by a cultivation-based method. In some cases, a genetic analysis based on known resistance determinants was also performed.

## **Data gaps**

Many of the studies included are descriptive, with small sample sizes and lacking quantitative and longitudinal perspectives. The lack of a quantitative focus, as well as limited information on the genetic basis for most of the resistance traits described in the studies, limits our understanding as well as current ability to identify exposure pathways and infer evolutionary trajectories of particular resistance determinants.

# Samandrag

Antimikrobiell resistens (AMR) vert sett på som ei av dei største helseutfordringane i vår tid. Utvikling av antimikrobiell resistens kan gå føre seg i naturen når mikroorganismar forsvarar seg mot antimikrobielle stoff som finst naturleg. Den viktigaste drivkrafta for auka førekomst og spreining av resistens hos bakteriar, er likevel trykket frå bruk av antimikrobielle stoff i human og veterinærmedisin, ved husdyr og planteproduksjon og i akvakultursamanheng. Sams bruk av leveområde kan gi opphav til overføring av resistente bakteriar mellom viltlevande dyr, dyr til matproduksjon og menneske. Bakteriepopulasjonar med ulik antimikrobiell resistens som kan overførast frå viltlevande dyr, vert rapporterte. Slike dyr er ei godt kjend kjelde til resistente bakteriar som kan ende opp i matkjeda, både ved produksjon av kjøt og planteprodukt. Samanlikna med andre kjelder til antimikrobiell resistens, er den relative tydinga av viltlevande dyr som reservoar og moglege overføringsvegar for antimikrobiell resistens til patogene bakteriar framleis uklare.

Miljødirektoratet ba Vitenskapskomiteen for mat og miljø (VKM) om å gjennomføre ei vurdering av kva rolle viltlevande dyr kan ha for spreining av antimikrobiell resistens. Miljødirektoratet ville at VKM skulle gje si vurdering av viltlevande dyr og antimikrobiell resistens på desse områda:

- Identifisere overførbar resistens hos bakteriar frå viltlevande dyr, både landlevande og akvatiske.
- Metodar for prøvetaking og dataanalyse i studiar som inngår.
- Antimikrobiell resistens hos bakteriar i viltlevande dyr som funksjon av leveområde (bynære område, grisgrendte strom, marine og ferskvassmiljø samt for dyr med lange vandringar).
- Moglege mekanismar for korleis restar av antimikrobielle stoff kan føre til resistente bakteriar i miljøet.
- Overføring av resistente bakteriar mellom viltlevande dyr og andre dyr, moglege mekanismar for spreining av resistente bakteriar til viltlevande dyr frå husdyr og vice versa, utveksling av antimikrobiell resistens mellom menneske og viltlevande dyr.

For å gjennomføre vurderinga, oppnemnde VKM ei arbeidsgruppe som var samansett med ein medlem frå kvar av faggruppene for mikrobiell økologi og genetisk modifiserte organismar, ein ekstern medlem og medlemmar frå VKM-sekretariatet. Faggruppa for mikrobiell økologi gjekk gjennom og reviderte utkastet som var utarbeidd av arbeidsgruppa.

I denne vurderinga samanfattar me storparten av tilgjengeleg forskning som er utført på antimikrobiell resistens hos viltlevande dyr, identifiserer kunnskapsmanglar og område der det er stor grad av uvisse, greier ut om kopplingsflatene mellom viltlevande dyr, husdyr og menneske når det gjeld resistensutvikling, stabilitet for resistenseigenskapar og overføring av desse.

## Metodikk

Denne vurderinga er basert på internasjonalt publiserte data identifiserte etter spesifikke søkjeternar og etter definerte kriterium for å inkludere eller ekskludere arbeida. Desse søkjedatabasane vart nytta: PubMed, EmBase, ScienceDirect og Web of Science. Informasjon frå Noreg er henta frå internasjonalt publiserte artiklar og data frå NORM/NORM-VET databasane.

## Resultat

Me har vurdert over 230 fagfelleverderte arbeid som framkom etter søk i dei ulike databasane. Desse artiklane viste variasjon i val av eksperimentelt oppsett, prøvetakingsstrategi og metodar for isolasjon og karakterisering av antimikrobiell resistens hos viltlevande dyr. I dei fleste tilfella vart isolat av resistente bakteriar fyrst karakterisert ved hjelp av dyrkingsbaserte metodar. I nokre tilfelle vart genetisk analyse og samanlikning med kjende resistensdeterminantar gjennomført. I storparten av studia rapporterer ein berre punktprevalens av antimikrobiell resistens i små populasjonar av viltlevande dyr. Publiserte arbeid undersøker sjeldan resistensutvikling i tid og rom. Direkte samanlikning mellom ulike studiar er vanskelege grunna lite innbyrdes metodisk samsvar, ulik prøvetakingstid, og stort sprik i rapporteringstilnærminga. Graden av menneskeskapt påverknad vert sjeldan diskutert. Desse manglane er til hinder for å bruke arbeida til å utleia moglegheiter for overføring av resistens mellom habitat, viltlevande dyr, husdyr og menneske. Det er trong for standardisering og større sams studiar for å kunne utforske epidemiologiske tilhøve ved antimikrobiell resistens hos viltlevande dyr.

Studiar som er inkluderte i denne vurderinga vart fyrst sorterte etter dyregruppe (eksempelvis amfibiar og krypdyr, fuglar, fisk og andre vasslevande dyr eller landlevande pattedyr), deretter vurderte etter dei fyljande kriteria: tal på studiar, tidsrom for publisering, geografisk område der studiet vart gjennomført og resistenstype med særleg fokus på enkelte viktige bakterieartar.

Dei fleste studia tok føre seg bakterieartar som er vanlege i mage/tarmkanalen til menneske og husdyr, og mange av artane er kjende for å kunne etablere seg i både menneske og dyr, inkludert hos viltlevande dyr. To bakteriegrupper vart studerte i 60 % av artiklane inkludert i denne vurderinga: *E. coli* og *Enterococcus* spp.

Mange interaksjonsflater der overføring av antimikrobiell resistens er mogleg mellom viltlevande dyr, menneske og husdyr er identifisert: a) Menneske og husdyr kan vere i direkte kontakt med eller tett på viltlevande dyr; b) Vatn er eit hovudmedium for overføring av antimikrobiell resistens (kloakk, elvar, vatningssystem, innsjøar og sjø); c) Jordsamfunn, kanskje særleg fordi dei er kjende for å huse enkelte soppar og bakteriar som kan produsere naturleg førekomande antibakterielle stoff. Hovudkjelda til antimikrobiell resistens i jordbruksareal er bruk av irrigasjonsvatn, direktetilførsel av faeces eller urin under beiting, gjødsling med gylle eller avløpsvatn. Sistnemnte inkluderer også faeces og urin frå husdyr som er handsama med antimikrobielle stoff.

Fuglar, særleg trekkfuglar, har truleg den høgste spreingskapasiteten for antimikrobiell resistente bakteriar sidan dei har sesongvandringar to gangar årleg mellom land, eller til og med mellom kontinent. Omnivore artar beitar ofte på menneskeskapt avfall og lever nær hus og gardsbruk. Dyr som t.d. gnagarar er å finna dei fleste stadar og kan derfor representere ei kopling mellom viltlevande dyr, husdyr og menneske. Vidare vil små byttedyr som gnagarar kunne representere ei kopling mellom menneske og rovfuglar, som på si side kan ha sesongvandringar.

I svært få av studiane har ein undersøkt korleis biletet av antimikrobiell resistens hos viltlevande dyr endrar seg over tid. Dei fleste arbeida representerer ein kortvarig småskala studie. Dette er grunnen til at tid- og rom-dynamikken for resistenseigenskapar hos populasjonar av viltlevande dyr er langt på veg ukjend.

Sett under eit, indikerer dei undersøkte studiane at populasjonar av viltlevande dyr som oppheld seg nær menneske har høgare nivå av antimikrobiell resistens, samanlikna med dei som har minimal kontakt med menneske eller restar av antimikrobielle stoff med opphav frå menneskeleg aktivitet. Denne observasjonen kan peike mot at det er ei retning (direksjonalitet) i utbreiing av antimikrobiell resistens. På den andre sida sett, er det ingen konkluderande observasjonar som klårt viser slik direksjonalitet, og dynamikken for interaksjonar mellom bakteriepopulasjonar hos viltlevande dyr, husdyr og menneske kan best karakteriserast som samansett og multidimensjonal.

## **Uvissefaktorar**

Me har identifisert ei rekkje usikkerhetsfaktorar knytt til vår forståing av utvikling og spreing av antimikrobiell resistens frå viltlevande dyr. Mange av desse kjem frå datamanglar, mangel på tilstrekkelege tidsseriar, mangel på kvantitative tilnærmingar og eit breiare teoretisk rammeverk som kan nyttast ved fastsetjing av eksperimentelle design. Sjølv om mange studiar på punktprevalens er tilgjengelege, har berre nokre vorte designa for å fastslå i kva retning resistensoverføringa har gått.

## **Konklusjon**

I ein One Health samanheng representerer antimikrobiell resistens eit samansett økologisk problem som vert påverka av ei heil rekkje faktorar, inkludert grad og type av selektivt trykk, mekanismar for overføring og stabilitet av resistenseigenskapar, samt moglege spreingsvegar.

Over 230 studiar er inkluderte i denne vurderinga, likevel er me framleis av ulike grunnar ikkje i stand til å påvise klår kopling og årsakssamanheng mellom antimikrobiell resistens hos viltlevande dyr, husdyr og menneske. Dei fleste studiar fokuserer på få bakterieartar hos ein art av viltlevande dyr i eit avgrensa geografisk område i eit kort tidsintervall. I eit fåtal studiar er det undersøkte geografiske området stort (administrativ region eller stat) og inkluderer ulike habitat, men med lite prøvetal og uklart nivå av menneskeleg påverknad.

Nokre få studiar set funna i samanheng med bruk av antimikrobielle stoff i studieområdet eller til førekomsten av antimikrobiell resistens i husdyr eller menneske i same region.

Direkte samanlikningar mellom eksperimentelle studiar er vanskeleg grunna ikkje standardisert metodebruk og rapportering. Mange ulike prøvetakingsstrategiar og metodar for isolering og karakterisering av antimikrobiell resistens er blitt nytta. I storparten av tilfella er resistente isolat fyrst karakterisert med dyrkingsbasert metodikk. I nokre tilfelle har også genetisk analyse av kjende resistensdeterminantar vore gjennomførte.

### **Datamanglar**

Mange av dei inkluderte studiane er deskriptive, har eit avgrensa prøvetal og manglar eit langsiktig kvantitativt perspektiv. Mangelfullt kvantitativt fokus og mangel på informasjon om det genetiske grunnlaget for storparten av resistenseigenskapane i dei inkluderte studiane, set grenser for vår forståing av og evne til å identifisere eksponeringsvegane og utlede utviklingsmessige spor for særne resistensdeterminantar.

# Abbreviations and/or glossary

## Abbreviations

AMR	Antimicrobial resistance
API	Analytical Profile Index
ARB	Antimicrobial-resistant bacteria
ARG	Antimicrobial resistance gene
BLAST	Basic Local Alignment Search Tool
BRICS	Brazil, Russia, India, China, South Africa
CA-MRSA	Community Acquired Methicilin Resistant <i>Staphylococcus aureus</i>
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase-negative staphylococci
CP	Carbapenem
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EHEC	Enterohaemorrhagic <i>Escherichia coli</i>
EMA	European Medicines Agency
EPEC	Enteropathogenic <i>Escherichia coli</i>
EPS	Extracellular polymeric substance
ESBL	Extended-Spectrum Beta-Lactamases
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FAO	Food and Agricultural Organisation of the United Nations
FF	Filter-feeding
HA-MRSA	Hospital Acquired Methicillin Resistant <i>Staphylococcus aureus</i>
HGT	Horizontal gene transfer



IUPAC	International Union of Pure and Applied Chemistry
LA-MRSA	Livestock Methicillin Resistant <i>Staphylococcus aureus</i>
MALDI-TOF	Matrix Assisted Laser Desorption/Ionisation Time-of-Flight
MCC	Minimum metal co-selective concentration
MDR	Multidrug resistant
MIC	Minimum inhibitory concentration
MLST	Muli Locus Sequence Typing
MLVA	Multilocus variable number of tandem repeats analysis
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-susceptible <i>Staphylococcus aureus</i>
MSC	Minimum Selective Concentration
NCCLS	National Committee for Clinical Laboratory Standards, USA
NDM-1	New Delhi Metallo-Beta-Lactamase 1
NORM	The Norwegian monitoring programme for AMR in human pathogens
NORM/VET	The Norwegian monitoring programme for AMR in veterinary pathogens
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PNEC	Predicted No Effect Concentrations
PMQR	Plasmid-Mediated Quinolone Resistance
QACs	Quaternary ammonium compounds
RAPD	Random Amplified Polymorphic DNA
rep-PCR	Repetitive Element Sequence-Based PCR
SNP	Single nucleotide polymorphism
STEC	Shiga-toxin producing <i>Escherichia coli</i>

ToR	Terms of reference
VDs	Virulence determinants
VKM	Norwegian Scientific Committee for Food and Environment
VRE	Vancomycin-resistant enterococci
WWTP	Wastewater treatment plant
WHO	World Health Organization

## Glossary

**Acquired resistance:** Resistance to a particular antimicrobial agent to which the microorganism was previously susceptible. The change in resistance level is the result of genetic changes in the microorganism due to mutation(s), the acquisition of foreign genetic material, or a combination of both mechanisms.

**Animal:** For the purpose of this report, an animal is defined as a mammal, bird, reptile, amphibian, fish, crustacean, mollusc, or bee (as defined by OIE).

**Antibiotics:** Traditionally refers to natural organic compounds produced by microorganisms that act in low concentrations against other microbial species, mostly bacteria. Sometimes, the terms “antibiotics” is used to refer to synthetic (chemotherapeutic) and semi-synthetic compounds (chemically modified antibiotics) with similar effects.

**Antimicrobial agents:** A general term for the drugs (antibiotics), chemicals, or other substances that either kill or inhibit the growth of microbes. The concept of **antimicrobial agents** applies to antibiotics, disinfectants, preservatives, sanitizing agents, and biocidal products in general. All antibiotics are **antimicrobial agents**, but not all **antimicrobial agents** are antibiotics.

**Antimicrobial resistance:** A property of microorganisms that confers the capacity to inactivate or exclude antimicrobials, or a mechanism that blocks the inhibitory or killing effects of antimicrobials.

**Bactericidal agent:** An antimicrobial agent capable of killing bacteria.

**Bacteriostatic agent:** An antimicrobial agent that inhibits the growth of bacteria.

**Biocide/Biocidal products:** Active substances and preparations containing one or more substances intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.

**Biocide resistance:** When non-antibiotic antimicrobial agents (i.e., biocides) are considered, the word “resistance” is used in a similar way when a strain is not killed or inhibited by a concentration attained in practice (the in-use concentration) and in a situation where: 1) a strain is not killed or inhibited by a concentration to which the majority of strains of an organism are susceptible, or 2) bacterial cells are not killed or inhibited by a concentration acting upon the majority of cells in that culture (SCENHR 2009).

**Biofilm:** Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid/liquid) and typically surrounded by an extracellular polymeric slime matrix.

**Captive wild animals:** Describes animals that have a phenotype not significantly affected by human selection but that is captive or otherwise lives under direct human supervision or control, including zoo animals and pets.

**Carnivore:** Animal species that has flesh as its primary food source, either through predation or scavenging.

**Chemotherapeutics:** In this context, compounds with antimicrobial effect that are synthesised in the laboratory and that have no natural reserve in the environment. In modern popular literature chemotherapeutics and antibiotics are commonly referred to as “antimicrobials”.

**Clone (bacteria):** Bacterial isolates that, although they may have been cultured independently from different sources in different locations and perhaps at different times, still have so many identical phenotypic and genotypic traits that the most likely explanation for these similarities is a common origin within a relevant timespan.

**Conjugation:** Transfer of genetic material between different bacterial cells by direct cell-to-cell contact.

**Co-resistance:** Resistance occurring when the genes specifying different resistant phenotypes are genetically linked, for example by being located together on a mobile genetic element (e.g., a plasmid, transposon, or integron).

**Cross-resistance:** Resistance occurring when the same or similar mechanism(s) of resistance applies to different antimicrobials.

**Feral animals:** Animals descended from domestic populations, but that have established a self-sustaining population in the wild (e.g., feral cats, feral pigeons, feral pigs).

**Fertilising product:** Describes a substance, mixture, microorganism or any other material, applied or intended to be applied, either on its own or mixed with other material, on plants or their rhizosphere for the purpose of providing plants with nutrients or improving their nutrition efficiency.

**Filter-feeder:** An aquatic animal that feeds on particles or small organisms extracted from water by circulation through its filtering system: includes most of the stationary feeders, such as clams, oysters, barnacles, corals, sea squirts, and sponges.

**Heavy metal:** Naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water.

**Heavy metal resistance:** Bacteria are considered to be resistant to heavy metals when: 1) a strain is not killed or inhibited by a concentration to which the majority of strains of a organism are susceptible, or 2) when bacterial cells that are not killed or inhibited by a concentration acting upon the majority of cells in that culture.

**Herbivores:** Animal species that has plants or plant-derived material as its primary food source.

**Horizontal gene transfer:** Transfer of genetic material between bacterial cells due to other processes than cell division. E.g. transduction, transformation, and transduction.

**Indicator bacteria:** Bacteria used to measure the hygienic conditions of food, water, processing environments etc. Indicator bacteria are not usually pathogenic, but their presence indicates that the product or environment tested may be contaminated with pathogenic bacteria, often originating from the same reservoirs as the indicator organisms.

**Integron:** Integrons are assembly platforms — DNA elements that acquire open reading frames embedded in exogenous gene cassettes and convert them to functional genes by enabling their expression.

**Intrinsic resistance:** A natural property of an organism resulting in the absence of or a decreased susceptibility to a particular antimicrobial agent.

**Isolate (bacteria):** A bacterial isolate is a single isolation in pure culture from a specimen.

**Microbiota:** Collective term for microflora (i.e., any type of microorganism) that may be found within a given environment.

**Minimum Inhibitory Concentration:** The lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions. MIC data can provide information about the activity of antimicrobials.

**Minimum Selective Concentration:** The lowest concentration of an antimicrobial agent that can select for AMR.

**Multi-drug resistant bacterium:** a bacterial isolate resistant against at least three antimicrobial agents.

**Natural environment:** The biotic and abiotic surroundings of a given individual, population, or species, human or animal. In this report, other humans, other animals or manmade constructions are not included in the concept of environment; thus the natural environment denotes the biotic and abiotic components of plants, soil, sediments, water, air etc. with which an individual, population, or species comes into contact.

**Normal flora:** Indigenous microbial flora of human/animal external and internal surfaces like the skin, mouth, and gastrointestinal tract, and the upper respiratory tract. The normal flora contains numerous bacterial species, and numerous strains within each species. Although some may contain opportunistic pathogens, most are symbiotic or commensals that contribute to general health, as well as to colonisation resistance.

**Omnivores:** Animal species that utilises both plants and animals as food sources.

**Prevalence:** The proportion of cells in a particular population with a specific trait. In this context AMR. Often reported as a percentage. Point prevalence is the proportion of cells at a given timepoint with the trait, and period prevalence is the proportion of cells with the trait over a specific time period. The term prevalence is usually understood and refers to a defined population. The term occurrence can describe observations in a larger community with different populations. However, the prevalence in a particular bacterial population (species) in that same environment may still remain low.

**Reservoir of AMR:** One or more epidemiologically connected populations (reservoir hosts) or environments (reservoir environments) in which a microbe with a certain AMR trait can be permanently maintained, and from which the trait may be transmitted to a defined target population.

**Sanitizer:** An agent that reduces microbiological contamination.

**Selection (bacteria):** A process by which some bacterial species or strains of bacteria in a population are selected for due to having a specific growth or survival advantage over other microorganisms. Antibacterial substances may provide a more resistant sub-population with such an advantage, enabling them to increase their relative prevalence.

**Sterilization:** The process of destroying all microorganisms (including spores).

**Strain (bacteria):** A subset of a bacterial species differing from other bacteria of the same species by some minor, but identifiable, difference.

**Susceptibility:** Describes the extent to which a target microorganism is affected by an antimicrobial agent.

**Transduction:** Transfer of genetic material from one bacterium to another via bacteriophages (viruses that infect bacteria and are integrated into the host genome).

**Transferable resistance:** Antimicrobial resistance that can be transferred between bacteria, and their mobile-encoded resistant genes can be next transferred to other bacteria.

**Transformation:** Direct uptake of fragments of naked DNA from the environment and their incorporation into the cell's own genome.

**Transposon:** A segment of DNA that is capable of moving into a new position within the same or another chromosome or plasmid. Also called jumping gene.

**Vector of AMR:** May broadly refer to any living creature or object that can transmit AMR from one host to another. In a stricter sense, the term may refer to arthropods that can transmit bacteria with AMR from one host or environment to a host. A vector can function only as a mechanical transmitter, but in other cases may itself be infected. In the latter case, it is a multiplicative vector (or host).

**Wild animals:** Animals with a phenotype unaffected by human selection and living independently of direct human supervision or control, i.e. living and roaming freely in their natural environment, and not domesticated or tamed.

**Wildlife:** Feral animals, captive wild animals and wild animals (OIE 2017).

# Background provided by the Norwegian Environment Agency

Development of antimicrobial resistance (AMR) is a fast-growing problem in the world. The national strategy against antibiotic resistance for 2015-2020 of the Norwegian Government highlights that this problem should be considered in a holistic perspective, where human and animal health and environment interact. Use of antibiotics results in development of resistance, but other factors may also play a role. The presence of resistant bacteria in different environments, such as soil, water, sediments, and wildlife, may all contribute to development of resistance in bacteria of pathological relevance. AMR in the environment is influenced by a variety of anthropogenic factors. In the strategy of the Norwegian Government, increased knowledge on development of antibiotic resistance is indicated as one goal. The strategy is built on the report "*Antibiotikaresistens – kunnskapshull og aktuelle tiltak (2014)*" compiled by an expert group.

An increasing number of papers have been published describing AMR in the environment. These studies vary according to type and amount of animals or environment analysed, as well as methods used. However, together, data from these studies provide important information regarding the role of wildlife in dissemination of AMR. Consequently, we hereby ask VKM to summarise relevant studies and, depending on data available, perform a risk assessment of the potential dissemination of AMR in wildlife in Norway. The overall goal of the current assignment is to achieve a better understanding of the potential for dissemination of AMR by wildlife. This will give valuable background for the next steps of the strategy against antibiotic resistance, regarding research, mapping, standardisation of methods, and relevant environmental measures.

Studies and scientific publications regarding AMR in all types of wildlife should be included. All varieties of transferable AMR are relevant, but those that are of clinical relevance are of particular interest. Studies from all part of the world may be included in this assessment, if considered relevant. The possible role of wildlife in dissemination of AMR should be discussed in general terms, but environment and wildlife of relevance in Norway may be considered in particular.

## Terms of reference:

1. List the transferable antimicrobial-resistant bacteria (ARB) identified in wildlife. The list should include type of animals, preferably divided into terrestrial and aquatic animals, in which AMR bacteria have been described.
2. List the different methods used for sampling and analysis of data. Based on information collected, evaluate the suitability of the different methods used.
3. Evaluate information on AMR in bacteria in wildlife, according to their habitat. Preferably, information should be grouped into animals living close to urban areas, living in rural areas, or that are migratory.

4. Based on the information collected, and if sufficient data are available, assess;
  - a. the possibility of ARB being transferred between wildlife and other hosts,
  - b. possible routes for antimicrobial residues to induce AMR bacteria in the environment,
  - c. possible routes for domestic animals to disseminate ARB to wildlife and vice versa,
  - d. the possible exchange routes for ARB between humans and wildlife.

Question 4 is limited to an overall assessment of each sub-question (a, b, c, d) and not expected to be answered in detail. An estimate of probability or thorough risk assessment is not part of this assignment.



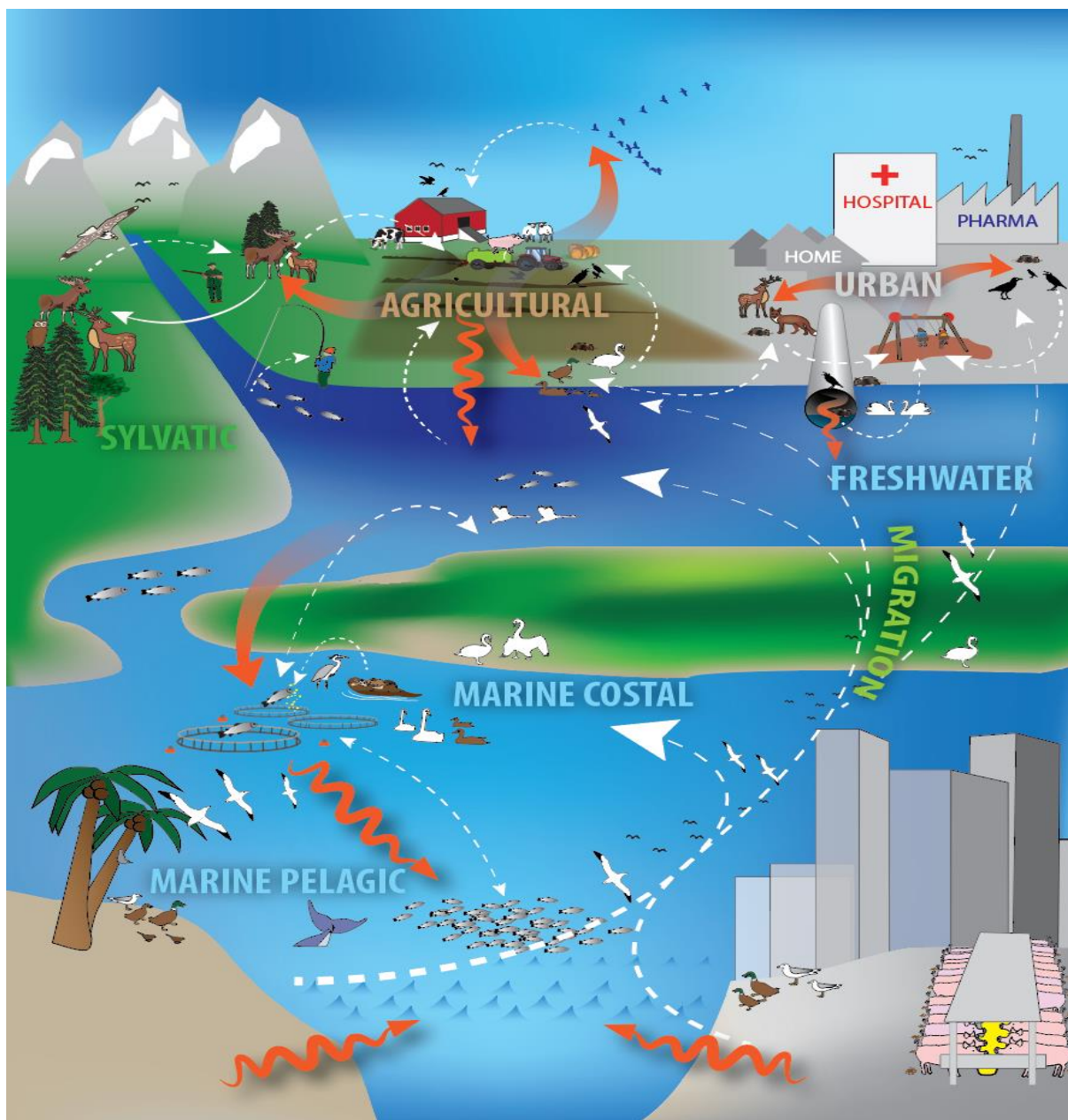
# 1 Introduction

Antimicrobial resistance (AMR) has become a global clinical and public health threat against the effective treatment of common infections caused by resistant pathogens, resulting in treatment failure and increased mortality. Development of AMR is a part of natural evolution of microbial populations, but widespread use and misuse of antibacterial agents in humans and animals has accelerated this process (WHO, 2014).

The development of AMR is expected to occur in nature as a defence by microbes against naturally occurring antibiotics. However, the much higher concentrations, and resulting selective pressure, generated by use of antimicrobial agents (in human medical, veterinary, husbandry, and agricultural practices) have been the major driving force leading to the broad emergence and spread of resistance traits among pathogenic bacteria, and which have been observed since the beginning of the antibiotic era (reviewed by Pallecchi et al. (2008)). Examples of acquired AMR (i.e., resistance to antimicrobials to which the bacterial species is intrinsically susceptible) have also been detected among commensal bacteria isolated from humans and wildlife that have not been subject to significant antimicrobial exposure and living in remote areas. These unexpected observations underscore the complexity of the mechanisms involved in the emergence and spread of AMR.

The review article of Vittecoq et al. (2016) summarises several articles that have focused on the presence of AMR-bacteria (ARB) in wildlife. The main measure against AMR currently being applied in European countries is reducing the use of antimicrobial agents in both human and domestic animals, as it is clear that these two compartments are closely linked. This measure is based on the assumption that acquired AMR is associated with fitness costs where there is no selection linked to antimicrobial drugs. Yet it appears that these costs are highly variable and can be reduced or turned into fitness benefits by compensatory mutations. Additionally, in some cases the same mechanism, or a mechanism found on the same genetic element, can confer resistance to both antimicrobial drugs and other pollutants. Thus, the general chemical pollution of environmental reservoirs can contribute to the development and maintenance of ARB. Finally, bacteria with resistance to some antimicrobials are naturally found in soils in the absence of exposure to anthropogenic antimicrobial drugs and assumed to be the result of exposure to antibiotic molecules produced by some bacteria and fungi. The latter exposure scenarios are, however, rarely quantifiable, and occur at many orders of magnitude lower than those associated with the anthropogenic sources of antimicrobials (Vittecoq et al. (2016)).

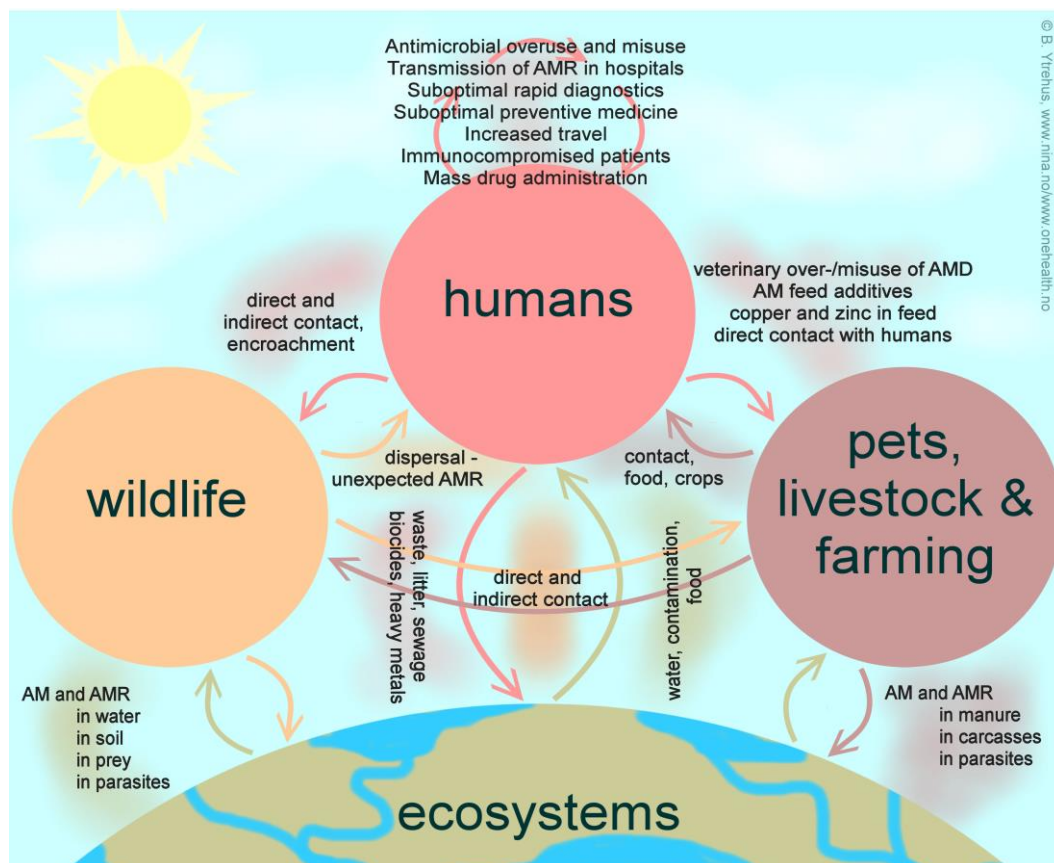
Direct exposure of antimicrobials to wildlife is rare, indicating that environmental contamination with antimicrobial agents and ARB, in association with sharing common habitats and water sources, is important (Wellington et al., 2013). This could result in transfer of AMR traits and bacteria between wildlife and domestic animals, with the potential for subsequent transmission to the food chain. In addition, AMR bacteria from wildlife may directly contaminate foods of plant origin (Jay et al., 2007). Wildlife populations have the



**Figure 1.** The figure illustrates important interfaces between wildlife, domestic animals, and humans. Most antimicrobial resistance (AMR) in bacteria is associated with anthropogenic use of antimicrobial drugs. Wildlife is exposed to resistant bacteria, resistance genes, and resistance-promoting substances in sewage, waste, pollution and manure via direct or indirect contact with other wildlife, humans, livestock, and pets (red arrows). Antimicrobial resistant bacteria has been reported from a variety of wildlife, with highest prevalences in those wildlife species that live in urban environments and close to sites of intensive terrestrial and aquatic animal production. Information on the importance of wildlife in transfer of AMR and ARB to human and domestic animal populations (including aquaculture) is limited. A number of interfaces between different environments and populations of wildlife, and between wildlife, humans, and domestic animals (white arrows) are known to exist and could facilitate transfer of AMR and ARB. Research is warranted, in particular, on the role of migratory wildlife species in long-distance spread of AMR from other parts of the world with other AMR patterns (illustrated in the lower lefthand corner) and from areas with higher human population densities and more intensive animal production (illustrated in the lower righthand corner).

potential to act as reservoirs for AMR and emerging resistant pathogens (**Figure 1**). A number of review articles are available on AMR and wildlife (Bonnedahl and Jarhult, 2014; da Costa et al., 2013; Greig et al., 2015; Guenther et al., 2011; Marinho et al., 2016; Pallecchi et al., 2008; Radhouani et al., 2014; Tusevljak et al., 2012; Vittecoq et al., 2016; Wang et al., 2017a).

For the purpose of this report, **environment** is defined as the **natural environment** (See glossary). Wildlife animals are categorised as an environmental compartment because they are not treated with antimicrobial agents, and their carriage of bacteria with transferable resistance traits is considered mainly to be the outcome of uptake of resistant bacteria from the natural environment (Huijbers et al., 2015), and possibly through the accidental exposure of their own microbiota to pharmaceutically produced antimicrobial drugs. The One Health concept incorporates the increasing awareness that pathogens and resistance genes flow between human, animal and wildlife populations (Figure 2).



**Figure 2.** The One Health approach: the concept that the health of the human population, the health of domestic animals, the health of wildlife, and the state of the ecosystems are intrinsically linked. Processes that affect one of these “systems” inevitably have an impact on the others. This conceptual figure describes some of the key factors for transmission in the interfaces between the systems and drivers for development of antimicrobial resistance (AMR) in the human population and domestic animals (the latter described by (Castro-Sánchez et al., 2016; Holmes et al., 2016; Robinson et al., 2016).) (Figure: B. Ytrehus, Norwegian institute for nature research). AM: antimicrobial; AMR: antimicrobial resistance; AMD: antimicrobial drugs.

## **1.1 Relationship between global consumption of antimicrobial agents and development of resistance**

According to the World Health Organization (WHO), AMR develops over time, usually through genetic changes. In many places, antimicrobials are overused and misused in people and animals, and often taken without professional recommendation. Examples of misuse include when they are taken by people with viral infections like colds and flu, are prescribed for prophylaxis, and when they are given as growth promoters in animals or used to prevent diseases in healthy animals.

All bacteria, including ARB, do not “respect” borders and resistance determinants may be transferred and disseminated between unrelated bacterial species from various animal species, including wildlife, and between environments. WHO has developed an action plan that underscores the need for an effective “One Health” approach, and involves coordination among numerous international sectors and actors, including human and veterinary medicine, agriculture, finance, environment, and consumers. This plan describes the key actions that the various actors should take, using an incremental approach over the next 5-10 years to combat AMR

([http://www.wpro.who.int/entity/drug\\_resistance/resources/global\\_action\\_plan\\_eng.pdf](http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf)).

## **1.2 Key emerging antimicrobial resistant bacteria (ARB)**

One challenge when assessing the risk of AMR, is that only a small fraction (approx. 1 %) of environmental bacteria are culturable, and this places a considerable limitation on our knowledge about the true diversity and composition of this reservoir (Finley et al., 2013). This limitation is addressed in the opinion paper by (Berendonk et al., 2015), which emphasises “current risk assessment models are inadequate to evaluate the effect of antimicrobials and antimicrobial resistance genes on resistance emergence and selection, especially in non-clinical environments”. Acknowledging these limitations, the VKM panel has given particular attention to some specific resistant bacterial species that have emerged at the animal-human interfaces in recent decades. In particular, high-risk clones of these pathogenic species seem to have the propensity for epidemic spread and are able to establish themselves in both animals and humans. These species have a zoonotic potential and there are limited alternatives for treatment of infections caused by these bacteria.

### **1.2.1 Vancomycin-resistant Enterococci (VRE)**

Resistance to the glycopeptide vancomycin emerged in enterococci (primarily *E. faecium*) in the late 1980s in both Europe and USA. VRE have intrinsic resistance to most of the commonly used antimicrobials and the ability to acquire resistance to most of the current available antimicrobial agents, either by mutation or by acquisition of foreign genetic material. Thus, they have a selective advantage in the intestinal flora during antibiotic exposure and pose a major therapeutic challenge. The potential of further transfer of

vancomycin-resistance genes to other Gram-positive organisms raises significant concerns about the emergence of vancomycin-resistant *S. aureus* (Cetinkaya et al., 2000).

### **1.2.2 Methicillin-resistant *S. aureus* (MRSA)**

Semi-synthetic penicillins, such as methicillin, were introduced in the late 1950s as a response to the rapid development of penicillinase-producing *S. aureus*. Subsequently, a wave of hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) strains emerged. From the mid-1990s and onwards, MRSA with novel properties also become more broadly dispersed in the community. This community-acquired MRSA (CA-MRSA) combined rapid spreading ability with resistance to antimicrobial agents. Livestock-associated MRSA (LA-MRSA) were detected for the first time a decade ago but are now globally distributed (Vanderhaeghen et al., 2010).

Through horizontal gene transfer (HGT), MRSA have acquired the gene *mecA* (or *mecC*). The *mecA/mecC* gene is located on a complex mobile genetic element, named the staphylococcal chromosomal cassette, *SCCmec*, which was probably acquired from coagulase-negative staphylococci (CoNS) (Hanssen and Ericson Sollid, 2006). *SCCmec* elements may be disseminated between staphylococcal species, but, to date, this element has not been detected in bacterial species other than staphylococci.

### **1.2.3 Quinolone-resistant (QR) Gram-negative bacteria**

Quinolone antimicrobials are potent, broad-spectrum antimicrobial agents commonly used to treat a range of infections. Resistance to these agents is mainly introduced by chromosomal mutations in the genes that encode the enzymes targeted by the antimicrobial. The resistance level can increase via production of multidrug-resistance efflux pumps, modifying enzymes, and/or target-protection proteins, or combinations of these. Resistance towards the synthetic quinolone, nalidixic acid, requires only one mutation, whereas resistance towards more broad-spectrum fluoroquinolones needs two or more mutations. Genes encoding for quinolone resistance are mainly located on the chromosome, but transferable genes located on plasmids have also been described (PMQR). These resistance mechanisms usually result in only a slight increase in the MIC of quinolones, but they have an additive effect and may facilitate acquisition of full quinolone resistance (Ruiz et al., 2012).

### **1.2.4 Extended-spectrum beta-lactamase (ESBL/pAmpC)-producing bacteria**

Resistance in Gram-negative bacteria to extended-spectrum cephalosporins, like cefuroxime, ceftazidime, and cefotaxime, has been developing over two decades. It is most often caused by extended-spectrum  $\beta$ -lactamases (ESBLs) (class A, termed ESBLA), but may also be facilitated by plasmid-mediated AmpC-type enzymes (Class C, called ESBLM or pAmpC). Hyper-production of AmpC-type enzymes due to chromosomal mutations can mediate resistance to cephalosporins. Plasmids that harbour ESBL and/or pAmpC genes may also

carry other resistance genes, meaning that ESBL/pAmpC-producing pathogens can also be resistant to other classes of antimicrobial agents (MacVane et al., 2014). The main bacterial family associated with ESBL/pAmpC production is the Enterobacteriaceae, of which *E. coli* and *Klebsiella* spp. are the most important. Further development of resistance genes encoding ESBL/pAmpC enzymes can occur either by emerging bacterial clones or by HGT, due to the spread of plasmids between bacteria of the same and/or different species (Brolund et al., 2014).

### **1.2.5 Carbapenemase-producing (CP) bacteria**

Carbapenemases are another emerging mechanism for resistance to  $\beta$ -lactams; these enzymes cause resistance to carbapenems, as well as other  $\beta$ -lactams (class B) (Nordmann, 2014). Carbapenem resistance is commonly associated with combined resistance to 3rd-generation cephalosporins, aminoglycosides, and fluoroquinolones (ECDC, 2012). Carbapenemases are biochemically diverse. It is also increasingly evident that carbapenem-resistance can be conferred through other mechanisms, like AmpC enzymes and beta-lactamases, in combination with mechanisms that limit carbapenem entry into bacterial cells (Sartelli et al., 2014). Therapy options are limited for patients with infections caused by carbapenemase-producing bacteria and there are significant limitations to the few existing alternatives to carbapenems. Multidrug resistant (MDR) Enterobacteriaceae, mostly *E. coli* and *Klebsiella pneumoniae*, with resistance to carbapenem conferred by New Delhi metallo- $\beta$ -lactamase 1 (NDM-1) have the potential to become a major human health problem globally.

### **1.2.6 Colistin resistance and plasmid-mediated colistin resistance (*mcr-1*)**

Enterobacteria containing NDM-1 are highly resistant to all antimicrobial agents, except tigecycline and colistin (polymyxin) (Kumarasamy et al., 2010). Until now, colistin resistance has emerged via chromosomal mutations and, although clonal outbreaks have been reported, the resistance is often unstable, imposes a fitness cost upon the bacterium, and is incapable of spreading to other bacteria (Falagas et al., 2011). A recently published paper (Liu et al., 2016) from China reported a major increase in colistin resistance in commensal *E. coli* from food animals in China. The authors found an *E. coli* strain isolated from a pig contained colistin resistance that could be transferred to another strain. The study resulted in the identification of the first plasmid-mediated polymyxin-resistance mechanism, MCR-1, in Enterobacteriaceae. Enterobacteriaceae containing *mcr-1* gene have now been isolated from food and humans in Denmark (Litrup et al., 2017; Roer et al., 2017).

## **1.3 Classification of antimicrobials according to their importance in human and veterinary medicine**

### **WHO**

In 2005, the WHO organised a consultation in Australia to develop a list of antimicrobial agents in human medicine. This list divided antimicrobial agents used in human medicine into three different categories. Each antimicrobial agent (or class) was assigned to one of three categories of importance on the basis of two criteria: **a.** the agent or class is the sole therapy or one of few alternatives to treat serious human disease; and **b.** the antimicrobial agent or class is used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources.

The 3 categories were:

Critically important antimicrobials - those that meet both criteria.

Highly important antimicrobials - those that meet 1 of the 2 criteria.

Important antimicrobials - those that do not meet either criterion.

This list was generated in an effort to provide a tool for developing risk-management strategies and to focus resources to address antimicrobial use in agriculture and veterinary medicine. Until that time, there had been no international consensus on the classification of different groups of antimicrobial agents according to importance. The WHO convened a second meeting in Copenhagen, Denmark, in 2007 to re-evaluate the classification and update the list on the basis of recent developments. Relatively few changes were needed. (See Appendix I for the list of the three categories of antimicrobial agents).

## **OIE**

Similarly, OIE has ranked veterinary antimicrobial agents as critically important, highly important, or important to animal health, according to the same criteria as used by the WHO. When the lists of critically important antimicrobials are compared, some classes appear only on the WHO list (carbapenems, ansamycins, glycopeptides, streptogramins, and oxazolidinones), whereas others appear only on the OIE list (phenicols, sulphonamides, diaminopyrimidines, and tetracyclines). However, for some classes there is an overlap, such that some classes of antimicrobial agents are listed as critically important for human health by WHO and critically important for animal health by OIE. These are 3<sup>rd</sup>- and 4<sup>th</sup>-generation cephalosporins, quinolones (including fluoroquinolones), macrolides, penicillins, and aminoglycosides. This overlap highlights the need for AMR surveillance, and to be able to identify and implement appropriate management measures in order to mitigate resistance dissemination and maintain the efficacy of the drugs. Prudent use of all antimicrobials is considered essential (FAO/WHO, 2008).

## **1.4 Literature assessed in this opinion/report**

### **1.5 Search strategy**

The search was conducted in PubMed, EmBase, ScienceDirect, and Web of Science using the terms listed in Table 1 [Title/Abstract] AND Antimicrobial resistance or Antibiotic resistance [Title/Abstract] AND Wild animals [Title/Abstract] using the Advanced Search Builder provided in above mentioned databases. The search resulted in 1267 studies (29. May-2. June 2017). The search term Wild animals was used instead of Wildlife as it identified more studies.

In addition, due to the focus on AMR in wildlife in Norway, the NORM-NORM/VET reports from 2002-2017 were also used.

#### **1.5.1 Inclusion criteria**

- AMR bacteria
- Occurrence in wild animals/wildlife

All relevant articles with no date restriction were included.

#### **1.5.2 Exclusion criteria**

Articles/reports describing development of resistance in microorganisms other than bacteria, such as viruses, fungi, and parasites, were excluded as these were not part of the mandate. Articles focusing only on zoo animals (wild but not wildlife), meat (from wildlife animals), and wild animals at live markets were excluded. Articles that were not in English or a Scandinavian language (Swedish, Danish, and Norwegian) were also excluded.

## **1.6 Literature**

Titles and abstracts of all citations identified were screened manually and those that did not relate to the terms of reference were excluded. Of those of potential relevance, the full text was obtained and assessed whether it was of relevance to this Opinion (Appendix II). Short descriptions of the original articles are found in Table 1 to Tables 7, in Appendix II.



**Table 1-** Search terms used to identify studies considered in this Opinion.

Database	Search terms 1	Search terms 2	Search terms 3	Sum	Comments
PubMed	Antimicrobial resistance AND Wild animals, AND Review  n=93	Antimicrobial resistance AND Wild animals, (Title and Abstract)  n=167	Antibiotic resistance AND Wild animals, (Title and Abstract)  n=270	n=530	
Science Direct	Not done (N. d.)	Antimicrobial resistance AND Wild animals, (Title and Abstract)  n=42	Antibiotic resistance AND Wild animals, (Title and Abstract)  n=5	n=47	
Embase	N. d.	Antimicrobial resistance (all fields) AND Wild animals, (Abstract)  n=52	Antibiotic resistance AND Wild animals, (Title and Abstract)  n=16	n=68	
Web of Science - Scopus	N. d.	Antimicrobial resistance (all fields) AND Wild animals, (Title/Abstract)  n=296	Antibiotic resistance AND Wild animals, (Title and Abstract)  n=326	n=622	
Sum				n=1267	
Duplicates				n=381	Removed
Excluded articles; irrelevant, other language than mentioned in inclusion criteria				n=504	Removed

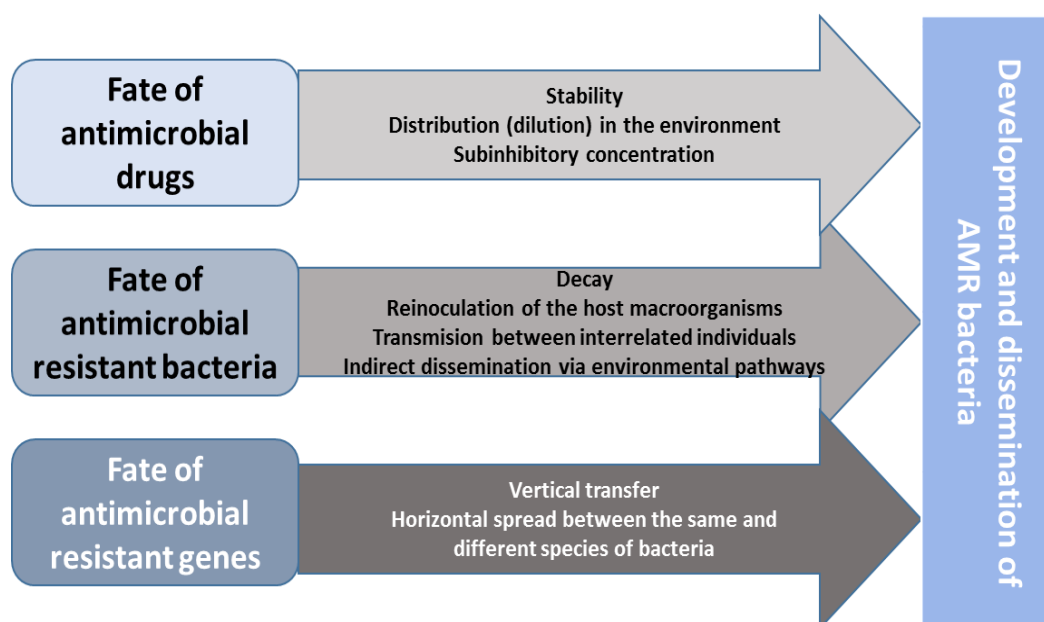
Remaining articles				n=342	
<b>Included articles:</b>					
Full-text articles provided and evaluated. Articles that fulfilled the criteria were included in this assessment: n=237. For details ; see Tables 1-7 in Appendix II. The total number of articles in appendix II is 264, since several articles were included in several animal groups. In addition, we included 6 reports (NORM/NORM-VET) describing AMR in wildlife in Norway.					

### Reports considered of particular relevance to Norwegian environments

- NORM/NORMVET reports (n=6, from 2002-2017)

## 2 Hazard identification

Hazard identification is implicit in the title of this opinion and in the terms of reference (ToR). The issue of AMR in the wildlife is addressed either as a **direct** hazard or as an **indirect** hazard through resistance formation and transfer. The direct hazard is caused by exposure to resistant bacteria with pathogenic properties in wildlife. The indirect hazard is caused by the presence of antimicrobial drugs with selective properties or genetic material with the potential for HGT. In both of the latter cases, previously susceptible bacteria with pathogenic properties may become resistant to antimicrobial drugs. These hazards may materialize into an adverse effect and hence have consequences for veterinary and public health by limiting treatment options for some bacterial infections. The fate of antimicrobial drugs, resistant bacteria, and their resistance genes is illustrated in **Figure 3**:



**Figure 3.** The development and dissemination of AMR in the environment is influenced by the release and stability of antimicrobial agents, the fate of ARB from humans and animal sources and the potential for HGT of resistance genes, modified after da Costa et al. (2013).

# 3 Hazard characterisation

## 3.1 Theoretical background

For a hazard to result in an adverse effect, the following steps must be considered:

1. **presence** of resistance genes, resistant bacteria, and/or anthropogenic antimicrobial drugs or other AMR-driving substances in the environment of the animal,
2. **exposure** of the wildlife resulting in uptake of the resistance genes, resistant bacteria, or AMR-driving substances,
3. **establishment** in the wildlife of a population of ARB that are viable for long enough for transmission of bacteria or resistance to other wildlife, humans, or domestic animals,
4. **contact** between individuals of the wildlife population and other wildlife, humans, or domestic animals sufficient to facilitate transmission.

### 3.1.1 General concepts of antimicrobial resistance

Development of AMR is increasingly used to explain treatment failure for infections caused by bacterial pathogens previously susceptible to the same antimicrobial agent. Thus, in a clinical context, AMR is understood as an emerged trait that leads to treatment failure and therapy changes.

In most cases, newly developed AMR is also considered an acquired trait that is transferable between bacterial cells, species, and populations. The genetic basis for the acquired resistance trait will determine its potential for further vertical and horizontal dissemination in and between bacterial populations.

In a clinical context, levels and changes in levels of AMR are measured *in vitro* as the minimum inhibitory concentration (MIC) ([http://www.eucast.org/mic\\_distributions\\_and\\_ecoffs/](http://www.eucast.org/mic_distributions_and_ecoffs/)). When strains of bacterial pathogens develop/acquire traits that enable them to withstand concentrations that would be used *in vivo* during drug therapy they will be described as resistant. If they remain susceptible to increased concentrations of the particular antimicrobial agent they are considered intermediate resistant. The exact concentrations of antimicrobial agents that are achieved *in vivo* vary by drug and hence the concentration level of each drug/pathogen combination that set the limits between sensitive, intermediate, and resistant are described as clinical breakpoints ([http://www.eucast.org/clinical\\_breakpoints/](http://www.eucast.org/clinical_breakpoints/)). For technical information regarding MIC-determination by disc diffusion testing and the broth microdilution method see

[http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Disk\\_test\\_documents/Versio n\\_5/Media\\_preparation\\_v\\_5.0\\_EUCAST\\_AST.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Versio n_5/Media_preparation_v_5.0_EUCAST_AST.pdf).

Experimental studies of changes in the resistance levels in non-pathogenic commensal bacteria, as well as bacteria found in natural environments, are also described using the same terminology. However, it should be kept in mind that these populations are not intentionally targeted with antimicrobials. Thus, only *in vitro* measures can be used. There are no systematic studies of resistance changes over time in such populations and breakpoints are not relevant or available due to the lack of *in vivo* infection models and treatment scenarios. Nevertheless, measurement of the effects of antimicrobials on any culturable bacterial species can be done in *in vitro* and the established methods to determine MIC are useful for descriptive studies of tolerance or resistance in non-pathogenic populations over time.

The selective effects of antimicrobials in bacterial species and communities present in non-clinical contexts are poorly understood. A precise understanding of such effects is challenging given the broad diversity of species potentially exposed, large tempospatial variations in exposure levels, variable growth dynamics of the populations involved, as well as the presence of both biotic and abiotic factors determining the degradation kinetics of antimicrobials. The term Predicted No Effect Concentrations (PNECs) was introduced by Bengtsson-Palme and Larsson (2016) based on EUCAST data, extrapolation, and modelling. The resulting PNECs for the antimicrobials considered ranged from 8 ng/L to 64 µg/L.

In addition to acquired resistance, many bacteria are not naturally susceptible to a particular antimicrobial compound or class of antimicrobial. This is due to the lack of the drug target or the presence of resistance mechanisms in the species. This phenomenon is called intrinsic resistance and is commonly observed and routinely considered in treatment of bacterial pathogens

([http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Expert\\_Rules/Expert\\_rules\\_i ntrinsic\\_exceptional\\_V3.1.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Expert_Rules/Expert_rules_i ntrinsic_exceptional_V3.1.pdf)).

The level and distribution of intrinsic resistance in complex bacterial communities are rarely known. Any experimental screen of resistance in complex bacterial communities, such as those found in the gut, soil, and water, will reveal a high number of non-susceptible isolates to the tested compound. Such phenotypic observations do not, however, indicate whether the resistance property was acquired at some timepoint and can be transferred further to other bacteria, or whether the property is due to intrinsic resistance and hence lack of initial susceptibility. The inability to discriminate between different types of phenotypic resistance in complex bacterial communities may confound the interpretation of resistance patterns seen in cultivation-based studies of natural populations.

Emergence of antimicrobial resistance is also considered a natural phenomenon occurring over thousands of years. It can be a mechanism of bacterial defence against natural antibiotics released by antibiotic-producing microorganisms. Whereas acquired resistance can be transferred both vertically and horizontally in bacterial populations, intrinsic resistance is largely limited to vertical transmission.

### **3.1.2 Resistance emergence, dissemination and persistence**

Acquired resistance to pharmaceutically produced antimicrobials is the outcome of a number of sequentially occurring steps occurring at the genetic, cellular, population, and community levels. These steps are governed by a range of biological and mechanistic factors, and are also affected by natural variation and randomness. The trajectories of new resistance traits emerging in a small proportion of cells in a larger bacterial population are complex and rarely fully amenable to detailed experimental analysis (Nielsen and Townsend, 2004; Pettersen et al., 2005). Thus, resistance emergence, dissemination, and persistence in bacterial populations are currently understood mainly through experimental sampling that is limited in time and space, combined with metagenomics, molecular epidemiology, and mathematical modelling.

Most analyses of resistance characteristics in bacterial populations focus on those bacterial species of clinical importance. Some countries also have annual monitoring systems in place (e.g., [NORM-NORM/VET website](#)). These systems focus mainly on a few key bacterial species that are culturable and are of clinical and/or veterinary importance. Few, if any, long-term monitoring systems are in place to catalogue changes in the levels of acquired resistance in bacterial populations unintentionally exposed to pharmaceutically produced antimicrobial agents.

Bacterial populations will, nevertheless, be exposed to resistant bacterial cells and their remains, as well as to antimicrobial agents from anthropogenic environments. Such directional exposure is considered to be pollution (Woegerbauer et al., 2015). Reciprocally, bacteria linked to anthropogenic environments will be exposed to bacteria from natural environments and possibly also to naturally occurring resistance traits and low levels of naturally produced antimicrobial agents. Exposure to resistance determinants should be considered multi-dimensional (Gatica and Cytryn, 2013).

Considering the quantitative properties of AMR bacteria, genes and selective compounds suggest exposure pathways are largely unidirectional (from human to natural environments). This expectation of directionality is caused by the use of high concentrations of pharmaceutically produced antimicrobial agents in anthropogenic environments, the release from human activities of a range of other compounds with selective and antimicrobial effects, as well as the massive release of bacteria with transferable resistance traits from agricultural production systems present in manure and sewage, and vesicles and extracellular DNA containing resistance determinants. See also section 3.2.2 in this opinion.

Below, we briefly introduce the key factors and processes behind resistance emergence, dissemination/dispersal, and persistence in previously susceptible bacterial populations.

#### **3.1.2.1 Emergence**

At the genetic level, both mutations and HGT events produce bacterial phenotypes with increased resistance levels. Mutations include both point mutations and larger intra-chromosomal rearrangements, such as relocation of mobile genetic elements within cells. HGT

events enable exchange of DNA between bacterial cells, populations, and species, and therefore lead to new resistance acquisitions. In many cases, multiple resistance traits can be acquired in a single HGT event (Domingues et al., 2012). HGT events can occur both in and between mobile genetic elements, such as plasmids, and in and between bacterial chromosomes (Domingues et al., 2015; Domingues et al., 2012; Thomas and Nielsen, 2005). The main mechanisms facilitating HGT are conjugation, transduction, and transformation. More recently, bacterial membrane vesicles have been described as possible important agents for HGT (Domingues and Nielsen, 2017; Fulsundar et al., 2014; Nemeth et al., 2017). Bacterial membrane vesicles are released by many bacterial species and can be found in natural environments (Biller et al., 2014; Domingues and Nielsen, 2017). The lumen of some vesicles may contain DNA that can contribute to HGT processes. The potential of membrane vesicles in HGT processes remains to be investigated in natural systems. However, they may be important because they exist independently from the donor bacterium in space and time, and some fuse with the membranes of different species of bacteria (Fulsundar et al., 2014; Nemeth et al., 2017).

Several studies suggest that microbial exposure to antimicrobials increases the mobility of mobile genetic elements and hence the potential for HGT of resistance determinants (Beaber et al., 2004; Lopatkin et al., 2016). However, the extent, type, and concentration ranges remain mostly unresolved (Lopatkin et al., 2016).

Gene flow within and between bacterial populations varies in direction, frequency, and in the amount and type of genetic material transferred. This is caused by differences in bacterial donor and recipient populations due to size, activity, proximity, selection intensity and directionality, genetic properties, and cellular and environmental factors. In many cases, randomness in the occurrence of processes means HGT events may occur in some cases, but not others (Pettersen et al., 2005). The long-term survival of small populations with newly acquired resistance is also affected by Allee effects. Allee effects occur when a fitness component has a positive association with population size.

### ***3.1.2.2 Dissemination / Stability***

Mutations / HGT that result in new resistant phenotypes that can spread further in the bacterial population depend on a range of factors. Importantly, directional (positive) selection can lead to rapid clonal expansion and hence an increased relative proportion in the overall population. During clonal expansion, secondary HGT events and mutations may compensate and offset fitness costs associated with the acquired traits (Johnsen et al., 2009).

Both the processes of random genetic drift and directional selection affect the population dynamics and fate of new resistant bacterial phenotypes (Pettersen et al., 2005; Townsend et al., 2012). The strength of directional selection of resistant phenotypes is expected to vary dramatically with time and space, because concentrations of antimicrobials are rarely uniformly distributed/degraded in structured environments. Directional positive selection for many bacterial species and resistance traits occurs at concentrations of antimicrobials well below the

levels that inhibit growth *in vitro*. Selective effects have been reported at concentrations 100-fold to 1000-fold below the MIC level, but the actual minimal selective concentration (MSC) may be difficult to quantify in natural bacterial communities due to the impact of other selective forces (Bengtsson-Palme and Larsson, 2016).

Selection intensity in natural environments is considered a complex phenomenon that is affected by both abiotic and biotic factors (Nielsen et al., 2007; Rizzi et al., 2012). Biotic factors include the populations and characteristics of other bacterial species present in the same environment. Abiotic factors include the concentrations and characteristics of other compounds present in the same environment, such as heavy metals, disinfectants, other pharmaceutically produced compounds, agricultural chemicals, preservatives etc. (Venter et al., 2017), as well as the compound-specific degradation kinetics of antimicrobials (Kallenborn et al., 2008). Thus, selection in natural environments affected by anthropogenic activities is highly complex and rarely amenable to quantification.

In addition to the population genetic factors governing the fate of bacterial cells, the horizontal gene pool of mobile genetic elements has its own population dynamics and governing factors for spread and persistence (Domingues et al., 2015). Thus, the potential for resistance dissemination in a given bacterial species/population should be considered from both the characteristics of the particular bacterial cells and the properties of the horizontal gene pool.

It should also be mentioned that changes in the prevalence of resistance in a specific bacterial population / context can occur through means other than resistance acquisition. In the process of population replacement, previously rare phenotypes pre-existing in the population can become dominant, or variants present in other populations/environments can, through migration/dispersal, become new dominant members of a given population. In both cases, sensitive variants can be replaced by more resistant phenotypes.

Acquired resistance traits that have disseminated in bacterial populations often remain in the population over time, even when selective pressure is reduced (Johnsen et al., 2009; Johnsen et al., 2011). This is due to adaptation conferred by compensatory mutations that reduce the initial cost of carrying resistance traits in the absence of antimicrobial agents (Starikova et al., 2013; Starikova et al., 2012). The reversal of resistance, e.g., by changes in prescription practices, is challenging, confounded by genetic linkage to other adaptive traits, and should be considered a long-term process.

### **3.1.3 Key factors limiting our ability to describe antimicrobial resistance in natural environments**

Our ability to understand and describe resistance development in bacterial populations is currently limited by financial and practical constraints on sampling and analysis. The global populations of bacteria are enormous and we are only able to sample a minute fraction, even of pathogenic bacteria. A further complication is that bacterial populations are continually

evolving and the dissemination pathways and patterns are not fully understood and are subject to chance and variation (Singer et al., 2016).

Any experimental analysis is therefore limited, and may be unable to capture the dynamic properties of resistance in the bacterial population (Nielsen et al., 2014). Rapid saturation of experimental sampling efforts precludes sensitive analyses, and only bacterial species and cells with the highest prevalence will be characterised. For instance, for PCR analyses there are limits to the number of bacterial genomes that can be analyzed in single reactions (Nielsen and Townsend, 2004). Currently, our ability to predict further developments is limited and based on mathematical modelling and descriptive data from selected populations, often with preference/bias for bacterial populations of immediate clinical importance.

The development of rapid DNA sequencing technologies has substantially advanced our understanding of the genetic structure of some important bacterial populations. Nevertheless, we are far from having a robust understanding of the genetic coherence and level of gene flow in bacterial populations. Metagenomics-based approaches are mostly suitable for investigating the occurrence of predefined genetic resistance determinants in a sample. Such approaches do not aim to reveal linkage between resistance traits and bacterial species identifiers. Thus, they are rarely quantitative although relative amplicon numbers can yield some insights.

The main limitations for studies of HGT include the current inability to determine conclusively the specific conditions, location, and time that result in the HGT event that has subsequently multiplied in a bacterial population to the current level. In most cases, we do not understand the past, current, and future evolutionary trajectory of such event. Current resistance characterisation is mainly descriptive, and often linked to available pathogenic species in clinical settings:

[http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Resistance\\_mechanisms/EUCAST\\_detection\\_of\\_resistance\\_mechanisms\\_170711.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf)

### **3.2 Dissemination of antimicrobials and AMR in the environment**

Development, dissemination, and persistence of AMR in the environment are considered either as direct or indirect hazards,:

- The direct hazard is where the AMR is caused by antimicrobial drugs or other resistance-driving agents, and the resistance may cause an adverse effect (treatment failure).
- The indirect hazard arises through resistance transfer. In this case, the hazard is the resistance gene and further transfers that may cause adverse effect.



- In some cases, both hazards may occur; an ARB may cause a direct adverse effect as well as transfer the resistance to other bacteria, enhancing the resistance level in the population and community.

### 3.2.1 Antimicrobial residues

Globally, approximately, one tonne of antimicrobials are used every 5 minutes, all too often without any prescription. The use, overuse, and misuse of antimicrobials select for resistance in numerous species of bacteria (Harbarth et al., 2015).

Information on the types and amounts of antimicrobial agents used for therapy and prophylaxis throughout the world is not readily obtainable. Furthermore, it can be anticipated that there is considerable uncertainty in those figures available. Van Boeckel et al. (Van Boeckel et al., 2014), estimated global antimicrobial consumption patterns in human medicine based on national sales data from 71 countries. The consumption was expressed as “standard units” defined as one pill, capsule, or ampoule. Between 2000 and 2010, the consumption of antimicrobial agents increased by 35 % (from  $5.2 \times 10^{10}$  to  $7.0 \times 10^{10}$  standard units). Large countries with emerging economies, mainly the BRICS nations (Brazil, Russia, India, China, and South Africa), were overrepresented in the growth in consumption of antimicrobials, accounting for 76% of the total increase. Antimicrobial consumption varied significantly through the year, being highest during the cold seasons in countries far from the equator, or during rainy seasons nearer to the equator. The most important relative increase in this period was for monobactams (2031 %), glycopeptides (232 %), cephalosporins (94 %), and fluoroquinolones (65 %). In addition, increases were also noted for the two “last-resort” antimicrobial agent classes, carbapenems (45 %) and polymyxins (13 %). The highest gross consumer of antimicrobial agents was India with  $1.2 \times 10^{10}$  units (10.7 units per capita), followed by China with  $1.0 \times 10^{10}$  units (7.5 units per capita) and USA with  $6.8 \times 10^9$  units (22.0 units per capita).

In addition to antimicrobials used in human medicine, considerable amounts are also used for companion or food producing animals. E.g., in USA, the antimicrobials used for farmed animals are estimated to account for 80 % of the total annual consumption. The global trends regarding use of antimicrobials in food producing animals were reviewed by Van Boeckel et al., in 2015 (Van Boeckel et al., 2015), and a conservative estimate of global consumption for livestock animals in 2010 was 63 151 tonnes. The average annual consumption per kilogram of animals produced was 45, 148, and 172 mg kg<sup>-1</sup> for cattle, chickens, and pigs, respectively. The five nations with the largest share of global consumption were China (23 %), USA (13 %), Brazil (9 %), India (3 %), and Germany (3 %). The authors predict a global increase in consumption of 67 % by 2030, and a near doubling in BRICS nations within the same period.

In Europe, trends in antimicrobial consumption is well documented. In a recent ECDC/EFSA/EMA joint report on consumption of antimicrobials and occurrence of AMR (ECDC et al., 2017), it is documented that during 2013 the total consumption of antimicrobials in

the EU/EEA member states was 3908 and 8063 tonnes for humans and animals, respectively. Furthermore, the overall average consumption of antimicrobials, expressed in mg kg<sup>-1</sup> biomass, was also lower in humans than in food-producing animals. In 18 of the 28 countries included in the report, consumption of antimicrobials was lower or much lower in food-producing animals than in humans. In two countries, consumption was similar, and in the eight remaining countries, the consumption was higher or much higher in food-producing animals than in humans. The highest production-corrected consumption (mg kg biomass<sup>-1</sup>) was in Cyprus (425.8), Spain (317.1), and Italy (301.6). The lowest production corrected consumption was in Norway with 3.7 mg kg biomass<sup>-1</sup>. This low consumption in Norway is due to high fish production in aquaculture with only minor consumption of antimicrobial agents. Overall, in the EU/EAA countries, penicillins, macrolides, and fluoroquinolones were the most consumed antimicrobial classes in human medicine, when expressed in milligrams per kilogram of estimated biomass, whereas in veterinary medicine, tetracyclines, penicillins, and sulphonamides were the most used classes.

After excretion from humans, chemically unchanged antimicrobials or their metabolites enter the sewage system, where they are only partially removed during treatment (Da Costa et al., 2013). From animals, antimicrobials and their metabolites reach the soil, either directly during grazing or via manure as a fertiliser, and subsequently end up in streams, rivers, and lakes, and, finally, the sea.

Consumption of therapeutic agents for animals and humans is well documented in Norway. In 2015, a total of 36 996 kg of antimicrobials were used in human medicine, 5862 kg for animals excluding fish, and 301 kg for fish (NORM/NORM-VET, 2015). Although slightly more than 1.3 million tonnes of farmed fish were produced in Norway in 2015 (Directorate of Fisheries, Bergen, 2017), consumption of antimicrobials was 194 kg florfenicol, 82 kg oxolinic acid, and 25 kg oxytetracycline, measured as pure substance (Norwegian Institute of Public Health, Oslo, 2017).

In addition to antimicrobials, heavy metals, and biocides are well known to drive AMR. Norwegian use of residues of heavy metals and biocides has been extensively reviewed (VKM, 2016). Although most biocides and heavy metals are known to be high volume products, exact data on the use of disinfectant agents and heavy metals could not be obtained. However, based on data provided by the Norwegian Environment Agency, it was concluded that approximately 14 000 tonnes of disinfectant agents were used in 2015. Those used in highest quantities were: ethanol, sodium hypochlorite, propan-2-ol, propan-1-ol, quaternary ammonium compounds (QACs) H<sub>2</sub>O<sub>2</sub>, peracetic acid, pentapotassium bis(peroxymonosulphate) bis(sulphate), glutaral, and 2-phenoxyethanol. These agents were used in a variety of products in healthcare and hospital settings, in consumer products such as cosmetics, in household items and textiles, in disinfection of food production environments, in animal husbandry, and in or on food products. Due to their broad field of application, residues of many biocides will inevitably end up in the environment.

Heavy metals are naturally found in highly variable concentrations in most environments, including crust minerals, soil, water, air, and in biota. Generally, concentrations in the abiotic environment and in plants and animals, show an increasing trend as a result of human activity involving extraction of the heavy metals from minerals. Some heavy metals have multiple industrial, domestic, agricultural, medical, and technological applications. Heavy metals, like lead, arsenic, mercury, aluminium, zinc, chromium, and iron, are found in a wide variety of personal-care products. The chemical forms of the metals, either elemental or as, e.g., oxides, will strongly influence their antibacterial activity. Available information indicates that the amounts of heavy metals used in various products in Norway add up to over 200 000 tonnes annually, and can be ranked by quantity as follows: Zn > Cu > Cd > As > Ag > Hg (VKM, 2016; VKM, 2017). In addition, Zn and Cu have applications as feed additives for some food producing animals like pigs and poultry, with consumption levels in 2012 of 119 232 kg and 18 866 kg, respectively.

### **3.2.2 Antimicrobial-resistant bacteria (ARB) and resistance genes**

The ubiquitous nature of AMR is exemplified by the presence of resistance genes in an 11<sup>th</sup> Century A.D. Andean mummy examined by 16S rRNA gene high-throughput sequencing (Santiago-Rodriguez et al., 2015). The authors reported finding genes possibly coding for beta-lactamases, penicillin-binding proteins, and resistance to phosphomycin, chloramphenicol, aminoglycosides, macrolides, sulphonamides, quinolones, tetracycline, and vancomycin.

Emission of antimicrobial agents to the environment may occur at any stage in the lifecycle of any agent, including during production and during disposal of unused drugs (Crane et al., 2008). When given orally, the bioavailability of antimicrobial agents is variable, and a proportion of the agents will not be absorbed and thus follow the faeces. The absorbed fraction may, depending on the agent, be partially metabolised and excreted via faeces or urine. However, the majority of metabolites still possess antimicrobial activity, although it is lower than that of the parent drug (Crane et al., 2008). Furthermore, for antimicrobials intended for urinary tract and gastrointestinal infections, a therapeutic prerequisite is that the agents are active in the faeces and urine. Overall, it has been estimated that between 20 and 80 % of the antimicrobials used globally (depending on substance), will be released in an active form to the environment via urine and faeces (Andersson and Hughes, 2014).

The stability of antimicrobial agents in the environment depends on many factors, including the chemical properties of the agent itself, the water, soil and sediment properties, the nature of the indigenous microbiota in the different environmental compartments, and weather conditions such as precipitation and sunlight exposure. According to several authors, antimicrobial agents are not readily degradable in soil, sediments, or aquatic systems (da Costa et al., 2013; Halling-Sorensen et al., 1998; Lunestad et al., 1995; Manzetti and Ghisi, 2014; Samuelsen et al., 1991). However, there are large variations in environmental stability dependent on the class of agents. Tetracyclines are known to adsorb onto clay, soil, and sediment particles and make complexes with divalent and trivalent

cations (Lunestad and Goksøyr, 1990); this makes them less antimicrobially active, but also less degradable. This group are prone to photo-oxidation, provided sufficient light exposure. When in environments without sunlight, such as soil, manure, and marine sediments, tetracyclines are stable (Lunestad et al., 1995; Pikkemaat et al., 2016). In marine systems, quinolones seem to be highly stable, whereas nitrofurans are unstable in sediments (Samuelsen et al., 1991). The stability of macrolides seems to depend highly on the substance. Pikkemaat et al. (2016) reported that 60 to 80% of tylosin was degraded within 24 h under anaerobic conditions. However, the authors refer to a study in which lincomycin and spectinomycin in manure were stable for up to 150 days in lagoons. Quinolones display a strong binding ability to soil and sediment particles, giving them high stability in dark environments. On the other hand, light exposure results in some degradation of quinolones (Pikkemaat et al., 2016). Beta-lactams are rapidly hydrolysed under mild acidic and alkaline conditions (Huang, 2001), and are considered biodegradable. Thus, beta-lactams are probably the most readily degradable class of antibacterial agents in use. Although photo-degradation may occur, sulphonamides should be considered stable and are expected to be found in the environment in an active form (Pikkemaat et al., 2016). Provided sufficient light exposure, tetracyclines and nitrofurans are degraded in seawater (Lunestad et al., 1996). Little information on the environmental stability of aminoglycosides is available.

Thus, studies indicate that metabolites of antimicrobial agents persist at background concentrations in the environment, and accumulate in foods and drinking water, including ground waters, which were originally expected to be protected from contamination. The major source of such contamination is wastewater, and as current sewage treatment decontamination approaches do not remove antimicrobials compounds fully, a low but steady supply to as well as concentration remain in the environment and represent a source for low-level human and animal exposure to antimicrobial agents via drinking water, food, or feed.

After leaving the body of humans and animals, antimicrobial agents will be diluted and, with time, decrease to sub-inhibitory concentrations. However, even such low concentrations are likely to enhance AMR development and resistome expansion (Friman et al., 2015; Ter Kuile et al., 2016; You and Silbergeld, 2014). Sub-inhibitory concentrations may act as selectors for pre-existing resistant bacteria, and as generators for genetic and phenotypic variability and signalling molecules resulting in increased gene expression (Andersson and Hughes, 2014).

The fate of resistant bacteria in the environment depends on several interacting factors related to the organism itself and the environment. Whether possession of AMR genes will influence the survival of particular bacteria in the environment has not been fully explored, but keeping antimicrobial resistance genes in an environment without a selective pressure is considered to be negative for bacterial survival over time (Martinez, 2009). Bacteria originating from human or other homeothermic animal sources will have highly variable environmental survival and possibilities for multiplication. Faecal material from humans and homeothermic animals, harbour more than  $10^{12}$  cells  $g^{-1}$ , representing approximately

16 000 species of which fewer than 20 % have been cultured (Guarner and Malagelada, 2003). The bacterial proportion constitutes approximately 50 % of the weight of the faeces (Guarner and Malagelada, 2003). The density of *E. coli* alone in human faeces normally varies from  $10^6$  to  $10^7$  cells  $g^{-1}$  (Forsythe, 2010). A substantial proportion of the indigenous intestinal microbiota of humans and homoeothermic animals, including *E. coli*, is considered unable to multiply, but may survive for days to months in the environment, depending on conditions. Other representatives of the gut microbiota, such as the spore formers (*Clostridium* sp.), will be able to survive or even multiply in certain environmental compartments. In the environment, resistant bacteria may interchange genes with other indigenous bacteria, making the environment a melting pot for resistome expansion. Once in the environment, the genes conferring resistance in ARB of human and animal origin will add to the reservoir of resistance genes among the autochthonous bacteria (native bacteria from a particular region), and HGT of these genes can occur.

### 3.3 Wildlife

The occurrence of AMR bacteria in mammals, birds, reptiles, amphibians, and fish is a result of the environment in which they live and the extent to which the bacteria there are exposed to antimicrobial substances.

The following steps are necessary for a wildlife population to contribute to dissemination of antimicrobial resistance:

1. **presence** of resistance genes, resistant bacteria, and/or antimicrobial drugs or other AMR-driving substances in the environment of the wildlife
2. **exposure** of wildlife resulting in uptake of the resistance genes, resistant bacteria, or AMR-driving substances
3. **establishment** in the wildlife of a population of ARB that remain viable for long enough to allow transmission of bacteria or resistance to other wildlife, humans, or domestic animals
4. **contact** between individuals of the wildlife population and other wildlife, humans, or domestic animals sufficient to facilitate transmission.

The extent to which these steps occur will depend on the ecology of the wildlife species in addition to those factors related to the use of antibiotics and other AMR-driving substances and factors related to bacterial release and survival in the environment.

Important factors determining the potential role of a given species for dissemination of AMR will be

- habitat preferences
- behaviour
- food choice

Categorising wildlife according to these factors is difficult as many species utilise different habitats and food sources and have different behaviour according to age, physiological status, or time of year. Below, wildlife has been grouped according to main habitat, migratory behaviour, and food source selection. However, grouping in one category does not

exclude being grouped in another, as many species have subpopulations that are found in different habitats or explore different ecological niches in different periods of the year. In this section, for the sake of feasibility and readability, only **Norwegian-relevant species** will be considered.

#### 1. Terrestrial wildlife

This group includes all mammals, reptiles, and birds that predominantly move and find their food on land or in the air above land. Among mammals, all artiodactyls, lagomorphs, bats, hedgehogs, and shrews, and all carnivores except polar bears (*Ursus maritimus*) and otters (*Lutra lutra*) belong to this group. The rodents, beaver (*Castor fiber*), the muskrat (*Ondatra zibethicus*), and the coypu (*Myocastor coypus*) are also excluded from the terrestrial group and categorised as aquatic. Among birds, all pigeons/doves, cuckoos, galliforms, nightjars, swifts, woodpeckers, owls, and passerines and all raptors apart from osprey (*Pandion haliaëtus*) and white-tailed eagle (*Haliaeëtus albicans*) are included in this group.

##### a. Urban

Many species of wildlife have adapted to a life in an urban habitat. Some of these mainly live in towns and cities, for example the house sparrow (*Passer domesticus*), whereas for other species subpopulations have adapted to an urban life. Mallards (*Anas platyrhynchos*) in urban areas, for example, may show a migratory pattern and behaviour that differ from those of mallards in less populated areas (Størkersen, 2006). Other species may utilise urban areas and anthropogenic food sources or manmade installations suitable for breeding, hibernation etc. in certain periods of the year, but remain out of sight of humans at other times. For example, the black-legged kittiwake (*Rissa tridactyla*) may hatch on buildings in central harbour areas in the summer, but search for food far out at sea during winter. The common bullfinch (*Pyrrhula pyrrhula*) is a frequent guest on bird-feeders in gardens during winter, but often lives in deep forests during summer.

With an urban lifestyle, wildlife is exposed to a wide variety of anthropogenic substances, especially species searching for food in waste or sewage like rats, red foxes, gulls, and corvids (crows, ravens, and magpies). Such wildlife may be readily exposed to ARB. Furthermore, with their relatively close relationship with humans, could also easily transmit ARB to humans, domestic animals, and food.

##### b. Agricultural

Several species of wildlife rely on agricultural crops for food, live in ecotones created in a mixed landscape, or have adapted to agricultural landscapes similar to their original habitat. In such landscapes, they will be exposed to pesticides, a wide range of pollutants, and waste from production animals. In addition, faeces or other excretions from these animals could readily contaminate crops intended for human consumption and pastures used by farm animals. Animals that may be classified in this group include red fox (*Vulpes vulpes*), badger (*Meles meles*), water vole (*Arvicola terrestris*), common vole (*Microtus arvensis*), common wood pigeon (*Columba palumbus*), crow (*Corvus cornix*), jackdaw (*Corvus monedula*), starling (*Sturnus vulgaris*),

sky lark (*Alauda arvensis*), barn swallow (*Hirundo rustica*), house martin (*Delichon urbicum*), and several others.

c. Sylvatic

Many birds and mammals try to avoid humans as far as possible. Other species do not come into contact with humans or domestic animals as they live in habitats not frequently visited by them, or utilise food sources that are associated with anthropogenic activity to only a minor extent. Wildlife in this group will be exposed to anthropogenic ARB to a lesser degree, and only occasionally have such close contact with humans and/or livestock that they may transmit ARB to them.

2. Aquatic wildlife

In this context, aquatic wildlife are those species that live, or mainly find their food, in water or on the shoreline. Included in this group are all fish and amphibians, molluscs, crustaceans, mammals like beavers, muskrat, coypu, polar bears, otters, pinnipeds and cetaceans, and birds such as the white-tailed eagle and osprey, grebes, loons, ducks, geese, swans, storks, herons, waders, crakes and rails (*Rallidae*), kingfishers, seabirds like petrels, skuas, gulls, terns, auks, and cormorants, and one passerine species, the dipper (*Cinclus cinclus*).

a. Freshwater

Here, freshwater species are defined as those that spend most of their life in or close to ponds, lakes, rivers, and streams and find their food in the water or in its close vicinity. All species of freshwater fish are included in this group. The anadromic species, such as the salmonids, which migrate from freshwater to sea when they reach a certain age, and come back as reproductive adults, and the catadromic European eel (*Anguilla anguilla*) that hatch in the Sargasso sea, live for years in rivers and lakes in Europe, before returning to the Sargasso sea for spawning, are also included in this group (Righton et al., 2016). This is because they constitute a much larger proportion of the biomass in the freshwater habitat than in the sea, and are thus quantitatively more important as a potential source of introduction of AMR in freshwater than in the marine environment. However, their migratory behaviour warrants particular consideration in this report.

The amphibians are also included in the freshwater group, although all Norwegian species live on land for long periods of their life. We have classified them as freshwater species as the potential for spread of AMR associated with these species is probably greater in water than on land. For reptiles, we have included the European grass snake (*Natrix natrix*) in this group, as it prefers areas close to water and often hunts in the water. Among mammals, the European beaver, the musk rat, and the coypu are included in this category, as they spend a large proportion of their time in water and rarely are seen more than 100 m from water bodies. Some aquatic birds only very rarely visit marine environments. These include the dipper, kingfisher, and osprey and perhaps crakes and rails. However, most species that breed inland in Norway, spend some time during the winter or during migration on tidal flats, shorelines, or in coastal waters, and

can be categorised as “periodic marine birds”. For example, many of the geese, ducks, and waders that spend their summer in Norway, are found at the Wadden Sea on the coast of the Netherlands, Germany, and South-Western Denmark for some periods of the year. As for the amphibians, these birds are assumed to have a larger potential for transmission of ARB in their freshwater habitats than their marine habitats, and are therefore categorised as freshwater birds.

b. Marine coastal

Included in this group are molluscs, crustaceans, and fish that are relatively stationary along the coastline of Norway for large parts of the year, i.e., living in the fjords and shallow waters along the coast, and round and between islands. Some species, for example the Atlantic cod (*Gadhus morhua*) are divided into populations of coastal stationary and offshore migratory ecotypes. Coastal fish populations are exposed to runoff water from land, sewage, and human waste and contamination deposited in coastal waters. Many coastal crustacean and fish species are harvested for human consumption. These wildlife species are also in close contact with farmed fish, as they live in the same medium and thus share the same environment. They thereby have the potential to act as both source and recipient of ARB to/from the commercial fish farm industry. In this context, particular consideration should be focused on wild fish populations used as cleaner fishes. Cleaner fishes are used in salmon farms against salmon lice (*Lepeophtheirus salmonis*). Fish commonly used include different species of wrasse (Goldsinny wrasse (*Ctenolabrus rupestris*), Ballan wrasse (*Labrus bergylta*), Corkwing wrasse (*Symphodus melops*) and Cuckoo wrasse (*Labrus bimaculatus*)) and lumpfish (*Cyclopterus lumpus*).

Some species of seals and whales prefer coastal waters, including harbour seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*). Other seal species of Norway are associated with sea ice and Arctic waters, and categorised with marine pelagic species (see below). Among whales, most species have a migratory pelagic lifestyle, but harbour porpoises (*Phocoena phocoena*) may be relatively stationary within a coastal area for a period (MAREFA, 2012). An additional mammal categorised in this group is the Eurasian otter (*Lutra lutra*), which currently is mostly found along the coast in Norway, although previously was also common in inland rivers (Bevanger, 2015).

Among birds, large populations of seabirds are associated with coastal waters, living and feeding in areas of the sea that are affected to some extent by anthropogenic activity and land effluents. Common birds in such areas include divers (*Gavia* spp.), cormorants (*Phalacrocorax* spp.), marine ducks like common eider (*Somateria mollissima*) and common shelduck (*Tadorna tadorna*), white-tailed eagle, grey heron (*Ardea cinerea*), some waders like Eurasian oystercatcher (*Haematopus ostralegus*) and turnstone (*Arenaria interpres*), gulls (*Larus* spp.), terns (*Sterna* spp.) and Arctic skua (*Stercorarius parasiticus*).

c. Marine pelagic



This group consists of wildlife that live and find their food in or on the ocean. Most pelagic saltwater fish are included in this group, as well as most whales except the harbour porpoise. Apart from the harbour seal and grey seal, the five other seal species in Norway, ringed seal (*Pusa hispida*), harp seal (*Pagophilus groenlandicus*) hooded seal (*Cystophora cristata*), bearded seal (*Erignathus barbatus*), and walrus (*Odobenus rosmarus*), are Arctic species that are associated with pack ice and/or the Arctic coasts and waters. Seabirds often have a highly pelagic lifestyle, only visiting land during the breeding season. Use of sophisticated light-level geolocators and GPS-tracking systems has demonstrated that many of these species roam over large areas. Arctic terns (*Sterna paradisaea*), for example, migrate from breeding locations on Greenland and Iceland to Antarctica and, on their journey, travel along the coasts of Africa and South America (Egevang et al., 2010); similar migration patterns should be expected for Arctic terns breeding in mainland Norway and Svalbard. The long-tailed skua (*Stercorarius longicaudus*) similarly migrates from Svalbard (and North-East Greenland) to wintering areas in the Benguela upwelling along the coast of southern Africa and into the Indian Ocean south of Madagascar, with staging areas near Newfoundland, the Azores, the Mauritanian coast, and the Lesser Antilles (Gilg et al., 2013). Another species of relevance is the northern fulmar (*Fulmar glacialis*), not only because these birds roam over large areas of ocean, but also because of their feeding habits. Fulmars find their food on the surface of the sea, and swallow floating litter as bycatch. It is therefore common to find relatively large amounts of plastic material in the stomachs of fulmars (van Franeker et al., 2011), and it has been suggested (and disputed) that these birds are also exposed to increased amounts of persistent organic pollutants, as the litter acts as a “floating artificial compartment within reach of marine life” (Herzke et al., 2016). It seems plausible that increased exposure to anthropogenic litter may increase exposure to ARB or resistance-driving substances. The majority of the pelagic seabirds breed in colonies, often together with more coastal species. The long migrations coupled with colony breeding habits together with multiple other species, suggest that pelagic seabirds may play an important role in dissemination of AMR between locations that are distant from each other.

Wildlife populations with different predominant migratory behaviours occur within each group:

A. Long-distance migratory

Long-distance migration, in this context, may be pragmatically defined as migration to areas outside the European Union. Although not particularly meaningful biologically, it indicates that these animals visit areas with a different legislation and practices regarding use of antimicrobial drugs and other AMR-driving substances. Seabirds, in particular, have very long migration routes, but terrestrial and freshwater birds can also travel long distances. Swedish ospreys have been shown to overwinter in tropical Africa, and individual birds have been found as far south as Mozambique (Hake et al., 2001). Multiple waders and passerines also cross the

Sahara to overwinter in Central or West-Africa. Notably, although a western Palearctic-African migratory route is most common for long-distance migrators from Norway, some species diverge from this pattern. Among the passerines, the red-spotted bluethroat (*Cyanecula svecica*) travels along the Indo-European flyway and overwinters in India (Lislevand et al., 2015) and the common rosefinch (*Carpodacus erythrinus*) spends the winter in Northern India and Central Asia (Stach et al., 2016). Among waders, the red-necked phalarope (*Phalaropus lobatus*) migrates to the Arabian Sea and has a pelagic lifestyle there (Bemmelen et al., 2016) and the broad-billed sandpiper (*Limicola falcinellus*) is believed to stay in the Red Sea, Persian Gulf, and along the coast of the Indian subcontinent (Cramp and Perrins, 1983). Among fish, the European eel is particularly interesting, with a long migration.

B. Intermediate-distance migratory

Similar to the definition of long-distance migration in the current context, in this report intermediate-distance migration is defined as migration within Europe. This includes many migratory bird species that hatch in Norway and many species that travel through Norway from the Arctic. Many Norwegian and Arctic waterbirds, such as ducks, geese, and waders, overwinter along the southern coasts of the North Sea, especially in the Wadden Sea along the coast of Denmark, Germany and The Netherlands, while many passerines (like thrushes, chaffinch (*Fringilla coelebs*), and several others) spend their winter in Great Britain or Continental Europe. Fish, like the economically important Atlantic cod and salmon, may also be defined as having intermediate-distance migrations.

C. Stationary

In this report, animals that stay within the borders of Norway the whole year are classified as stationary. Nevertheless, these species may also travel long distances in search of food, vacant home ranges, or mates, or simply to avoid adverse weather. Young carnivores, like wolves, wolverines, and red foxes, can wander several thousand kilometres over relatively short timespans. Cervid species, like red deer (*Cervus elaphus*) and moose (*Alces alces*), typically show partial migration, with some individuals annually travelling long distances between their summer and winter pastures, while other individuals in the same population stay in the same areas throughout the year. Birds like Bohemian waxwing (*Bombycilla garrulus*), redpolls (*Acanthis* spp.), crossbills (*Loxia* spp.), spotted nutcracker (*Nucifraga caryocatactes*), and other species may regularly migrate apparently randomly and be seen in large flocks far south of their breeding grounds. Others, for example hooded crows (*Corvus cornix*), mute swans (*Cygnus olor*), and grey heron may show opportunistic migration, depending on the weather conditions during the winter.

### 3.3.1 Interfaces between wildlife, domestic animals, and humans

Below are listed some important exposure scenarios where ARB, AMR traits and/or resistance-drivers are likely to be present at interfaces between wildlife and humans:

**Waste:** Waste from hospitals, nursing homes, the pharmaceutical industry, and veterinary clinics may represent sources of antibacterial agents and, potentially, ARB.

Household waste and industrial waste from non-pharmaceutical industries may also contain minor amounts of antibacterial agents and bacteria, but other AMR-promoting chemicals may be as important. Adequate removal and disposal of refuse will, in well-developed societies, minimise exposure of wildlife to litter, but where procedures fail or are inadequate, many species will exploit this source of easily accessible food.

In Norway, waste disposal systems have improved in recent decades and disposal of waste in landfill sites has become less common

<http://www.environment.no/Topics/Waste/>. However, where dustbins are easily accessible, disposal services fail, and where landfills are found, gulls (*Laridae*) and corvids will take the opportunity for searching the waste for food. In streets, parks, and gardens, gulls and corvids will be accompanied by sparrows and feral pigeons (*Columba livia*) and feed on litter and food remains. In less developed countries, access to waste may be easier, and provide a very reliable food source for these birds.

Where access is available, mammals, such as rats, mice, foxes, and badgers, may also investigate litter, dust bins, rubbish skips, and landfill sites for food.

**Sewage system:** Sewage contain enormous amounts of bacteria and low concentrations of antibacterial agents excreted in urine and faeces from humans, and grey water from hospitals, industries and households where numerous AMR-promoting substances are used (Kummerer et al., 2000). Consequently, animals using sewage systems as a part of their habitat, will be exposed to AMR/ARB. In Norway, as in most of the world, the brown rat (*Rattus norvegicus*) is commonly found in sewage systems. According to pest control companies, the population is increasing, but population estimates or population surveillance in Norway are lacking (Malmo, 2018). Given the exposure of rats to human excrement and waste, and that their intestinal environment is physiologically similar to that of humans, rats are suggested to constitute an important potential reservoir for human pathogens and AMR. A recent study of rats residing in sewage systems receiving wastewater from hospitals in Copenhagen, Hong Kong, and Malaysia indicated that the transfer of ARB from hospital environments to rodents is a strong possibility, as rat faeces from hospital environments contained more vancomycin resistance than similar samples from other areas in the same cities (Hansen et al., 2016). This study describes the scenario as follows: "a high-risk situation that might be driven by the elevated number of antibiotic resistance genes and influx of antibiotics, as seen in the sewage from the hospital use, in combination with an environmental connectivity between wastewater, rats and humans". Another study showed a higher prevalence of ESBL-producing *E. coli* in rats living near hospital wastewater (Guenther et al., 2013).

**Contact with pets:** Pets like dogs and cats are occasionally treated with antimicrobial drugs and may consequently contribute to propagation of AMR in their environment. Treatment patterns in pets are currently very similar to those in

humans (VKM, 2015b). In addition, pets are in close physical contact with their owners, and may consequently share parts of their microbiome. Many pets are fed dried or canned feed, which is unlikely to constitute major contribution to transmission of AMR. However, some pets are fed raw, frozen feed made from offal, both from domestic sources and imported. This could provide a route by which pets are exposed to AMR from livestock production. Dogs may eat anything edible and sniff anything outside, edible or not, and may thereby be exposed to AMR from a variety of sources. Cats prey on rodents and birds, and may as such be exposed to any AMR found in these species. AMR in pets like dogs and cats may be transmitted to wildlife through direct contact, but indirect contact through saliva, urine, and faeces is probably more common. Wildlife living in urban areas may be exposed to small amounts of antimicrobial drugs and AMR from pets, and there is a potential for transmission of AMR from these pet species to sympatric foxes, badgers, hedgehogs, rodents, crows, sparrows, magpies, crows, gulls, etc.

**Arthropod vectors:** Insects and acarids can transmit a wide variety of pathogens, both as mechanical vectors that have been contaminated incidentally and as true multiplicative vectors that host the pathogen and allow reproduction.

Insects and other arthropods that visit and/or feed on the skin, fur, blood, excretions, or carcasses of pets and humans may transmit any pathogen or AMR-carrying microorganism present on/in one host to a new host, including wildlife. Flies, for example, are shown to carry AMR (Schaumburg et al., 2016). Through this route, insectivorous wildlife species, like many birds, shrews and bats, can be exposed to AMR.

Below are listed situations where there is the potential for contact and transmission of AMR from wildlife to humans:

**Food storage and food transport:** Storehouses and food stores in shops, restaurants, and homes can be visited by wildlife. Modern packaging and storage procedures, vermin control programmes, and better building construction has probably limited the access of wildlife to human food, but the possibility of wildlife contaminating food in stores cannot be totally eliminated. Transmission of AMR can occur when food is contaminated with saliva, faeces, urine, or animal carcasses. Relevant bird species are house sparrows, which can both nest and search for food in storehouses, but also long-distance migrators such as barn swallows (*Hirundo rustica*), common house martin (*Delichon urbicum*), common swift (*Apus apus*), and other less prevalent species can nest in buildings with easy access through doors, ventilation, or windows. In contrast to the house sparrow, that finds food in the streets and also feeds on human foods, hence providing a link between waste, animal excrement, and human food, the latter group of birds are insectivores that catch insects in the air. They should therefore present a lesser risk for transmission of AMR. Corvids and gulls can feed on transported and stored food if accessible, but normally

do not go into buildings. However, there is very little knowledge about the prevalence and characteristics of AMR in urban birds, and little information about the probability of transmission.

Rodents are considered a major pest in food stores and during transport. In Norway, one of the most important species is the brown rat. The black rat (*Rattus rattus*) is not found in the country, but is frequently found in ships. Both rat species can contaminate food and thereby constitute a link between sources of AMR, for example, sewage, and humans. Among mice, the house mouse (*Mus musculus*) lives closely associated with humans and the whole life of a house mouse may be spent in, for example, storehouses. Other species, like yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), Northern red-back vole (*Myodes rutilus*), and bank vole (*Myodes glareolus*) frequently overwinter in buildings, but do normally not breed there. These mouse and vole species are probably less exposed to sewage than rats, but may come in contact with a wide variety of substances, such as manure, and may consequently spread AMR to the human food chain.

**Outdoor restaurants and food markets:** Places where food is served or presented for sale outside represent an area of food opportunities for opportunistic feeders like house sparrows, feral pigeons, and gulls. In urban areas, these birds can become quite bold and it is not uncommon that such birds land on tables and eat directly from the plates, or try to take food from market stalls. This can result in direct contamination of drinks and fresh food. As house sparrows also feed on horse dung and waste they could transmit AMR from the environment to humans. Gulls (*Larus* spp.) travel widely and feed on a variety of food sources. They are attracted to landfill sites, investigate dustbins in urban areas for food, swim in harbours and close to sewage outlets, and hunt earthworms on agricultural land. Gulls may also have long migrations along the Eastern Atlantic Coast down to Northern Africa (and some species, for example the Northern subspecies of the lesser black-backed gull (*L. fusca fusca*), even down to Middle East and great lakes of Central Africa.) In addition, gulls share their marine environment with other seabirds with even longer migrations and may receive AMR from these sympatric species, in, for example, bird colonies. The gulls scavenging the left-overs on the restaurant table, does hence represent a link to a wide specter of environments.

**Bird feeding:** Feeding garden birds has become increasingly popular and many people feed birds throughout the year. This attracts a wide range of species, (see <https://www.fuglevennen.no/aktiviteter/foringsplassen/resultater/?sort=antal> a for an overview of registrations of garden birds in Norway). Bird feeding sites can also be visited by squirrels, rodents, hedgehogs, and carnivores, like foxes, raptors, and domestic cats. Bird feeding stations can thus provide a meeting point where pathogens and AMR can be transmitted between individuals, populations, and species. In addition, they provide a point where people can get in close contact with birds and their excreta, either directly when they handle or clean the feeding station,

or indirectly when the birds contaminate for example berries, fruit or salad in the garden, or when their pet cat catches, handles, and eat birds with AMR.

**Indirect contact in playgrounds, gardens, and parks:** Incidental contact with wildlife occurs when children play outside, when people are in their gardens, and when they visit parks and other green spaces in cities. The population density of many bird species can be relatively high in such areas, and they can often be visited by opportunistic feeders like gulls, corvids, rodents, foxes, badgers etc. when human activity is low, for example during the night and early morning. Furthermore, people may prepare food and eat without ordinary hygiene precautions, providing an opportunity for contamination of food and hands with wildlife faeces and other excreta, and they may feed birds like sparrows, pigeons, and ducks, providing relatively close contact between human, wildlife, and everything with which the wild animals have been in contact.

**Indirect contact through pets:** As stated in (VKM, 2015b), humans are exposed to the microflora acquired by the pet in parks and streets and will share a considerable part of their microbial flora, including resistant bacteria and resistance genes. Dogs and cat thus provide an indirect interface with wildlife. Dogs lick and sniff anything with an interesting odour, and can thus transmit bacteria from any excreta from any species visiting an area, while cats that prey on wild birds, rodents, and shrews provide an interface with these species.

Below are listed situations where ARB/AMR and/or AMR-drivers are likely to be present and wildlife exposed mainly in the terrestrial agricultural environment:

**Manure and direct contact with livestock:** Livestock treated with antimicrobial compounds can excrete small amounts of these substances in urine and faeces. This promotes the development of AMR (Heuer et al., 2011). In addition, pig herds and poultry flocks can act as reservoirs of AMR that is not necessarily associated with treatment; for example LA-MRSA in pigs and quinolone-resistant *E. coli* in poultry (VKM, 2015a). Manure can also contain relatively large amounts of metals like copper and zinc, that may promote AMR (Singer et al., 2016). Exposure to manure, either when it is being stored or when it is spread on the field, can hence expose wildlife to AMR. Direct contact between livestock and wildlife, either in barns or on the pasture, may also facilitate transmission of AMR. A Canadian study, for example, found increased occurrence of AMR in small animals (rodents and shrews) living on swine farms than in residential areas, landfill sites, or natural environments (Allen et al., 2011). In contrast, house sparrows and house martins living on cow farms seemed unlikely to share AMR *E. coli* isolates with their sympatric bovines (Dolejska et al., 2008; Rybarikova et al., 2010). However, flies on these cow farms carried *E. coli* with AMR of similar phenotypes and genotypes as those found in the cattle, indicating the potential for transmission of AMR from livestock to insectivorous wildlife through flies.

**Sewage sludge:** VKM assessed the risks associated with using sewage sludge as fertiliser and soil conditioner and concluded that it was unlikely that AMR was promoted in the processing and application of the sludge increased concentrations of Cd, Hg, Cu and Zn, and partly also Pb, in the soil. The risk of development of AMR related to increasing concentrations of heavy metals in soil has been assessed in another recent report from VKM (VKM, 2017).

Manure and sewage sludge may also contain biocides. Barns and stables, for example, are frequently and thoroughly cleaned with disinfectants, such as when stock is replaced in all-in-all-out production systems for pigs and poultry, and households, food industry, and healthcare institutions may use large amounts of biocides. In animal production buildings, runoff from cleaning and disinfection often flows off to the manure handling system of the farm rather than going into a sewage system. As some resistance mechanisms are common to biocides and antimicrobial agents, there is concern that biocides in soil can lead to increased AMR in the agricultural environment. This concern has been addressed in an assessment from European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2009).

Water sources for irrigation can be contaminated with manure, runoff from agricultural production, sewage etc., and can be a source of ARB or AMR-promoting substances.

Prophylactic use of antimicrobials in plant production to combat bacterial plant diseases is not allowed in Norway, but may be used in other countries as a prophylactic treatment against bacterial diseases in orchards when disease risk is regarded as high. Data on use of antimicrobials on crops are difficult to retrieve, but seem to be still permitted in various countries, such as Germany, Austria, and Switzerland, on an emergency basis and under tightly restricted conditions (Stockwell and Duffy, 2012), and we might assume that use is more common in other, less-regulated countries. Thus, migratory birds travelling through countries where large areas of orchards are sprayed with antibiotics could be accidentally exposed to antibacterial agents and associated AMR.

Below are listed situations where there is the potential for contact and transmission of AMR from wildlife to humans through crops and livestock:

**Faecal contamination of crops:** Produce that is consumed with little or no treatment, such as various vegetables and fruits, are the most significant potential vehicle of transmission of ARB. Cereals that are used without heat treatment, for example used as breakfast cereals, may also constitute a vehicle. Many bird species, mice, voles, lagomorphs, shrews, frogs, toads, predators of these animals, and larger animals such as roe deer, red deer, and moose may live in or visit vegetable fields and orchards or the ecotones at their margins.

**Direct contact with livestock on pastures and in barns:** This could result in establishment of wildlife ARB or transfer of AMR genes to food-producing animals.

**Wildlife visiting feed storage rooms and barns:** Wildlife searching for food or shelter may contaminate livestock feed and thus transmit AMR to food-producing animals.

**Supplementary salt licks used by both wildlife and livestock:** Indirect contact through salt licks is especially important as both wildlife and livestock will consume the salt-laden soil around the salt stone. This soil will also be contaminated by bacteria present in the the faeces and urine of all animals that visit the lick. Supplementary salt licks are used in several sorts of animal production, but are most important in sheep production. Mountains and forests used for sheep can have high numbers of salt licks, used over many years, providing a hot spot for faecal-oral transmission of bacteria between wildlife and sheep. The most obvious wildlife at such places are cervids (roe deer, red deer, reindeer, and moose) but more inconspicuous wildlife, like voles, mice, shrews, and birds, attracted to the salt, the faeces, and the insects that gather there, may also be important both numerically and with respect to biomass of bacteria.

Below are listed situations where ARB/AMR and/or AMR-drivers are likely to be present and wildlife exposed in the sylvatic environment:

In the sylvatic environment, contact between wildlife, humans, and our pets and livestock is limited. However, human encroachment and fragmentation of landscapes is increasing globally, and there are few environments that are truly pristine and without anthropogenic disturbance. Tourism, cabins, cruise ships, and infrastructure all bring spill-over from our civilization to those wildlife that try to avoid humans. Garbage, latrines, and outhouses constitute sources of transmission of AMR, even in these environments.

In addition, pollution may be carried over long distances as particles and droplets in the air, with water in streams and rivers, and with currents in the sea. The antimicrobial substances themselves, ARB, and heavy metals, biocides, and other promoters of AMR may be introduced to pristine environments through long-distance dispersal of pollution. Garbage containing small amounts of antimicrobial agents or promoters can also be widely spread over the globe, particularly through the sea. As a consequence, no environment can be truly sheltered from anthropogenic AMR.

Migratory birds and mammals dwelling in or travelling through more anthropogenically disturbed areas will also probably bring AMR with them and disperse AMR to pristine sylvatic environments, as described below for geese and swans in freshwater environments.



Below are listed situations where there is the potential for contact and transmission of AMR from wildlife to humans in the terrestrial sylvatic environment:

**Hunting:** This activity and the resulting dressing, preparation, and consumption of wildlife represents the most intimate interface between sylvatic wildlife and humans, and thus represents one of the most plausible ways of transmission of AMR in these environments.

**Water:** ARB can also be transmitted by wildlife contaminating a water source and humans drinking untreated water.

**Other outdoor activities:** Various outdoor activities can be associated with occasional direct contact with wildlife or their excreta and thus constitute a mode of transmission. For example, transmission can occur when humans pick contaminated wild berries, mushrooms, or herbs and eat them. However, the probability seems relatively low.

Contact via pets is described above.

**Arthropod vectors:** There are only a few known bacterial infections that are transmitted from wildlife to humans with true multiplicative arthropod vectors in Norway. In the forest Ticks (*Ixodes ricinus* and other *Ixodes* spp.) transmit *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Candidatus Neohhrlichia mikurensis* and possibly other species. Mosquitos are known to transmit *Francisella tularensis*, at least in Sweden. Deer keds (*Lipoptena cervi*) harbour *Bartonella* spp. and might transmit these. Little, however, has been published on AMR or occurrence of AMR genes in these species of bacteria, although *B. burgdorferi* s.l. is known to have an extremely complex genome compared with other bacteria, consisting of numerous linear and circular plasmids (Brisson et al., 2012).

There is also a surprising lack of information on the role of arthropods as mechanical vectors of AMR bacteria. For example, flies can easily transmit bacteria from live wildlife or wildlife excreta or carcasses to humans or human food sources (Schaumburg et al., 2016).

Below are listed situations where ARB/AMR and/or AMR-drivers are likely to be present and wildlife exposed in freshwater environments:

As stated by Vaz-Moreira (Vaz-Moreira et al., 2014), water is one of the most important bacterial habitats on Earth, is a major way of dissemination of microorganisms in nature, and is recognised as a significant reservoir of antibiotic resistance. Lakes, rivers, and streams are recipients for sewage, pollution and runoff from urban areas, cultivated land and pastures, and can hence receive AMR-promoting agents, ARB, and resistance genes from all these sources. In addition,

some studies indicate that algae, that comprise the greatest abundance of plant biomass in aquatic environments, may produce antimicrobial compounds (Nagarajan et al., 2013). Algal biomass is expected to be highest in eutrophic lakes, that also may receive agricultural effluents and have a high content of diverse bacteria. Some lakes, especially eutrophic ones, may be used as resting areas for migratory birds, and thus provide a melting pot of both local AMR and transported from distant places, as well as being a place where various wildlife can pick up AMR and transport it further. Many waterbirds coming to Norway have rested or overwintered in shallow coastal waters that receive effluents and sewage from areas with a much higher population density and a more intensive agriculture production than any area in Norway. Several studies have suggested that geese and swans could act as potential long-distance dispersers of AMR from such environments (Agnew et al., 2016; Elmberg et al., 2017; Middleton and Ambrose, 2005a; Mohamed Hatha et al., 2013).

Below are listed situations where there is the potential for contact and transmission of AMR from wildlife to humans in freshwater environments:

Wildlife AMR can be transmitted from freshwater ecosystems to humans through drinking water, water used as an ingredient in food production, or when it is used as drinking water for livestock. Transmission can also occur when water sources with AMR are used for irrigation and when humans consume freshwater fish or crustaceans. In addition, aquatic birds, such as geese and ducks, frequently forage and defecate on cultivated fields or public amenity areas (e.g, parks), and thus create a link between agricultural, urban terrestrial, and aquatic environments. There is also a potential risk when humans bathe in lakes or streams, especially when the local wildlife population density and bacterial content is high.

Below are listed situations where ARB/AMR and/or AMR-drivers are likely to be present and wildlife are exposed in the marine (coastal and pelagic) environment:

**From water:** Rivers and streams, as well as treated or untreated sewage, eventually end up in the sea. This water contains residues of antibacterial agents derived from human and animal therapeutic applications, a wide range of microorganisms from humans, animals, and soil, as well as contaminants, such as heavy metals and biocides, which are known as drivers for AMR. In the sea, these substances and microorganisms come into close contact with microorganisms naturally found in the marine environment, making HGT possible. Coastal and shallow waters near densely populated urban areas and agricultural areas with high livestock densities are presumably hot spots for such phenomena.

**Marine-associated activities:** Shipping, leisure boating, and public transport, as well as aquaculture, inevitably add waste to the marine environment. This could include macro- and micro-plastics, small-scale sewage discharge, antifouling agents and paint from boats, and components for anti-oxidation treatments from marine installations.

**Migration:** Migratory marine mammals, such as the Northern minke whale (*Balaenoptera acutorostrata*) and other whales, show long haul movements between Arctic and Antarctic waters during feeding, and will carry microbiota, possibly with AMR, over long distances. The killer whale (*Orcinus orca*) is commonly known to assemble around fishing vessels to prey on the catch. During migration, birds may use marine areas for resting and will inevitably be exposed to the local resistome.

**Seabird colonies:** Some seabirds make large colonies, particularly during nesting. Such birds collect prey animals of various origins and bring them for feeding in the nest. Consequently, faeces and associated microbiota will be shed in the colony area. Bird colonies also act as a hub, where coastal birds and birds that have close contact with humans, for example gulls, meet and exchange microflora with birds that have a less intimate interface with humans, their livestock, or food production chains. In bird colonies, new variants of AMR can potentially be introduced from all the vast areas covered by Norwegian wild birds, disseminated among the individuals in the colony, and dispersed wherever these birds travel.

**Aquaculture:** The feed used in Norwegian aquaculture has traditionally been composed of marine oil and proteins (fish meal), but a major trend is to include vegetable components. The components of fish feed have recently been shown to facilitate resistance-gene propagation in marine aquaculture sediments (Han et al., 2017), with residues of antimicrobial agents and resistance genes detected in fish meal. Uneaten fish feed and faeces occur in the environment and are utilised by a wide range of wild animals.

Below are listed situations where there is the potential for contact and transmission of AMR from wildlife to humans in marine environments

**Seafood:** For many seafood products, heat treatment sufficient to inactivate possible pathogens is applied. However, cross contamination during handling or food preparation may occur. Marine filter feeders, such as the bivalves, actively concentrate microorganisms from the seawater. Commonly, a blue mussel (*Mytilus edulis*) may filter and retain particles from approximately 70 litre of seawater daily. Some bivalves, such as oysters are consumed raw, but in Norwegian cuisine other shellfish and crustaceans, such as lobster and shrimps, are commonly consumed after simmering at temperatures reaching 70°C (shellfish) or boiled (crab, shrimp, lobster). Farmed and wild-caught fish are widely consumed in Norway, the majority in a heat treated form. However, raw consumption is gaining increasing popularity.

Marine mammals and, to a much lesser extent, marine birds are used as food in Norway. Marine mammal meat is either eaten raw or heat treated, whereas meat from marine birds is cooked before eating. Several human pathogens are regularly detected in marine mammals (Tryland et al., 2014), but little is known about the prevalence of AMR genes in the microbiota of these animals.

Marine birds carry several pathogens of concern for humans, including those in the genera of *Campylobacter* and *Salmonella*. Contamination of drinking water by

*Salmonella* from seagulls has been described. Likewise, contamination of farmed fish in net pens and seafood organisms during catch and handling cannot be excluded. As an example, large flocks of gannets (*Morus bassanus*) follow vessels during trawling and purse seine fishing, and prey upon the catch during the hauls.

## **The interface between wildlife and aquaculture**

In 2016, a total of 1.6 million tonnes of dry fish feed were used in Norwegian aquaculture to produce approximately 1.3 million tonnes of farmed fish, mainly Atlantic salmon (*Salmo salar*). Although most of the feed is utilised by the fish to gain weight, some feed is not eaten and will be eaten by wild animals living near the net pens. Furthermore, a considerable amount of fish faeces will end up in the environment. Aquaculture attracts wild animals, such as birds (gulls, cormorants, and herons), mammals (mink and otters), and a wide variety of marine animals, such as fish, crustaceans, polychaetes, and echinoderms (Samuelsen et al., 2015). Wild animals will thus be in close contact with the farmed fish and the farm installations and microbiota interchange inevitably occurs.

In addition, 27.3 million individual wild wrasses were caught in 2017 (Fiskeridirektoratet, 2017), transported, and introduced to salmon and rainbow trout enclosures as cleaner fish. A large proportion of these are fished along the southern coast of Norway and western coast of Sweden and transported to fish farms in Northern Norway, where wild wrasses are less abundant. These coastal species, which feed on molluscs, crustaceans etc., live in shallow waters and may easily be exposed to AMR.

## **3.4 Antimicrobial resistance in different wildlife animals**

In this report, an animal is defined as a mammal, bird, reptile, amphibian, fish, crustacean, mollusc, or bee (defined by OIE). However, we have also included articles referring to insects other than bees, as they may contribute to transmission of bacteria and AMR bacteria to different animal species; only three such articles were included (See 3.6.9).

Animal species are divided in three main groups; herbivores, carnivores, and omnivores.

In Appendix II, we have evaluated original articles belong to different animal groups and a summary of these evaluations follows below. In addition, review articles, which were not included in Appendix II, are used in different parts of this opinion.

### **3.4.1 Amphibians and reptiles**

#### ***Number of studies***

There were 13 relevant studies covering amphibians and reptiles in the timespan from 1991 to 2017. One article covers animals from two regions (Ref Appendix II, Table 1).

#### ***Geographical area***

The geographical areas covered include North America (9), South America (3), Africa, and Europe (one each). Several bacterial species and animal groups were examined in these studies.

### ***Bacterial species***

The majority of studies have been conducted on members of the Enterobacteriaceae family, including the genera *Escherichia*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Kluyvera*, *Serratia*, *Pantoea*, *Providencia*, *Shigella* and *Salmonella*. A few authors report on obligate anaerobes (*Bacteroides fragilis*), *Stenotrophomonas maltophilia*, *Pseudomonas* spp., *Aeromonas* spp., or coagulase-negative staphylococci (predominantly *Staphylococcus sciuri* and *Staphylococcus xylosus*).

### ***Resistance types***

Resistance to a variety of antimicrobial agents was encountered in bacteria from amphibians and reptiles.

Resistance against antimicrobial agents was not detected in bacterial isolates in 3 (of 13) articles (Mayorga et al., 2015; Sylvester et al., 2014; Thaller et al., 2010).

Wild-living gray treefrogs (*Hyla chrysoscelis*) were found to harbour inordinately large numbers of methicillin-resistant non-*S. aureus* staphylococci as part of their normal flora; the mechanism of methicillin resistance in these strains may be independent of the *mecA* gene (Slaughter et al., 2001).

Resistance to imipenem was seen in 30 % of *Klebsiella* spp. isolated from wild turtles and snakes in USA (Liu et al., 2013).

Resistance to cephalosporins and carbapenems were found in bacteria from several animal species, whereas VRE and colistin resistance have not been reported.

Reptiles accounts for a considerable proportion of imported animals, and it has been estimated that approximately 10 % of live animal shipments to USA constitute reptiles for pet purposes (Smith et al., 2012). They may represent possible vectors for ARB.

For more information, see Table 1 in Appendix II.

### ***Diets (herbivorous, carnivorous, omnivorous)***

Amphibians and reptiles feed in a variety of ways, and herbivorous, carnivorous and omnivorous animals occur in both groups.

One important group of reptiles are the iguanas. These animals are predominantly herbivores. Two of the reviewed publications on iguanas, showed an absence of AMR among bacteria in the intestine of these animals (Sylvester et al., 2014; Thaller et al., 2010).

## **Conclusion**

A high rate of resistance to penicillin G and oxacillin (a clinical substitute for methicillin) in wild amphibians was reported (Slaughter et al., 2001), suggesting that natural populations may be a reservoir for AMR staphylococci.

It seems to be a general trend that for the same bacterial species found in several compartments, the prevalence of phenotypic resistance and resistance genes is lower in wild animals than in food-producing animals and pets (Mayorga et al., 2015). This conclusion was strengthened by (Wheeler et al., 2012) who found resistance towards doxycycline, tetracycline, and trimethoprim/sulphamethoxazole in bacteria from various Galapagos reptiles sampled in tourist areas, but not in pristine areas.

Due to the low number of studies, it is difficult to draw firm conclusion regarding any relationship between diets and the resistance profiles in bacterial isolates from amphibians and reptiles included in this Opinion. Although resistance against antimicrobials was reported as not detected in bacterial isolates in some publications, resistance against broad-spectrum antimicrobials like 3<sup>rd</sup>-generation cephalosporin, combination of amoxicillin-clavulanic acid, chloramphenicol, and tetracyclines was observed in others.

### **3.4.2 Birds, including migratory birds**

#### ***Number of studies***

A total of 99 articles were included. The bird species include both stationary and migratory birds. The studies were from 1992-2017, with approximately 80 of the studies published during the last 7 years (since 2010).

#### ***Geographical area***

Most studies were performed in Europe (54), followed by North America (18), and Asia (12). For more information see Table 2.

#### ***Bacterial species***

Faeces was the most common sample material examined. Bacterial species belonging to the family Enterobacteriaceae were predominant, in particular *E. coli*, *Klebsiella* spp., and *Salmonella*. Among the other bacterial species detected in the faecal samples were *Enterococcus* spp., *Campylobacter* spp., and *Pseudomonas* spp. The most commonly isolated bacterial species from nasal samples were *Staphylococcus* spp., both *S. aureus* and CoNS.

#### ***Resistance types***

A high number of publications in this animal group reported resistance against most commonly used antimicrobial agents in veterinary and human medicine. Multidrug resistance (MDR) was observed in the majority of bacteria in the family Enterobacteriaceae, in

particular *E. coli* and *Klebsiella*. Although many of these species were resistant against 3<sup>rd</sup>-generation cephalosporins (ESBL-producing isolates), some studies reported resistance against carbapenems (carbapenemase-producing bacteria, NDM-1 isolates). A few studies reported resistance against colistin; also plasmid-mediated resistance: *mcr-1* carrying isolates.

Generally, the prevalence of resistance and MDR in *Salmonella* was lower than in other bacterial species in the Enterobacteriaceae family.

Resistance against quinolones, like ciprofloxacin, was widespread among *Campylobacter* isolates.

In the studies investigating enterococci, a high prevalence of resistance against vancomycin and MDR was observed.

A few studies examined staphylococci. Resistance against methicillin was reported in several of these articles, both in *S. aureus* and in CoN-isolates.

For more information, see Table 2 in Appendix II.

### ***Diets (herbivorous, carnivorous, omnivorous)***

All diets were represented among the birds in these studies, but the majority were omnivores.

### ***Conclusion***

The origin of the migratory birds was usually not stated in the studies. All types of resistance were detected, including resistance against important, clinically important, and critically important antimicrobials, based on WHO/OIE definitions (see section 1.3 and Appendix I). Most birds in the studies were omnivores and the prevalence of MDR bacteria was high in comparison with other animal groups examined in this opinion. The diets of birds may be the reason for high variation in resistance types including MDR. However, due to the lack of specific studies comparing AMR in relation to diets in this animal group, it is difficult to draw firm conclusions. Migratory birds with their high mobility can carry ARB over long distances, even between continents, and this may be partly responsible for the global transmission of ARB (Wang et al., 2017a). Birds play an important role in the ecology, circulation, and dissemination of microorganisms with various levels of resistance and can therefore be considered as sentinel species and environmental health indicators (Foti et al., 2011). Several studies included in this section reported the recovery of genetically similar bacterial isolates from wild birds, food-producing animals, and humans, suggesting shared ancestry.

### 3.4.3 Fish and other water-living animals; vertebrates, and invertebrates (marine and fresh water)

#### ***Number of studies***

There were 18 relevant studies in fish and other water-living animals in the timespan from 1996 to 2017.

#### ***Geographical area***

The geographical area covered includes Europe excluding Norway (7), Norway (1), North America (6), South America (2), Oceania (1), and Asia (1).

#### ***Bacterial species***

The Enterobacteriaceae family were included in several studies, including the genera *Escherichia*, *Klebsiella*, *Hafnia*, *Morganella*, and *Salmonella*.

The enterococci were represented by *E. faecalis*, *E. faecium*, *E. durans*, *E. hirae*, *E. casseliflavus*, *E. gallinarum*, and the streptococci included *Streptococcus agalactiae*.

In the family Vibronaceae, *V. cholera*, *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus* and *Aeromonas* were examined.

In addition, *Shewanella putrefaciens*, *Acromonas* spp., *Bordetella* spp., *Arcanobacterium* spp., *Erysipelothrix* spp., *Chromobacterium violaceum*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Plesiomonas shigelloides* were included.

#### ***Resistance types***

Resistance to a variety of agents has been encountered in bacteria from fish and other aquatic organisms. Some are probably often intrinsic for the bacterial species.

MDR was identified in bivalve-associated microbiota. In one study (Grevskott et al., 2017), MDR was recorded in 5% of 199 isolates in the Enterobacteriaceae family. Phenotypic resistance was seen for 75 of these 199 isolates. Of these, resistances towards extended-spectrum penicillins (83%), aminoglycosides (16%), trimethoprim (13%), sulphonamides (11%), tetracyclines (8%), third-generation cephalosporins (ESBL-producer) (7%), amphenicols (5%), nitrofurans (5%), and quinolones (5%), were observed. Genes for resistance towards extended-spectrum penicillins (*bla<sub>TEM-1</sub>*), third-generation cephalosporins (*bla<sub>CTX-M-14</sub>*, *bla<sub>CTX-M-15</sub>*), aminoglycosides [*strA-strB*, *aadA5*, *aac(3)-IIId*, *aph(3)-Ia*], trimethoprim (*dfiA17*, *dfiA5*, *dfiA14*), sulphonamides (*su1*, *su2*), tetracyclines [*tet(A)*, *tet(B)*, *tet(D)*], amphenicols (*catA1*), quinolones (*qnrS1*), and macrolides (*mphA*), were detected.



MDR *E. coli*, *Aeromonas* spp, *Vibrio* spp., *Bacteriodes* spp., staphylococci, enterococci, including vancomycin-resistant *Enterococcus* were reported in several of the studies in this animal group.

For more information, see Table 3 in Appendix II.

### ***Diets (herbivorous, carnivorous, omnivorous)***

Fish and water-living animals comprise a very diverse group, and include herbivorous, carnivorous, and omnivorous species. A particular feeding behaviour occurs among filter-feeding species; this group is omnivorous as they feed non-selectively on particles that could belong to plants (phytoplankton) or animals (zooplankton), bacteria, or debris. They are therefore concomitantly exposed to antimicrobial agents that find their way to the marine environment.

### ***Conclusion***

Bacteria with AMR reach the sea through sewage and runoff from land, and may subsequently pose a risk to humans through seafood.

Among the water-dwelling organisms, the filter feeders, particularly bivalve molluscs, showed the highest prevalence of resistant bacteria. This could be due to their feeding behaviour, in which they filter large volumes of water and actively retain particles, including bacteria. Resistance in many bacterial species like *Aeromonas* spp. and *Vibrio* spp. from the aquatic environment may also be due to use of antimicrobial agents as feed additive and potentially toxic metals, like Cu, being used in aquaculture.

Migratory fish, mammals, and birds may also contribute to spreading AMR bacteria between geographical areas. Due to the low number of studies it is not possible to draw a firm conclusion regarding a relationship between diet and the occurrence of AMR.

## **3.4.4 Insects**

### ***Number of studies***

Only 3 relevant studies were identified, published between 2012 and 2017.

### ***Geographical area***

The studies came from Asia (1), Europe (1), and North America (1).

### ***Bacterial species***

Only *E. coli* was studied.

### ***Resistance types***

$\beta$ -lactamase producing *E. coli* isolates resistant to ampicillin were reported from mosquitoes. While colistin resistance and carbapenem resistance was observed in flies. The third study also reported a high incidence of tetracycline and oxytetracycline resistance in bees.

For information see Table 4, Appendix II.

### ***Diets (herbivorous, carnivorous, omnivorous)***

There is a variation in diet types among insects. Mosquitoes can be herbivorous or omnivorous depending on species and gender. It has been observed that only females feed on blood, and will do so preferentially, over sugar sources, subsequent to mating. The most prevalent feeding behaviour among flies is that they are detritivores, consuming decaying organic matter.

### ***Conclusion***

Although relatively few studies have been performed in this animal group, resistance against some of the most clinically important antimicrobial agents, such as tetracyclines,  $\beta$ -lactams, and colistin, has been observed.

Insects are ubiquitous and therefore potentially important in the spread of AMR. Insects have the potential to carry ARB from one point to another; for example from wildlife animals, food-animal farms, pets, and wastewater treatment facilities, to, for example, urban areas. Some insects, such as some mosquitoes, directly access both animal or human blood.

Thus, more studies are needed to determine the role of insects in the persistence, transfer, and dissemination of resistance.

## **3.4.5 Wild terrestrial mammals**

Mammals represent one of the most diverse classes in the animal kingdom, from the smallest bumblebee bat to the gigantic blue whale. The largest proportion is rodents and this is reflected by the number and division of studies included in this Opinion.

We have divided the studies on wild mammals into three groups: a) wild boars, b) large terrestrial animals (other than wild boars), and c) small terrestrial animals.

### ***3.4.5.1 Wild boars***

Wild boars are presented as a separate group animal within wild terrestrial mammals, because of their invasiveness from Sweden in some south-eastern counties in Norway.

### ***Number of studies***

A total 24 studies were included in this category, with a timespan of 10 years (from 2007 to 2017).

### ***Geographical area***

Except one study from Asia (Japan), one from South America (Brazil), and one study from North America (USA), the other studies were from Europe, with the majority from Portugal and Spain.

### ***Bacterial species***

Bacterial species belong to the Enterobacteriaceae family (*E. coli*, *Salmonella*, *Yersinia* spp.,) were the most frequently studied. Other bacterial species included staphylococci and enterococci, but also bacterial species like *Brucella* spp. and *Clostridium* spp.

### ***Resistance types***

In several studies, bacteria resistant against the tested antimicrobial agents were not observed. However, in other studies MDR was reported in several bacterial species from several studies. For observed MDR clinically important bacteria like MRSA, VRE, quinolone resistant *Campylobacter*, cephalosporin resistant Gram-negative bacteria, including ESBL-producing bacteria, and colistin resistant Gram-negative bacteria were observed, although the prevalence of these isolates was considered low. Resistance against linezolid, a synthetic antimicrobial that has been marketed during of last ten years, is important to note. A high prevalence of resistance against antimicrobial agents like erythromycin, streptomycin, tetracycline, was reported in some bacterial species, like *Enterococcus* spp.

For more information, see Table 5 in Appendix II.

### ***Diets (herbivorous, carnivorous, omnivorous)***

The wild boar is a highly adaptable animal, as it is found in a variety of habitats and eats almost anything that will fit in its mouth. Wild boars are omnivores.

### ***Conclusion***

Wild boar are distributed worldwide and can thrive in areas that are heavily influenced by human activity (Schley and Roper, 2003). Wild boar habituation to urban areas has occurred in certain cities and countries, and may give rise to public health concerns. Groups of wild boars feed and defecate in public parks, private gardens and other green areas, and drink from surface drinking water sources. This underscores the need for knowledge about such populations, as pathogens can be transmitted either through direct contact or indirectly via infected urine or faeces in places frequented by people or pets. Also, transmission may occur from people to wild boars through the consumption of domestic rubbish and other uncontrolled waste (Navarro-Gonzalez et al., 2013a).

Wild boars are omnivores and may live in agricultural areas, close to livestock, but also in areas with low human density and activity. This may be one reason for the variable observations of resistance types in bacterial species isolated from wild boars. Due to

habituation of wild boars to agriculture and urban areas, the transmission of ARB from food-producing animals, pets, and humans to this animal group and vice versa is possible, but the probability and frequency of such transmission needs to be evaluated. This exposure should be assessed in different geographical areas, based on the density of wild boars and the prevalence of ARB compared with the prevalence of ARB in other wildlife, food-producing animals, pets, and humans in the same area.

#### **3.4.5.2 Large terrestrial animals**

##### ***Number of studies***

A total of 60 studies were included in this category, published over a time span of 26 years (from 1991 to 2017). This category of animals includes a wide range of animals from elephants, non-human primates, lions, and tigers to polar bears, foxes, raccoons, deer, wolves, Iberian ibexes, buffalo, etc.

##### ***Geographical area***

The studies were from all areas of the world, but most studies concerned with non-human primates, elephants and tigers were from different countries in Africa. Most of the studies were from Europe and were dominated by Portugal and Spain.

##### ***Bacterial species***

Bacteria belonging to the family Enterobacteriaceae, mainly *E. coli*, and *Salmonella* spp. were examined in many of these studies. However, other bacterial species, *Campylobacter* spp., *Staphylococcus* spp., *Enterococcus* spp., and *Clostridium* spp., were also investigated.

##### ***Resistance types***

Resistance towards a range of different antimicrobial agents was observed, although the prevalence was generally low. MDR and ESBL-producing *E. coli* were isolated in several studies, although the prevalence was low. Enterohemorrhagic *E. coli* (EHEC) was isolated in several studies and examined isolates were generally pan-susceptible.

Resistance against tetracycline and or erythromycin in *Campylobacter* was detected in several studies in different animal species.

A few studies reported resistance against vancomycin in enterococci.

MDR and resistance against methicillin in staphylococci were reported in several studies.

For more information see Appendix II, Table 6.

##### ***Diets (herbivorous, carnivorous, omnivorous)***

All diet groups were represented.

### ***Conclusion***

Wild non-human primates in frequent contact with human waste had a higher proportion of enteric ARB than do conspecifics without such contact. The findings further suggest that such groups of wild animals may constitute an overlooked source of antibiotic resistance in the natural environment.

Some studies report evidence of high frequency of AMR, but most studies reported rather low frequencies of resistance in isolates. In studies where wild mammals were compared with domestic animals, there appeared to be less resistance in wildlife populations, probably due to low exposure to antibiotics, ARB and/or spatial separation. A possible correlation between diets and type of AMR bacteria could not be considered due to high variation in different animal species and variable level of antropogenic influence in this category.

### **3.4.6 Small terrestrial mammals**

#### ***Number of studies***

A total of 47 studies were included in this category with from 1988 to 2017. Of these, 43 studies have been performed since 2005. Many of the studies focused on mice, rabbits, and rodents.

#### ***Geographical area***

The relevant publications had a global distribution, with most from Europe (27), predominantly Portugal and Spain, followed by North America (10).

#### ***Bacterial species***

The experimental design most often focused on culturable bacteria from faecal samples. Most studies investigated human/animal associated (zoonotic) bacteria, predominantly *E. coli* and other enterobacteria, as well as *Enterococcus* and *Streptococcus* in some cases. Very few studies were on bacteria with no implications for human health. Only one study included anaerobic bacteria, and one study conducted repeat sampling of a rodent population over time.

#### ***Resistance types***

A high variation in prevalence of ARB in small terrestrial animals was found in the articles included in this Opinion. Some studies showed a low prevalence of ARB, but high prevalences of resistance were also observed in several other studies. Resistance against many different antimicrobial agents was observed. Resistance against tetracyclines were reported in many studies, in both Gram-negative and Gram-positive bacteria. MDR were

reported in several species. VRE, MRSA, and ESBL-producing bacteria were reported in only a few studies.

For more information see Appendix II, Table 7

### ***Diets (herbivorous, carnivorous, omnivorous)***

All diet groups were represented, but the majority were omnivores.

### ***Conclusion***

Some studies indicated higher rates of resistance and more multiresistance in small animals living near farms. However, there were only a few studies that explored the effects of the level of anthropogenic exposure that had a clear design. One longitudinal study sampled rodents over 2 years (repeated sampling). Although AMR in small terrestrial mammals is relatively low, it is remarkable that detection of MDR, MRSA, VRE, and multi-resistant ESBL-producing *E. coli* was higher in small terrestrial mammals than large terrestrial mammals. A correlation between diet and type of ARB could not be identified due to the high variation in different animal species in this category. Small mammals appear to be useful bioindicators of fine-scale spatial variation in the distribution of AMR and, potentially, of the risks of AMR transmission to mammalian hosts, including humans (Furness et al., 2017).

## **3.4.7 Antimicrobial resistance in wildlife in Norway**

A limited number of studies have examined AMR in Norwegian wildlife, the majority of which have been performed as part of the NORM-NORM/VET programme:

### **Red deer, moose, roe deer (NORM/NORM-VET, 2002)**

A total of 137 isolates of *E. coli* from wild cervids were susceptibility tested; 48 from moose, 45 from red deer, and 44 from roe deer. Also, a total of 19 enterococcal isolates were susceptibility tested; 13 *E. faecalis* – three from moose, five from red deer, and five from roe deer, and six *E. faecium* – three from red deer and three from roe deer. The antimicrobials to which resistance was observed were oxytetracycline, trimethoprim, sulphamethoxazole and streptomycin. These four drugs are commonly used for therapy in Norwegian husbandry. The *E. coli* isolates from moose were susceptible to all antimicrobials included. One of the *E. coli* isolates from red deer was resistant to two antimicrobials (sulphamethoxazole and streptomycin). Of the *E. coli* isolates from roe deer, one was resistant to oxytetracycline, and another was resistant to oxytetracycline, sulphamethoxazole, and trimethoprim. The number of enterococcal isolates included was too low to obtain a good indication of the resistance situation.

### **Wild reindeer (NORM/NORM-VET, 2003)**

Faecal samples from 50 wild reindeer were investigated. *E. coli* was isolated from 42 of the samples, and these isolates were susceptibility tested and indicated moderate occurrence of resistance. In total, 76.2 % of the isolates were susceptible to all antimicrobials tested and 23.8% were resistant to at least one of the antimicrobials. Six isolates (14.3%) were resistant to one antimicrobial (streptomycin), one (2.4%) to two (streptomycin and sulphamethoxazole), and three (7.1%) to three antimicrobials (streptomycin, oxytetracycline and sulphamethoxazole).

### **Red foxes (NORM/NORM-VET, 2010)**

A total of 88 intestinal samples from wild red foxes from Hedmark county shot during the 2010 hunting season were investigated. *E. coli* was isolated from 55 (62.5%) of samples. One isolate per positive sample was susceptibility tested. Resistant *E. coli* isolates was identified from eight out of 88 animals examined. Resistance to sulphonamides was most commonly observed, followed by resistance to tetracycline and streptomycin, then to ampicillin, trimethoprim, quinolones, and gentamicin. Multi-resistant strains (resistant to more than two antimicrobial agents) were isolated from four animals. From two animals, isolates with different resistance profiles were obtained. These isolates produced distinct pulsed-field gel electrophoresis (PFGE) patterns, thus indicating the presence of several different genotypes of resistant *E. coli* isolates in the intestinal microbiota. Resistance to broad-spectrum and critically important antimicrobials agents such as fluoroquinolones and gentamicin was also detected. This study shows that wild foxes in Norway can be colonised with resistant *E. coli*.

### **Wild reindeer (NORM/NORM-VET, 2012)**

Faecal samples from 134 wild reindeer in three different populations were investigated. *E. coli* was isolated from 107 of the samples, and these isolates were susceptibility tested and indicated moderate-low occurrence of resistance. Compared with the 2003 results, there was a significant increase in susceptibility as 89.7% of the isolates in 2012 were susceptible to all antimicrobial agents tested, compared to 76.2% in 2003 ( $p=0.03$ ). However, the results from 2003 were based on only 42 isolates and the confidence intervals slightly overlap. The highest prevalence of resistance was for streptomycin with 6.5% in 2012, compared with 24% in 2003.

### **Red foxes, wild birds (NORM/NORM-VET, 2016)**

Faecal swab samples from a total of 528 red foxes and 357 wild birds were examined and *E. coli* isolates were obtained from 434 (82.2%) foxes and 303 (84.9%) wild birds. One isolate per positive sample was susceptibility tested.

### **Foxes**

The samples were divided into three different groups based on human population density to reflect possible exposure to drivers for antimicrobial resistance related to human activity. *E.*

*coli* was investigated for the occurrence of AMR. Additionally, resistance to some selected critically important antimicrobial agents was investigated by the use of selective screening. The occurrence of resistance in *E. coli* was low, with 92.3% susceptible to all antimicrobials included in the test panel. However, the occurrence of AMR differed significantly between the medium and high population density areas with 4.7% and 15.2% resistant to at least one antimicrobial substance, respectively. MDR (i.e. isolates resistant to  $\geq 3$  antimicrobial substances) was only detected in (2.4%) of the isolates, but none of these were in the high human population density areas. Occurrence of both quinolone-resistant *E. coli* and *E. coli* resistant to 3rd generation cephalosporins were uncommon in the low population density areas, whereas these resistance forms were more frequently detected in foxes in areas with medium and high population density. (<https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2017/antimicrobial-resistance-in-the-norwegian-environment-red-fox-as-an-indicator>).

### **Wild birds**

A low occurrence of resistance among *E. coli* from faecal samples of wild birds was found. In total, 91.4% of the isolates were susceptible to all antimicrobial agents included. Altogether, 4.6% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin or tetracycline), 1.0% to two, 1.7% to three, and 1.3% to four or more antimicrobial agents. Resistance to ampicillin and tetracycline were the most frequently identified resistance determinants, followed by resistance to sulphamethoxazole, ciprofloxacin, and nalidixic acid. One isolate showed decreased sensitivity to colistin. Neither of the plasmid-mediated genes *mcr-1* and *mcr-2* were identified, indicating that the resistance phenotype is due to chromosomal mutations. None of the isolates displayed resistance to 3rd generation cephalosporins, cefotaxime or ceftazidime, indicating a prevalence below 1.2%. Resistance to quinolones (i.e., ciprofloxacin and/or nalidixic acid) was identified in 2.3% [95% CI:0.9-4.7 %] of the isolates.

### **Carbapenemase-producing Enterobacteriaceae from red fox and wild birds**

A total of 514 red fox and 357 wild bird samples were screened for the presence of carbapenemase-producing Enterobacteriaceae. One isolate, an *Enterobacter asburiae*, from wild birds was resistant to the carbapenems, meropenem, imipenem, and ertapenem.

### **Colistin-resistant *E. coli* from wild birds**

Selective screening for detection of colistin-resistant *E. coli* was performed on samples from wild birds. A total of 358 wild bird samples were screened and colistin-resistant *E. coli* were not detected.

### **Original articles**

Several articles describe investigation antimicrobial resistance from wildlife in Norway. See Appendix II: wild birds, including migratory birds (Literak et al., 2014) (**Table 2**); wild fish



and aquatic animals (Grevskott et al., 2017) (Table 3 ); large terrestrial mammals (Glad et al., 2010; Lillehaug et al., 2005) (Table 6).

## **Conclusion**

A total of 10 studies (six reports and four original articles published between 2002 to 2017 regarding ARB in wildlife in Norway were considered in this Opinion. Most of the studies included large terrestrial animals, some focused on birds, and one study was on animals belonging to the category fish and aquatic animals. Enterobacteriaceae and enterococci were the most commonly studied bacteria from different animal groups from different geographical areas in Norway. Resistance against a range of different antimicrobial agents like streptomycin, tetracyclines, sulphamethoxazole, quinolones, vancomycin, and cephalosporines was identified. Resistance against carbapenems, and colistin was also detected in some bacterial isolates. The same resistance traits can also be observed in isolates from animal husbandry, pets, or humans in Norway.

### **3.6.8 Geographical distribution of studies**

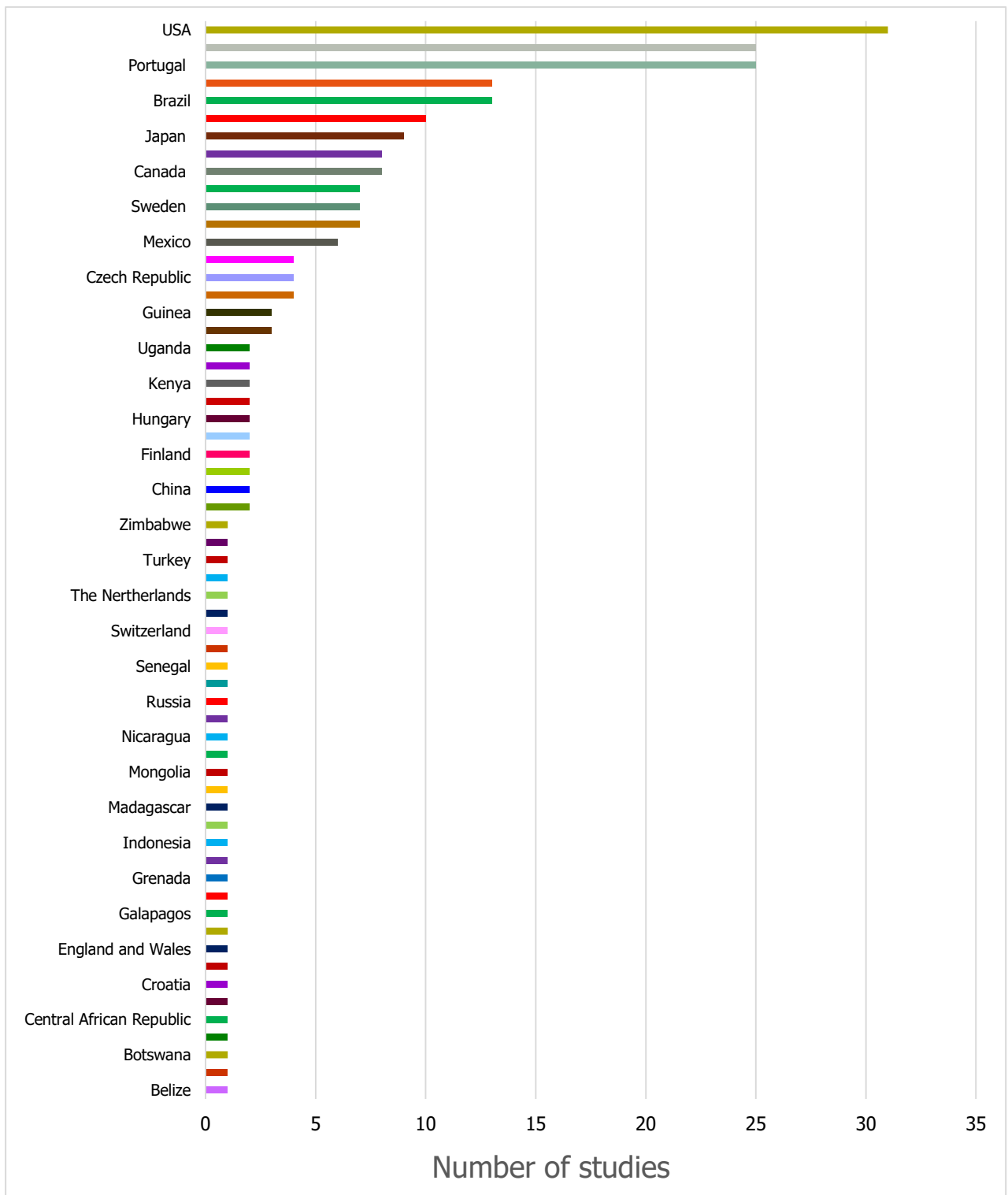
**Table 2** shows the host groups of animals in the articles/reports included in this Opinion divided by different geographical regions and **Figure 4** illustrates the number of articles from different countries.

**Table 2.** Host groups and regions in the articles that fulfilled the inclusion criteria in this assessment\*.

Region	Amphibians and reptiles	Birds including migratory birds	Water-living animals, including fishes	Insects	Terrestrial mammals			Total
					Boars	Large mammals	Small mammals	
<b>Africa</b>	1	0	0	0	0	13	4	<b>18</b>
<b>Asia</b>	0	12	1	1	1	6	6	<b>27</b>
<b>Europe (except Norway)</b>	1	56	7	1	21	30	26	<b>142</b>
<b>North America</b>	7	20	6	1	1	4	10	<b>49</b>
<b>Oceania</b>	0	2	1	0	0	1	0	<b>4</b>
<b>Polar regions</b>	0	1	0	0	0	0	0	<b>1</b>
<b>South America</b>	5	12	2	0	1	7	1	<b>28</b>
<b>Norway</b>	0	2	1	0	0	8	0	<b>11*</b>
<b>Total</b>	<b>14</b>	<b>105</b>	<b>18</b>	<b>3</b>	<b>24</b>	<b>69</b>	<b>47</b>	<b>280**</b>

\*6 reports from NORM-NORM/VET about fox, reindeer, moose, wild birds (NORM/NORM-VET, 2002; NORM/NORM-VET, 2003; NORM/NORM-VET, 2010; NORM/NORM-VET, 2012; NORM/NORM-VET, 2015; NORM/NORM-VET, 2016).

\*\* 237 original articles. In several cases, the same article included different animal species and the samples were from different countries and continents.



**Figure 4.** Distribution of scientific articles reporting on AMR bacteria in wildlife in different countries in the world. The number of countries is lower than the number of articles. For Norway 4 scientific articles and 6 scientific reports were found.

## 3.5 Methodology used in the evaluated studies

### Sampling

The experimental design and sampling methods used in the studies included is not harmonised, and shows a wide variety of approaches, even for the same sample material, animal, or group of bacteria. For smaller animals, such as insects and marine bivalves, the whole animal or pooled samples, consisting of several animals, were commonly examined. For most larger animals, samples of faeces or rectal/cloacal swabs were frequently included. In addition, intestinal content from several sections of the gastro-intestinal tract have been included in some studies. For non-enteric bacteria, tissue samples from viscera, lymph nodes, or tonsils, as well as urine and blood were included in some studies. Whole body swabbing, or swabbing of specific sites, such as eyes and ears, or the mucosa of the mouth, nasopharynx and genitalia were also conducted.

The samples were obtained either by collecting diseased or dead animals in the wild, by hunting or fishing, or by collection of sessile animals, such as marine bivalves. In addition, non-lethal sampling of animals captured in nets or traps was performed.

### Identification of bacterial species and strains

A variety of cultivation-based methods were used to obtain groups of bacteria or single-species isolates. The methods were largely non-harmonised between studies. The methods include growth on or in general agar and broth media, or by selective isolation on specific media designed to support the growth of certain bacteria or bacterial groups. Identification of pure isolates was done by biochemical methods (e.g. API), phage typing, serological methods, DNA-based methods, and or by Matrix Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS).

- Serology: Usually involves detection of surface antigens by direct agglutination, or indirectly by enzyme or fluorescence immunoassays.
- Phage typing: the viruses that infect bacteria are called bacteriophages and some of these can only infect a single strain of bacteria. These phages are used to identify different strains of bacteria within a single species (Wikipedia).
- MALDI TOF MS: A high-throughput protein sequencing method based on embedding samples in a matrix from which they are desorbed by laser light followed by fragment analysis.

A variety of culture-independent methods have also been used in the described studies. However, these have usually been performed on DNA extracted from pure isolates obtained after initial an intial culturing stage. Many of these methods are well known and include detection of housekeeping genes by PCR, gel electrophoresis, and amplicon match analysis, whole genome sequencing after DNA amplification by PCR, and followed by alignment

analysis by BLAST, PCR with DNA probes for specific genes, 16S RNA gene analysis, and examination of plasmids by agarose gel electrophoresis.

- RAPD: Analysis of DNA fragments from PCR amplification of random segments of genomic DNA, using single primers with arbitrary nucleotide sequences.
- Repetitive sequence-based PCR (rep-PCR): rep-PCR relies on amplifying repetitive sequences to produce amplicons of varying lengths that can be separated by electrophoresis giving a fingerprint comprised of bands that fluoresce at different intensities after binding with an intercalating dye.
- Ribotyping: based on normally occurring varieties in the sequence of the 16S and 23S rRNA genes coding for ribosomal RNA. The genes are cut using restriction enzymes and the DNA fragments separated by electrophoresis. The resulting fingerprint is visualised using fluorescent probes.
- PFGE: electrophoretic separation of low numbers of large DNA fragments produced using restriction enzymes to generate a highly discriminatory genetic fingerprint.
- MLST: sequencing 400-500 base pair fragments of DNA at seven different conserved genes allows small variations within a species to be detected. Quite time consuming and costly, but can be highly discriminatory if appropriate genes are chosen.
- MLVA: a technique related to MLST based on PCR amplification and sequencing of rapidly mutating repetitive DNA sequences called tandem repeats
- Metagenomics or whole genome sequencing: sequencing the entire genome of a microbial isolate to provide definitive typing

Data regarding genotyping methods; ribotyping, MLVA, MLST, PFGE, rep-PCR, and whole genome sequencing can be found in website of Rapid Microbiology (<http://www.rapidmicrobiology.com/test-method/molecular-techniques-for-microbial-identification-and-typing/>).

Data from typing methods can be used to generate a dendrogram tree that depicts the relationship between bacterial species, illustrating evolutionary changes from ancestral to descendant forms, based on shared characteristics.

## **Measurement and detection of antimicrobial resistance**

### **Susceptibility tests**

The susceptibility to antimicrobial agents in the isolated strains, in the studies examined here was investigated by four different methods: agar diffusion, agar dilution, broth dilution, and E-tests. In most cases, the overall goal was to classify isolates as sensitive, intermediate, or resistant, as compared with known phenotypes.

In the agar diffusion method, paper discs or tablets with a fixed amount of the antimicrobial to be tested, are placed on top of a lawn of bacteria inoculated on the agar. After incubation, the diameter of the diffusion zones with no or reduced growth will be compared with pre-established values, and the susceptibility and the Minimum Inhibitory Concentration (MIC) established. Correlation schemes to estimate the MIC from information on inhibition size from agar diffusion are available for many bacterial pathogens. As these schemes depend on the species, this method is not useful as a rapid screen of bacterial species that do not have an established scheme.

The agar dilution method is based on an inoculation of the test strain on agar with increasing concentrations of the tested antimicrobial. In broth dilution tests, increasing concentrations of the antimicrobial to be tested are added to broth in reagent tubes or in multi-well trays inoculated by the test strain. In both cases, the MIC value can be determined from the lowest concentration of a given agent that inhibits growth of the microorganism after incubation. The E-test is based on inhibition zones obtained when placing a paper strip with a concentration gradient on the surface of an agar inoculated with the strain to be tested. After incubation, a leaf-shaped inhibition zone can be seen. The MIC value can be read directly.

The experimental setups to assess susceptibility were mostly performed as recommended by international bodies, such as Clinical and Laboratory Standards Institute (CLSI), European Committee for Antimicrobial Susceptibility Testing (EUCAST), or National Committee for Clinical Laboratory Standards, USA (NCCLS). In addition to giving recommendations on methods, these institutions also propose the classification of individual strains into "sensitive", "intermediate" or "resistant" according to the observed MIC or inhibition zone size ("breakpoints").

### **DNA-based methods for identification of resistance traits**

A number of DNA-based methods are available to identify the presence of particular resistance genes/determinants in the bacterial genome. These include detection of resistance genes by PCR, gel electrophoresis and amplicon match analysis, whole genome sequencing followed by bioinformatics, and further confirmation by PCR for specific genes.

## **3.6 Summary of hazard characterisation**

AMR is often an acquired trait with the potential for further transfer between bacterial cells, species, and populations. Acquired resistance to pharmaceutically produced antimicrobial agents is the outcome of a number of sequentially occurring steps taking place at the genetic, cellular, population, and community levels. Several key factors and processes behind resistance emergence, dissemination/dispersal, and persistence in previously susceptible bacterial populations have been identified and characterised. The specific genetic basis for an acquired resistance trait will determine the likelihood for further vertical and horizontal dissemination in a bacterial population.

The occurrence of ARB in wild mammals, birds, reptiles, amphibians, and fish is the result of the environment in which they live and the extent to which bacteria there are exposed to antimicrobial substances and resistance traits. Environmental bacterial populations are expected to be exposed to resistant bacterial cells and their remains, as well as antimicrobial agents from anthropogenic environments. Directional exposure from anthropogenic environments is identified as a hazard that can materialise in wildlife depending on the following steps:

**presence** of resistance genes, resistant bacteria, and/or anthropogenic antimicrobial drugs or other AMR-driving substances in the environment of relevant wildlife,

**exposure** of wildlife resulting in uptake of resistance genes, ARB, or AMR-driving substances,

**establishment** in the wildlife of a population of ARB that are viable long enough to allow transmission of bacteria or resistance to other wildlife, humans, or domestic animals,

**contact** between individuals/bacteria of the wildlife population and other wildlife, humans, or domestic animals sufficient to facilitate transmission of bacteria with pathogenic properties.

The end result and adverse outcome are reduced options for antimicrobial drug therapy and thus increased morbidity and mortality.

A wide range of sampling strategies and methods for isolation and characterisation of AMR have been used. In most cases, resistant isolates were first characterised by cultivation-based methods. In some cases, a genetic analysis based on known resistance determinants was also performed. Direct comparisons between studies are often difficult due to lack of uniformity in methodological approaches and reporting.

The studies considered in this report were divided according to the various animal groups (e.g. amphibians & reptiles, birds, fish and other water-living animals, and terrestrial mammals) and further evaluated based on the following criteria: number of studies, timespan of publications, geographical areas where the studies performed, resistance types with focus on specific resistant bacterial species. The large majority of studies focused on bacterial species that are common in humans and domestic animals. Thus, the focus on these bacterial species in the literature enables a glimpse into the interfaces between wild-domestic animal-humans in recent decades. Many of the bacteria studied are of fecal origin that are able to establish themselves in animals, including wildlife, and humans.

Although many point prevalence studies are available, few have been able to establish directionality of resistance transfer. There is overall a tendency for higher resistance levels in animals with greater exposure to anthropogenic sources. Sampling size was limited in many of the studies, and longitudinal perspectives were lacking. Most studies were descriptive rather than quantitative. The lack of a quantitative focus, as well as limited information on the genetic basis for the resistance traits observed, prevent further examination of exposure pathways and the evolutionary trajectories of particular resistance determinants.

## 4 Exposure assessment

The high number of potential sources and routes that can lead to unwanted exposure of people and domesticated animals to ARB from wildlife, creates complexity and scenario uncertainty in the analysis. Incomplete identification of relevant resistance determinants hinders our understanding of the evolutionary trajectories of particular resistance determinants. The lack of quantitative resolution precludes a quantitative assessment of exposure. A quantitative exposure assessment is not a part of the mandate for this Opinion, and is therefore not considered further

## 5 Risk characterisation

Risk characterisation has been defined by the WHO as follows: "Integration of hazard identification, hazard characterization and exposure assessment into an estimation of the adverse effects likely to occur in a given population, including attendant uncertainties." The definition includes quantitative risk assessment, which emphasizes reliance on numerical expressions of risk, and also qualitative expressions of risk, as well as an indication of the attendant uncertainties (<http://www.who.int/foodsafety/risk-analysis/riskassessment/en/>).

Since we are unable to determine the exposure levels and consequences thereof quantitatively, we cannot estimate the risk, neither quantitatively nor qualitatively.

Due to the lack a quantitative risk assessment, a full risk characterization cannot be provided and is considered not applicable for this Opinion.

Below we discuss the development of ARB in wildlife and onward transmission.

### 5.1 AMR in wildlife and possible transmission

#### Available data

The currently available data regarding AMR in wildlife were collected from four databases (see literature search), resulting in more than 230 peer-reviewed articles, excluding 10 review articles. In addition, we included 6 reports from NORM-NORM/VET reporting on AMR in wildlife in Norway. Most studies focused to birds and terrestrial mammals in Europe, mainly central Europe, followed by North America (**Table 2**). Studies from developing countries are largely lacking. Most studies were observational/descriptive studies conducted in a defined geographical area over a limited timespan, and with no follow-up. Most studies of AMR in wildlife are survey based and/or small scale, providing limited understanding of the sources and sinks of AMR in the environment investigated. The general lack of quantitative understanding and data on the flow of AMR genes and ARB through natural environments could reflect methodological challenges due to the high number of AMR



sources and amplifiers in the populated world (Arnold et al., 2014). In this opinion, we summarise the majority of research conducted on AMR in wildlife, identify knowledge gaps and areas of uncertainty, and explore the interfaces between wildlife, domestic animals, and humans in the context of resistance emergence, persistence, and transmission.

### **Sampling methods and bacterial species**

The sampling methods in the literature are not harmonised, and vary even for the same sample material, animal, or group of bacteria. From smaller animals, such as insects and marine bivalves, the whole animal or pooled samples consisting of several animals were commonly examined. For most larger animals, samples of faeces or rectal/cloacal swabs were frequently examined. The large majority of these studies used culture-dependent methods for species identification and resistance determination. A minority of studies used additional methods, such as PCR and sequencing. Challenges in assigning particular resistance traits to specific bacterial species limits the utility of metagenomic approaches. For obvious reasons, data regarding ARB present in wildlife in biological materials other than faecal samples were limited.

The large majority of the studies have focused on bacterial species that are also found in clinical settings. This often skews the analysis to easily culturable bacteria of faecal origin and those that thrive in the gut system of different animal species. It should be noted that only a small fraction (approx. 1 %) of environmental bacteria is culturable, and the bias towards culturable faecal bacteria in these studies places a considerable limit on our knowledge about the true diversity and composition of AMR in non-clinical environments (Finley et al., 2013). Due to the limitations in study design and short sampling periods, the available studies do usually not reveal if the bacterial isolates found are temporary residents or permanent colonizers and have caused infection in the hosts. Most studies of AMR in wildlife are descriptive and sample oriented, and focused on identifying bacteria or resistance mechanisms that have known human health concerns.

While various bacterial species are important in terms of multi-resistance and nosocomial infections in human and veterinary medicine, we consider the Gram-positive vancomycin-resistant enterococci (VRE) and Extended-spectrum Beta-Lactamases producing Gram-negative bacteria like *E. coli* (ESBL-*E. coli*) as being key indicator pathogens to trace the evolution of multi-resistant bacteria in the environment and wildlife (Radhouani et al., 2014). In the studies included in this Opinion, two bacterial groups were studied in 60 % of the studies: *E. coli* (120 studies) and *Enterococcus* spp. (43 studies). In the **Table 3**, we summarise the different bacterial species examined in studies included in this Opinion.

**Table 3.** The most numeric bacterial species considered in the studies examined in this opinion. Most of these bacterial species are well-described in humans and also found routinely in various terrestrial animals:

Bacterial species	Number of studies	% of the total studies
<i>E. coli</i>	120*	44%
<i>Enterococcus</i> spp.	43**	16%
<i>Salmonella</i> spp.	43	16%
<i>Campylobacter</i> spp.	14	5.5%
<i>S. aureus</i>	15	5.5%

Other (10 or fewer studies per microbe): *Klebsiella* spp., *Shigella* spp., *Citrobacter* spp., *Proteus* spp., *Enterobacter* spp., *Yersinia* spp., *Escherichia* other than *coli*, *Hafnia* spp., *Serratia* spp., *Shewanella*, *Pseudomonas*, Coagulase-negative staphylococci, *Micrococcus* spp., *Corynebacterium* spp., *Providencia* spp., *Burkholderia* spp., *Streptococcus* spp., *Brucella* spp., *Mycobacterium* spp., *Listeria*, *Bacteriodes*, *Pantoea agglomerans*, *Arcanobacterium*, *Erysipelothrix*, *Aeromonas*, *Vibrio* spp., *Brachyospira*, *Actinobacteria*, *Proteobacteria*, *Frimicutes*, *Catelicoccus* spp., *Alcaligenes*, *Troprella pyogenes*, *Rahnella*, *Kluyvera*, *Plesiomonas*, *Lactobacillus*, *Acinetobacter*, *Hafnia*, *Morganella*, *Clostridium difficile*, *Psychrobacter* spp., *Brachyospira* spp., *Pasteurella*, Broad groupings e. g. Enterobacteriaceae, Gram-negative, Gram-positive, Obligate anaerobes.

\*114 international published articles and 6 reports from Norway (NORM-NORM/VET).

\*\* (42 international published articles and 1 report from Norway).

## Potential sources of AMR in wildlife

Parts of the current resistome pre-date human use of antimicrobial agents. Resistance arises in nature as a mechanism of bacterial defence against naturally produced antibiotics. Thus, AMR in the bacterial microbiota of wildlife is not simply a matter of recent anthropogenic contamination or selection. Our knowledge regarding the prevalence of AMR due to «natural antibiotics» in wildlife is, nevertheless, limited as there are few truly pristine environments. A bacterial collection pre-dating the antibiotic era is the Murray collection, including clinical isolates of Enterobacteriaceae from widely separated areas, collected during the period 1917–1954. In these isolates, the presence of acquired resistance traits common today was found to be negligible. This observation emphasizes the impact of the use of pharmaceutically-produced antimicrobial agents in promoting dissemination of acquired resistance among pathogenic bacteria (Hughes and Datta, 1983).

The selective pressure generated by the use of antimicrobials in clinical, veterinary, and agricultural practices has been the major driving force leading to the emergence and spread of AMR, as reviewed by (Pallecchi et al., 2008). The rapidly expanding aquaculture industry is another source of AMR and antimicrobials entering the environment: fish and seafood farmed in some countries where antimicrobial usage is high and poorly regulated are particularly likely to carry medically significant resistant pathogens (Cabello et al., 2013; Greig et al., 2015). Wildlife is not intentionally exposed to clinically used antimicrobials, but they can acquire resistance through exposure to resistant bacteria from humans, domestic animals, and polluted environments, where water polluted with faeces seems to be the most important vector (Guenther et al., 2011).

In a review article by (Arnold et al., 2014), the exchange of the ARB and resistance genes in the environment, including wildlife animal species, is summarised. Tracking the molecular ecology of AMR is complicated by the horizontal spread of genes encoding AMR through communities of different species and even genera of bacteria, via mobile genetic elements such as plasmids. These mobile genetic elements often encode multiple genes, providing resistance to antimicrobials and, indeed, other environmental chemical stressors, including metals and disinfectants. Consequently, exposure to one antimicrobial (or other stressor) can select for all co-encoded genes and thus facilitate the rapid emergence of MDR. Bacteria from wildlife and other environmental bacteria that have never been found to infect humans can, through horizontal gene transfer, exchange resistance mechanisms with human pathogens (Arnold et al., 2014).

AMR should be viewed as a complex ecological problem and also addressed by applying a landscape ecology approach, which takes into account the multitude of factors affecting this phenomenon (e.g., type and level of selective pressure, routes of transmission, and mechanisms of persistence) (Singer et al., 2006).

### **Resistance in the absence of antimicrobial exposure**

Acquired AMR has been detected among commensal bacteria isolated from humans and wildlife not subject to significant antimicrobial exposure and living in remote areas of the planet. These observations underscore the complexity of the mechanisms involved in the emergence and spread of AMR (Pallecchi et al., 2008). The review article of (Pallecchi et al., 2008) summarises the presence of AMR in the absence of selection pressure as follows: 'Whichever the mechanism responsible for the introduction of resistance genes in remote settings, the reasons for their maintenance in the absence of an evident selective pressure remain presently unclear'.

### **Resistance types**

Despite some differences in the methodological procedures and in the characteristics of the studied populations, results from the most recent studies are remarkably consistent in showing considerable rates of acquired resistance to older natural antibiotics (i.e., phenoxymethylpenicillin, tetracycline, and chloramphenicol), semisynthetic (e.g., ampicillin), and synthetic antimicrobial agents (e.g., trimethoprim–sulphamethoxazole) being found among commensal enterobacteria from subjects living in remote settings who have experienced low or minimal exposure to antimicrobials (Pallecchi et al., 2008).

**Table 4** highlights important resistant bacterial species that have emerged at animal-human interfaces during recent decades. These resistant strains seem to have the propensity for interspecies spread and are able to establish in animals, including wildlife, and humans. The proportion of isolates with resistance profiles in these categories is low when considering the overall number of studies and isolates examined in this opinion. The prevalence was highest for ESBL-producing Enterobacteriaceae (n=43) for which resistance was found in all animal groups, followed by VRE (n=15).

**Table 4.** Distribution of the clinically most important resistance types found in different animal groups. Wild boars are also large terrestrial animals, but are presented separately in this Opinion due to their increased presence as an invasive species in some counties in southern Norway.

Resistance types	Amphibian and reptiles	Birds	Fish and aquatic animals**	Insects***	Terrestrial mammals		
					Wild boars	Large	Small
	N	N	N	N	N	N	
<b>Enterobacteriaceae *</b> (mainly <i>E. coli</i> and <i>Klebsiella</i> )							
<b>ESBL-producing</b>	2	21	1	1	2	11	5
<b>Carbapenemase-producing</b>	1	2	0	1	1	0	0
<b>Colistin-resistant</b>	0	2	0	1	0	0	1
<b><i>Campylobacter</i> spp. (Quinolone-resistant)</b>	0	6	0	0	1	2	1
<b><i>Enterococcus</i> spp. (VRE)</b>	2	6	0	0	2	3	2
<b><i>Staphylococcus</i> spp. (Methicillin-resistant)</b>	1	1	0	0	2	4	5

N: Number of studies.

\*Resistance against cephalosporins, carbapenems, and colistin has also been identified in other bacterial families than Enterobacteriaceae.

\*\* Other bacteria than Enterobacteriaceae, *Campylobacter*, *Enterococcus*, and *Staphylococcus* were examined in this animal group.

\*\*\* Insects group contains only 3 articles. ESBL-producing, carbapenemase-producing, and colistin-resistant *E. coli* were from the same study.

## Diets

Studies offering a basis for comparison of the prevalence of AMR in species with different diets within a habitat are scarce. For information regarding diets in the studies included in this Opinion, see Tables 1-7: Appendix II. Carnivores include also piscivores, insectivores, and scavengers. None of the articles provided specific information regarding the diets of the

wildlife included in the studies. Some studies do not list the exact species they sampled (sometimes just referred to as “wild birds” or “wild mammals”) or do not give the number of individuals sampled, only referring to those that yielded resistance results.

The evaluated studies when categorized according to diet suggest that omnivores are generally more likely to carry ARB. Omnivorous species often feed on anthropogenic waste and near human habitations and farms, and could thereby represent a major epidemiological link between domestic animals, humans, and wildlife. Data in Tables 1-7 in Appendix II also support this suggestion.

**Table 5.** Wildlife animal categorized according to diets and the occurrence of important resistance types in bacteria isolated from these animals.

<b>Resistance types</b>	<b>Herbivores</b>	<b>Carnivores</b>	<b>Omnivores</b>	<b>Not identified diets*</b>
<b>ESBL-producing** Enterobacteriaceae</b>	5	6	25	9**
<b>Carbapenemase- producing bacteria</b>	0	0	3	1
<b>Colistin-resistance</b>	1	0	2	0
<b>Vancomycin- resistant enterococci (VRE)</b>	2	0	9	2
<b>Quinolone-resistant <i>Campylobacter</i></b>	3	0	6	1
<b>Methicillin-resistant staphylococci (MRSA)</b>	3	0	4	7
<b>Total number of studies</b>	14	6	49	20

\*Not identified diets: a) several animal species with different diets were included in the studies and no specific information was given regarding the resistance status of the bacteria isolated from the different animal species, or b) diet could not be identified. \*\*Two ESBL producing bacteria belonged to marine filter feeding wildlife.

## Habitats

The studies suggest AMR is widely distributed in natural ecosystems, although their concentrations and relative proportions have often not been quantified. The review of (Vittecoq et al., 2016) lists a number of niches with different animal species where transmission of AMR may occur:

Humans and domestic animals can be in direct contact with wildlife species and their feces when they live or feed near habitations.

Water is considered as a major transmission medium for antimicrobials and ARB as indicated by the occurrence of agents and bacteria in sewage effluent reaching rivers, in lakes and even in seawater. Furthermore, there is clear evidence of exchange of resistance genes between environmental bacteria and human pathogens in aquatic systems. ARB in water originate from humans, domestic animals, and wild animals. In numerical terms, water represent an unparalleled massive arena for physical contact between fecal bacteria from different sources.

AMR occurs naturally in soil communities, notably due to the production of antibiotics by some soil bacteria and fungi. But the presence of ARB in soil can also be due to the use of irrigation water, direct deposition of faeces or urine (e.g., in pastures), or the use of manure or from effluents. This complex interface is likely to provide an important route for multidirectional exchange of AMR genes and ARB in an agricultural context.

Wildlife populations living in close proximity to humans may be reported to have a higher levels of AMR than wild populations with virtually no contact with humans or anthropogenic antimicrobial sources (Osterblad et al., 2001). However, other studies suggest a less clear relationship. Distant use might have caused a multiresistant organism/trait to evolve and then spread within different ecological niches (O'Brien, 2002).

Although some tendencies were seen, overall, despite the large numbers of studies examined, we were un able to draw firm conclusions concerning differences in prevalence of AMR in wildlife across different environmental habitats, or to conclude on directions for resistance transmission. Most studies focused either on only one kind of habitat, or different sites but without detail or statistical power. In certain cases, the study areas were also very large (an administrative region or a state) and included various habitats, but with the extent of anthropogenic exposure poorly described.

### **Consequences of AMR in wildlife**

Direct exposure to antimicrobials is rare in wildlife, indicating that sharing common habitats, and water sources, along with environmental contamination (Wellington et al., 2013) could result in transfer of ARB between wildlife and food-producing animals, both terrestrial and aquatic, with potential for subsequent transmission to the food chain. Wildlife harbouring AMR may also contaminate foods of plant origin (Jay et al., 2007). Although wildlife could provide a reservoir of genetic determinants for resistance, it has usually been assumed that AMR in wildlife samples is acquired AMR, resulting directly or indirectly from humans or livestock (including aquaculture) treated with antimicrobials (Marshall and Levy, 2011).

The biological consequences for wildlife from the evolution of AMR in commensal, or even pathogenic, bacteria have not been studied. Unlike avian influenza, for example, AMR is not a disease and does not appear to reduce the survival or dispersal capacity of resistance

carrying animals, although this has not been explicitly examined, reviewed by (Arnold et al., 2014).

ARB in wildlife may be transferred within and between species, and also between wildlife and food-producing animals in terrestrial and aquatic environments, and humans. Genetic similarity between human clinical, livestock, companion animal, and wildlife AMR bacterial isolates has been noted, but there is a need to gain further insights into the clonal relatedness of isolates from all these groups. Methodologically, MLST paired with PFGE is an ideal tool to reveal clonal relatedness or even clonal identity of epidemiologically unrelated isolates. This approach has already been used in a small number of studies of wild birds, all clearly indicating that similar sequence types or clonal groups are present in humans, domestic animals, and wild birds (Guenther et al., 2011).

## 5.2 Summary of risk characterisation

The development of a quantitative model to characterize the complex routes of formation of AMR bacteria in wildlife animal species is currently not feasible. As we are unable to assess exposure quantitatively in this assessment, the risk cannot be estimated, either quantitatively or qualitatively. However, we have considered the transmission and dissemination of ARB in and from wildlife based on available data. The experimental design and sampling methods used in the reviewed literature are not harmonised, varying between studies even on the same sample material, animals, or group of bacteria. For smaller animals, such as insects and marine bivalves, the whole animal or pooled samples, consisting of several animals, were commonly examined. For most larger animals, samples of faeces or rectal/cloacal swabs were frequently used. Two of the most important indicator bacteria, *E. coli* and *Enterococcus* spp., were studied in 60 % of the studies. To the extent examined in wildlife, identical resistance genes has been observed in wildlife and in human isolates. This suggest shared ancestry and recent transfers, in an evolutionary context.

Our knowledge regarding the prevalence of AMR due to the exposure to presence of antimicrobials in the environment and wildlife is limited. This Opinion does not focus on the evolution of AMR due to the presence of «natural antibiotics», but on resistance that have emerged as a consequence of antimicrobial usage. In recent decades, ARB have evolved at animal-human interfaces, and many bacteria have the propensity for interspecies spread. They can establish in both humans and animals, including wildlife. Few studies in the examined studies reported MDR profiles; the highest prevalence was for ESBL-producing Enterobacteriaceae (n=43), which were identified in all animal groups, followed by VRE (n=15). Regarding a possible link between diets and types of resistance in bacteria, omnivorous species, rather than herbivores and carnivores, are more frequently identified.

A number of interfaces with different animal species where transmission of AMR is likely to occur have been identified:

- a) Humans and domestic animals can be in contact with wildlife species that live or feed near habitations,
- b) Water is a vast environment where AMR bacteria that are present in considerable numbers in runoff- or treated water, rivers, lakes, and even sea water,
- c) Soils and agricultural practices due to the presence of wildlife and the use of irrigation water, direct faeces or urine deposition (e.g., in pastures) or manure use.



# 6 Uncertainties

The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions identified in all the previous steps. Discrimination between uncertainty and variability is important in the subsequent selection of risk management options. Biological variation includes for instance the differences in resistance levels that exist in microbiological populations over time, and between hosts and environments, including random fluctuations.

In this assessment, a number of uncertainties have been identified related to our understanding of the the probability of development of AMR, and dissemination of AMR from wildlife. Many of these uncertainties are caused by data gaps and a lack of quantitative framework leading to a high number of point prevalence oriented studies with little coherence between studies.

Some sources of the uncertainties identified are limitations in the understanding of the:

- Pathways for AMR development due to lack of knowledge on the genetic interactions that occur in environmental bacteria.
- Mechanisms responsible for maintenance of resistance in bacterial populations in the absence of selective pressure of significant antimicrobial agents.
- Transfer potential of AMR from unculturable bacteria to culturable bacteria and vice versa.
- True diversity and composition of bacteria as potential resistance carriers in non-clinical environments, because most environmental bacteria cannot be cultured.
- Identification of the specific conditions and circumstances that gave rise to known HGT events, as they can rarely be traced back.
- Representativeness of samples, as sample sizes are limited to a minute fraction of the environment being investigated.
- Sampling approach, which needs to take into account that only those bacteria with highest prevalence (concentration) can be described.
- Anthropogenic and natural factors causing migration of AMR bacteria between environment and wildlife.
- Ecological roles of antimicrobial agents in the natural environment, in particular the effects of sub-inhibitory levels.
- Extent the isolated bacteria from various samples that are transiently or permanently colonizing the wildlife examined.

For uncertainties regarding the technical aspects of laboratory methods, see data gaps.

We recommend a broader uncertainty analysis (EFSA et al., 2018) to identify critical uncertainties and urgent research gaps.

# 7 Conclusions (with answers to the terms of reference)

**1- List the transferable antimicrobial resistant bacteria identified in wildlife. The list should include type of animals, preferably divided into terrestrial and aquatic animals, where AMR bacteria have been described.**

Only a small fraction (approx. 1 %) of environmental bacteria can be cultured, and this places a considerable limit on our knowledge about the true diversity and composition of the antimicrobial resistance profiles of the total population of bacteria.

**Table 3** lists all bacterial species that have been recorded in the studies referenced in this Opinion. The great majority of studies focused on well-described bacteria found in the gastrointestinal tract of humans and animals. In these studies, two bacterial groups were investigated in 60 % of the studies: *E. coli* and *Enterococcus* spp. Although various bacterial species are important in terms of multiresistance and nosocomial infections in human and veterinary medicine, we consider the Gram-positive vancomycin-resistant enterococci (VRE) and Extended-spectrum Beta-Lactamase producing Gram-negative bacteria like *E. coli* (ESBL-*E. coli*) as being key indicator pathogens for tracing the distribution of multiresistant bacteria, with pathogenic properties, in wildlife. The number of studies with resistance profiles in these categories is low. The prevalence was highest for ESBL-producing Enterobacteriaceae (n=43), presented in all animal groups, followed by VRE (n=15). Other important resistant bacterial strains and species were MRSA (n=13), and quinolone-resistant *Campylobacter* (n=10). Distribution of the clinically most important resistance types found in different animal groups, both aquatic and terrestrial, are provided in **Table 4**.

**2- List the different methods used for sampling and analysis of data. Based on information collected, evaluate the suitability of different methods used.**

Chapter 3.7 summarize the different methods used for sampling, isolation, and identification of bacteria, as well as those used for determination and characterisation of antimicrobial resistance. A wide range of sampling strategies, isolation techniques for bacteria, and methods for characterisation of antimicrobial resistance have been used. The following protocol was commonly used: dead or live wild animals or their faeces (other samples include skin, mucosal swabs, tissue, or urine) were collected, from these samples, bacterial groups or single species, (e.g., coliforms or *Salmonella*) were isolated and subsequently identified. Thereafter, their antimicrobial resistance were first characterized by cultivation-based methods, and then, in some cases, a molecular based technique was conducted to obtain genetic information on the presence of particular resistance determinants.

The sampling strategies used lacks standardisation, were not harmonised, and showed variation even for the same sample material, animal, or group of bacteria to be examined.

Sampling is often done over a limited period, and only few publications investigate resistance dynamics over time. The methods for sample storage may also bias the results. In the older publications, especially, a variety of cultivation-based methods have been used to obtain and identify groups of bacteria or single-species bacterial isolates. Methodologically, MLST paired with PFGE is an ideal tool to reveal clonal relatedness or even clonal identity of epidemiologically unrelated isolates.

This also partially applies for cultivation-based characterisation of antimicrobial resistance. However, several authors rely on standardised methods and breakpoints to define resistance (EUCAST, NCCLS) after pure isolates have been obtained. Only a few of the examined studies have determined the genetic basis of AMR and these are even less standardised. When positive, they show that the same resistance genes can be found in animals and humans. Direct comparison of resistance patterns obtained in different studies is hampered by the diversity of experimental design, sampling to data processing and presentation of resistance results.

**3- Evaluate information on AMR in bacteria in wildlife, according to their habitat. Preferably, information should be grouped into animals that living close to urban areas, to urban areas, living in rural areas, or are migratory.**

Our knowledge on AMR in bacteria in wildlife is largely limited to the results from cross-sectional prevalence-based studies of the occurrence of AMR in particular wildlife species in various locations over limited periods of time. Few of these studies have been able to establish the directionality of resistance transfer. Long-term, longitudinal studies that are based on the known ecology, food habits, and spatial movement patterns of different wildlife species could provide more in-depth knowledge on which populations and subpopulations are exposed to AMR, the extent to which AMR establish and persist in populations, and the degree to which these wildlife species share an interface with humans that could promote transmission.

The available scientific literature indicates that ARB are carried by both wild and feral animals, although the reported prevalences are usually lower than in domestic animals. The literature also indicates some support to the view that wild and feral animals living in areas with high human population densities (urban areas) or areas with intensive agricultural production, for example swine farms, have a higher prevalence of ARB than wildlife living in environments less affected by anthropogenic activity.

The highest prevalences of AMR and/or ARB have been found in birds, many of which are migratory. This emphasises the potential role of birds as dispersers of AMR between environments and areas, even over long distances.

Unfortunately, only limited data is available on the prevalence of AMR in Norwegian wildlife.

Further studies are needed. Some scenarios where wildlife might contribute to the transfer of AMR to other animals and humans have been identified by the panel:

i) Urban animals that feed opportunistically on human sewage or waste, and thereafter visit places where food is stored, marketed, prepared, or consumed or where people eat and children play, may constitute a risk of transmission of ARB. These wildlife species might be exposed to bacteria originating from a human reservoir and that are well adapted to humans. The Panel considers important species in Norway to be brown rats, house sparrows, corvids, feral pigeons, and gulls.

ii) Wildlife that are associated with intensive terrestrial animal production and animals that are exposed to manure may constitute a risk of transfer of AMR and ARB if they later contaminate food, especially food that is eaten raw, or if they later visit urban environments. The Panel considers important species in Norway in this context are Brown rats, voles, house sparrows, corvids, gulls, and foxes.

iii) In the marine environment, birds and mammals visiting aquaculture sites could constitute a risk of introduction of AMR to aquaculture, with an onward transmission potential to humans. The Panel considers important species in Norway are cormorants, gulls, grey herons, otters, and mink but very little is known about the prevalence of AMR in these species. The potential importance of the wrasse industry, in which many millions of wrasses are fished annually in areas of Norway and Sweden with a relatively high human population densities, and transported over long distances before introduction to aquaculture enclosures in locations further north. The potential for this trade to introduce AMR from human sewage to aquaculture is unknown.

iv) Wildlife species moving over long distances provide a link between different areas and environments, and consequently represent a potential for transport and introduction of AMR and ARB to food chains or humans. Such introduction could be both direct and indirect, as the long-distance migratory species could transmit AMR and ARB to wildlife living closer to humans. The Panel considers important species in Norway of particular concern are geese, swans, and ducks that may overwinter in areas with high human population densities or high livestock densities, and subsequently visit lakes, agricultural land, and parks in Norway. Another concern expressed by the Panel is the possible role of long-distance migratory seabirds that are in contact with gulls, which could contaminate urban and agricultural environments with ARB.

In conclusion, the literature clearly indicates that wildlife can carry AMR, and molecular-based studies suggest that some resistance traits found in bacteria in wildlife are identical to those found in pathogenic bacteria in humans. There is a trend towards the probability of carriage of AMR being highest in wildlife living close to humans and sites of intensive animal production, but the data are insufficient to determine statistical significance and reach a firm conclusion. As long-distance migrators may also carry AMR and ARB, they may provide transmission pathways to humans, food chains, or animal production. However, few studies have established the directionality of resistance transfer.

#### **4- Based on the information collected, and if sufficient data are available, assess a, b, c and d;**

The information collected has identified a high number of potential sources, pathways, and routes that could lead to unwanted exposure and transfer of AMR in and between pathogenic populations of bacteria. These interfaces are characterized by complexity and, in part, stochasticity. Prediction of resistance transfer paths and trajectories is rarely possible. We have identified various types of uncertainty (Chapter 6) and a range of data gaps (chapter 8). The lack of quantitative resolution precludes quantitative assessment of the effects of various sources of exposure at this time.

Incomplete identification of relevant resistance determinants in the examined literature hinders our understanding of the current evolutionary trajectories of particular resistance determinants. The few studies that have examined the genetic basis for resistance show that wildlife and humans can share the same resistance determinants.

Although this Opinion has considered a large number of peer-reviewed studies concerned with AMR in wildlife, the lack of sufficient source data means that we cannot reach a conclusion on the relative role of wildlife in the current dynamic of resistance traits affecting humans and animals. Such roles should further be considered according to the specific therapeutic agent of concern; pharmaceutically produced antimicrobials are a highly heterogeneous group of chemicals, with different modes of action and different ecological footprints.

##### **a. the possibility of AMR-bacteria being transferred between wildlife and other hosts**

A range of interfaces between wildlife and domestic animals and humans have been described in this Opinion. Possible exchange of bacteria, as well as transfer of resistance between bacteria, is expected to occur at these interfaces. Key interfaces are found in urban settings, agricultural areas, and in waterways exposed to faecal material and chemical pollution, including antimicrobials. The type, extent, and direction of transfer of ARB, as well as their resistance genes, remains to be investigated further and quantified at these interfaces.

Knowledge of AMR genes (resistome) abundance and mobility in wildlife is not readily available or systematically collected. Environmental monitoring programmes regarding resistance development in bacteria in wildlife are largely lacking, in contrast to the routine collection of such data in many clinically oriented settings that systematically monitor resistance in selected pathogens over time.

Thus, it is currently not feasible to distinguish between the natural resistome and elevated abundance and transmission of AMR genes in samples from wildlife due to anthropogenic influence. Available studies on resistance in wildlife is biased towards developed countries, and often to a common set of a few well-characterised bacterial species of known clinical

importance. Many studies only examine the phenotype, precluding molecular epidemiological studies. In most cases, bacteria known to inhabit the gastrointestinal tract of both animals and humans are studied through the collection of faecal material.

The challenges in reaching a quantitative understanding of transfer potential and effects are illustrated by the high number of migratory bird species that can transport faecal bacteria over large geographical areas. Many of these species are in contact with a number of agricultural and urban areas over the course of biannual migrations.

It is important to appreciate the exposure scenarios leading to opportunities for transfer, as well as the factors determining whether transferred bacteria will successfully establish across interfaces, and the relevant temporal dynamics. In some cases, limited temporary colonisation and transfer would be expected. However, most studies do not provide information on the long-term dynamics of bacteria or genes transferred across interfaces.

#### **b. possible routes of antimicrobial residues to induce AMR bacteria in the environment**

Antimicrobial residues can select for AMR in the environment if present over time in sufficient concentrations. Most often, the release of antimicrobials to the environment follows the wastestreams of treated or untreated faecal matter from domestic animals, pets and companion animals, as well as human sewage systems. Some horticultural systems also use antimicrobials, thus they may be present on plant material. Incomplete understanding of concentration ranges and the evolutionary trajectories of bacteria impacted by selection of particular antimicrobials precludes quantitative assessment of the impact of exposure.

#### **c. possible routes of domestic animals for dissemination of AMR bacteria to wildlife and vice versa**

Domestic animals, include production livestock, as well as pets and companion animals. Key interfaces with wildlife include water and agricultural use of manure, as well as direct contact in pastures and barns. Various wild animals, such as rodents and birds, live in close contact with domestic animals. Omnivorous species (e.g. urban birds and rodents) often feed on anthropogenic waste and near human habitations and farms, meaning that they could represent a link between domestic animals, humans, and wildlife. Few studies have been able to establish directionality of resistance transfer, although a tendency towards higher resistance levels occurring in animals with higher exposure to anthropogenic sources is apparent.

#### **d. the possible exchange routes for AMR bacteria between human and wildlife**

As illustrated in **Figure 3**, there are many potential exchange routes that can lead to exposure to ARB with pathogenic properties in wildlife and humans. The lack of a quantitative focus in available studies, as well as limited information on the genetic basis for the resistance traits observed in wildlife, limits our ability to identify specific exposure

pathways to and from humans. In conclusion, only few studies are available with a study design suitable to explore transmission of AMR bacteria and genes between habitats and between wildlife, domestic animals and humans.

**Question 4 is limited to an overall assessment of each sub question (a, b, c, d) and not expected to be answered in detail. An estimation on probability or thorough risk assessment is not part of this assignment.**

# 8 Data gaps

- Only a small fraction of the environmental bacteria can be cultured, and this places a considerable limit on our knowledge about the true diversity and composition of this reservoir.
- Information on the abundance of AMR genes (resistome) in the different samples from wildlife is not readily available or systematically collected. Thus, it is often not feasible to distinguish between the natural resistome and the abundance of AMR genes in samples from wildlife.
- Standardised sampling methods for the design of studies and collection of biological materials for isolation of bacteria are lacking.
- Limited numbers of whole genomes and metagenomics studies are currently available. Such studies may provide more detailed information on resistance characteristics if traits can be assigned to host genomes.
- Limited number of studies of AMR in anaerobic bacteria.
- Limited data regarding bacterial species other than the commonly studied species from faecal samples
- Lack of data on commensal bacteria in wildlife.
- Limited data regarding AMR in bacteria from insects.
- Lack of information regarding several important bacterial species in animals in aquatic environments; *Listeria* spp., *Aeromonas* spp., *Vibrio* spp.
- Limited information regarding the relationship between AMR and the habitat of wildlife.
- Limited understanding of genetic coherence and activity of various gene transfer mechanisms in bacterial populations under natural conditions (most studies done with monocultures in the laboratory).
- Limited availability of quantitative data regarding the transmission of AMR bacteria inter and intra wildlife animals and from domestic animals to wildlife and vice versa.
- Lack of directly comparable studies regarding occurrence of AMR in the same animal species in different habitats.



- Lack of studies using new tools, such as animal-tracking technologies and high-throughput sequencing of resistance genes and mobilomes.
- Lack of tools to identify patterns in large and complex data sets.
- Data regarding ARB in wildlife in developing countries are lacking.
- Lack of longitudinal studies.
- Lack of detailed knowledge about the biology of species important in this context, especially the movement patterns, food preferences and population dynamics of species frequently having close contacts with humans, such as rats, gulls, corvids etc.

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# 10 Appendix I

**Table 1. WHO listing (3<sup>rd</sup> revision, 2012) of antimicrobials for human medicine** (Doyle, 2015)

Critically important	Highly important	Important	Unclassified
Aminoglycosides	Amdinopenicillins	Aminocyclitols	Ionophores
Carbapenems and other penems	Amphenicols	Cyclic polypeptides	Bambermycins
Cephalosporins (3rd and 4th generation)*	Cephalosporins (1st and 2nd generation)	Nitrofurantoin	Carbadox
Cyclic esters	Licomsamides	Nitroimidazoles	
Fluoro and other quinolones*	Penicillins (anti-staphylococcal)		
Glycopeptides*	Pleuromutilins		
Glycylcyclines	Riminofenazines		
Lipopeptides	Steroid antibacterials		
Macrolides and ketolides*	Streptogramins		
Monobactams	Sulfonamides		
Oxazolidinones	Sulfones		
Penicillins (natural aminopenicillins and antipseudomonal)	Tetracyclines		
Polymyxins			
Rifamycins			
Tuberculosis and other mycobacterial drugs			

\*The top 4 critically important antimicrobials are prioritized based on: (1) high absolute number of people affected by diseases for which the antimicrobial is the sole or one of few alternatives to treat serious human disease, and (2) high frequency of use of the antimicrobial for any indication in human medicine, since usage may favor selection of resistance. In addition, a focusing criterion for the above classifications is that there is a greater degree of confidence that there are nonhuman sources that result in transmission of bacteria or their resistance genes to humans (WHO 2012).

# 11 Appendix II

**Important information:** Below we provide an overview of the key findings in the considered literature identified in the database searches. The tables are divided according to animal group. Due to the large amount of data provided in the tables, unintentional errors such as spelling errors, omissions and other sources of inaccuracies may occur. The reader should consider the tables to be a draft representation and consult the cited studies when exact data are needed.

**11.1.1 Table 1. Antimicrobial resistance in amphibians and reptiles.**

Antimicrobial agents	Bacterial species	Methodology for sampling and determination of resistance	Animal species	Omnivorous (O) Carnivorous (C) Herbivorous (H)	Country	Results	Reference
Ampicillin Ciprofloxacin Chloramphenicol Erythromycin Gentamycin Nitrofurantoin Norfloxacin Streptomycin Tetracycline Rifampicin Vancomycin	443 isolates of <i>E. faecalis</i> <i>E. faecium</i>	Culture method: Faecal samples  Susceptibility test: agar diffusion (CLSI)  PCR for detection of resistance genes	<b>Seabirds</b> (n=12): Magellanic penguin ( <i>Spheniscus magellanicus</i> ) (n=10), Snow-crowned tern ( <i>Sterna trudeaui</i> ) (n=1), White-backed stilt <i>Himantopus melanurus</i> (n=1)  <b>Turtles</b> (n=8): Hawksbill turtle ( <i>Eretmochelys imbricate</i> ) (n=6), green sea turtle <i>Chelonia mydas</i> (n=2)	C  O C  O O C	Brazil	Among the 443 enterococci isolated, 62 distinct antimicrobial patterns were observed. This classification was created for grouping strains with similar antimicrobial susceptibility and resistance patterns. Of the 62 observed patterns, 49 (79.03%) enterococcal strains profiles were resistant to one or more of the tested antibiotics. Single,	(Prichula et al., 2016)



			<p><b>Mammals</b> (n=3):  Minke whale (<i>Balaenoptera acutorostrata</i>) (n=1),  Humpback whale (<i>Megaptera novaeangliae</i>) (n=1),  and Risso's dolphin (<i>Grampus griseus</i>) (n=1),  found alive or dead in southern coastal Brazil.</p>	<p>C</p> <p>C</p>		<p>double, and multiple antibiotic resistance patterns were observed in 37.1%, 25.8%, and 16.1% of the isolates, respectively. Resistance to rifampicin occurred most frequently.</p>	
<p>Amikacin  Streptomycin  Gentamicin  Kanamycin  Ampicillin  Amoxicillin/clavulanic acid  Cefoxitin  Ceftriaxone  Ceftiofur  Ciprofloxacin  Nalidixic acid  Suphfoxazole  Trimethoprim/sulfamethoxazole  Chloramphenicol,  Tetracycline</p>	<p><i>Enterobacteriaceae</i> isolates (n=189) were recovered from both individuals and combined groups of animals. 137 isolates of seven genera from the 110 individually housed animals. <i>Citrobacter</i> spp. <i>Klebsiella</i> spp.</p>	<p>Culture method: faecal samples</p> <p>Susceptibility test: microdilution (CLSI)</p> <p>Typing method: Not stated</p>	<p>Tokay geckos (<i>Gekko gecko</i>) (n=110), imported and destined for the pet trade.</p> <p>Animals captured from rural locations.</p>	<p>C</p>	<p>USA</p>	<p>Resistance against some antibiotics including: ampicillin, amoxicillin/clavulanic acid, cefoxitin, chloramphenicol, kanamycin and tetracycline.</p> <p>chloramphenicol, kanamycin and tetracycline.</p> <p>Geckos acquire antibiotic resistant bacteria in their home range, may be directly through exposure to human or livestock waste, or indirectly through consumption of prey which harbour resistant bacteria. to human or livestock waste, or</p>	<p>(Casey et al., 2015)</p>

	<i>Enterobacter</i> spp. <i>Kluyvera</i> spp. <i>E. coli</i> <i>Serratia</i> spp. <i>Pantoea</i> spp. <i>S. arizonae</i>					indirectly through consumption of prey which harbour resistant bacteria.	
Metronidazole Ciprofloxacin Clindamycin Amoxicillin Tetracycline Enrofloxacin Cephalotin Trimethoprim/sulphamethoxazole	81 clinical isolates of obligate anaerobes, of which two were <i>Bacteroides fragilis</i>	Culture method: Materials from various sites in clinically diseased animals  Susceptibility test: Resistance determination by E-test strips and CLSI breakpoints for human strains  Typing method: Resistance genes assessed by PCR	Wild snake, Species name not given.	C	Costa Rica	Phenotypic resistance seen for ciprofloxacin, amoxicillin and cephalothin. Resistance-conferring genes found in 85% of bacteria isolated from food-producing animals and in 65% of pet isolates, but none in samples of wild animals.	(Mayorga et al., 2015)
Amoxicillin + clavulanic acid Ampicillin Cefotaxime Ceftazidime	<i>S. enterica</i> (n=27), 7 serovars	Culture method: cloacal swab samples  Susceptibility test: disc diffusion and breakpoints according to CLSI  Typing methods:	Wild Green iguanas ( <i>Iguana iguana</i> ) (n=47)	H	Grenada	No resistance was seen in any of the isolates.	(Sylvester et al., 2014)

Ciprofloxacin Enrofloxacin Gentamicin Nalidixic acid Streptomycin Tetracycline Trimethoprim + sulphamethoxazole		Not stated.					
Amikacin Amoxicillin + clavulanic acid Ampicillin Ceftriaxone Cephalothin Chloramphenicol Ciprofloxacin Gentamicin Kanamycin Streptomycin Tetracycline Trimethoprim + sulphamethoxazole	<i>Salmonella enterica</i> (n=106), 22 serovars	Culture method: swab from cloaca and ventral part of animal, whole body washing in sterile broth. Total sample number = 1144.  Susceptibility test: disc diffusion (FDA, National Antimicrobial Monitoring System)  Typing method: repetitive-element PCR, PFGE	Frogs (n=331) Lizards (n=59) Newts (n=5) Salamanders (n=6) Snakes (n=39) Toads (n=20) Water (n=119) A paired 1-L surface water sample was collected at each sampling event.  Because of the multiple sampling of individuals, 460 animals were trapped to make up 1025 samples. With	C	USA	38% sensitive to all antimicrobial agents.  Twenty-three isolates resistant to more than one class of antibiotic, and six isolates were resistant to three classes.  One strain from snake resistant to amikacin, ampicillin, cephalothin, gentamicin and streptomycin.  Resistant <i>Salmonella</i> from these animals may serve as reservoirs	(Gorski et al., 2013)

			the addition of 119 water samples, the sample total was 1144.			to contaminate leafy produce.	
Ampicillin Ampicillin + sulbactam Cefazolin Cephalothin Ciprofloxacin Gentamicin Imipenem Levofloxacin Trimethoprim + sulphamethoxazole	203 isolates identified to the genus level, <i>Klebsiella</i> (n=57) <i>Enterobacter</i> (n=23) <i>Escherichia</i> (n=18) <i>Shigella</i> (n=52) <i>Salmonella</i> (n=20) <i>Pseudomonas</i> (n=33)	Culture method: cloacal swab samples  Susceptibility test: disk diffusion method (CLSI)  Typing method: Not stated	Free ranging turtles, Red-eared slider: ( <i>Trachemys scripta elegans</i> ) (n=29)	H/O	USA	All strains were sensitive to gentamicin and levofloxacin,  Imipenem resistance seen in 30% of <i>Klebsiella</i> strains,  Widespread resistance towards ampicillin, ampicillin-sulbactam, cefazolin and ciprofloxacin among <i>Salmonella</i> isolates.	(Liu et al., 2013)
Cefoxitin Amikacin Chloramphenicol Tetracycline Ceftriaxone Amoxicillin + clavulanic acid Ciprofloxacin Gentamicin	<i>S. enterica</i> (n=88), belonging to 17 serogroups	Culture method on faecal samples,  Susceptibility test: Resistance determination by microdilution and breakpoints according to CLSI  Typing method: real-time PCR, serotyping	Tokay geckos ( <i>Gekko gecko</i> ) (n=110)	C	USA, Kansas	Six isolates (6.8%) expressed resistance to more than one antibiotic. All <i>S. enterica</i> subsp. <i>enterica</i> Adelaide isolates were resistant to nalidixic acid and sulphisoxazole, one <i>S. enterica</i> subsp. <i>arizonae</i> was resistant to	(Smith et al., 2012)

Nalidixic acid Ceftiofur Sulphisoxazole Trimethoprim + sulphamethoxazole Kanamycin Ampicillin Streptomycin						ampicillin and sulphisoxazole, and another isolate was resistant to streptomycin and sulphisoxazole. Forty-three additional isolates expressed resistance only to sulphisoxazole.	
Ampicillin Amoxicillin + clavulanic acid Ciprofloxacin Cefazolin Cefuroxime Chloramfenicol Doxycycline Tetracycline Gentamicin Trimethoprim + sulphamethoxazole	<i>E. coli</i> (n=18) <i>S. enterica</i> (n=5)	Culture method on faecal samples  Susceptibility test: Disc diffusion and breakpoints according to National Committee for Clinical Laboratory Standards (NCCLS)  Typing method: Detection of resistance genes (PCR, gel electrophoresis and amplicon match analysis)	Land iguanas: ( <i>Conolophus</i> sp.) Marine iguanas: ( <i>Amblyrhynchus christatus</i> )  Giant tortoise ( <i>Geochelone nigra</i> )	H	Ecuador	Resistance towards ampicillin, doxycycline, tetracycline, and trimethoprim/sulphamethoxazole commonly seen in 23 examined isolates.  <i>tetA</i> and <i>tetE</i> found in <i>E. coli</i>	(Wheeler et al., 2012)
Genes	<i>Pseudomonas aeruginosa</i> , (n=83, domestic	Culture method: faecal samples  Susceptibility test: VITEC2 system and Microdilution with CLSI breakpoints	Pet snake (France) (n=83) and Wild snake (Guinea) (n=23),  23 species	C	France and Guinea	All except one isolate had a wild-type resistance profile. One persistent clone isolated from	(Colinon et al., 2010)

	and n=3, wild)	Genetic examination by combining PCR for resistance genes and cassettes, sequence analysis and Southern blot hybridization of restriction fragments  Typing method: PCR, PFGE				both snakes and preys harboured multiple antimicrobial resistance genes mediated by an integron carrying the <i>qacH</i> , <i>aadB</i> , <i>aadA2</i> and <i>cmA10</i> cassettes, and a <i>tetA(C)</i> -carrying transposon.	
Ampicillin Tetracycline Chloramphenicol Streptomycin Kanamycin Gentamicin Amikacin Nalidixic acid Trimethoprim + sulphamethoxazole	Enterobacteriaceae (n=88), <i>E. coli</i> (n=68) <i>Citrobacter</i> spp. (n=13) <i>Klebsiella</i> spp. (n=7)	Culture method: cloacal swabs  Susceptibility test: Resistance determination by disk diffusion and breakpoints from CSLI  Typing method: Not stated	Land iguana ( <i>Conolophus pallidus</i> )(n=96)	C	Ecuador and Italy	Acquired resistance not seen in any isolate from the dominant Enterobacteriaceae.	(Thaller et al., 2010)
Penicillin G Oxacillin Erythromycin Clindamycin Trimethoprim + Sulphamethoxazole	222 isolates of coagulase-negative staphylococci, predominantly	Culture method: fecal sample  Susceptibility test: Disk diffusion method and breakpoints according to National Committee for Clinical Laboratory Standards (NCCLS).  Typing method: PCR with DNA probes for <i>mecA</i> gene, gel	Cope's gray tree frogs ( <i>Hyla chrysoscelis</i> ) (n=not specified)	C/O	USA	99% of isolates resistant to penicillin G and 59% resistant to oxacillin (a clinical substitute of methicillin).  <i>mecA</i> gene found in 4 of 10 examined	(Slaughter et al., 2001)

	<i>S. sciuri</i> and <i>S. xylosus</i>	electrophoresis and BLAST analysis.				oxacillin resistant strains.	
Sulphonamides, Class 1 integrons	<i>E. coli</i> , 134 from wild animals and 114 of clinical origin.	Culture method: Isolates from a culture collection  Susceptibility test: Unspecified disc diffusion method,  Typing method: PCR method for integrons	Isolates from a culture collection, including strains from wild reptiles, birds and mammals	Probably O, C, H	USA, Mexico, Antarctica	The prevalence of class 1 integrons, as revealed by <i>intI1</i> PCR, decreased dramatically, going from 24% among clinical strains (44% of sulphonamide-resistant strains) to 0% among isolates from wild animals.	(Diaz-Mejia et al., 2008)
Ampicillin Kanamycin Chloramphenicol Streptomycin Tetracycline Nalidixic acid	Aerobic Gram-negative bacteria, n=420, in the genera <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Klebsiella</i> , <i>Providencia</i> , <i>Escherichia</i> , <i>Proteus</i> , <i>Aeromonas</i> and <i>Salmonella</i>	Culture method: Faecal samples  Susceptibility test: MIC determination by serial dilution in broth.  Typing method: Examination for plasmids by agarose gel electrophoresis  Mating with sensitive <i>E. coli</i> and DNA-DNA hybridization to probes.	Wild amphibians (n=20), nine species from the genera <i>Adenomera</i> , <i>Hyla</i> , <i>Leptodactylus</i> or <i>Sphaenorhynchus</i>	C	Brazil	No resistance seen for Kanamycin, Chloramphenicol or Nalidixic acid.  Resistance to Ampicillin, Streptomycin and Tetracycline found, but breakpoints not applied to interpret results.  29% of isolates carried plasmids of 23MDa or above, but no conjugational gene transfer demonstrated <i>in vitro</i> .	(Ioshimoto et al., 1991)

						Among the plasmid-containing isolates, 41% showed resistance against ampicillin, 6.5% against streptomycin, and 6.5% against tetracycline.	
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### 11.1.2 Table 2. Antimicrobial resistance in birds (including migratory birds).

Antimicrobial agents	Bacterial species	Methodology for sampling and determination of resistance	Animal species	Omnivorous (O) Carnivorous (C) Herbivorous (H)	Country	Results	Reference
amikacin amoxicillin-clavulanic acid ampicillin cefotaxime ceftriaxone chloramphenicol ciprofloxacin gentamicin kanamycin nifuroxime	<i>E. coli</i> Extra-intestinal pathogenic <i>E. coli</i> (ExPEC) (n=9)	Culture method: sample from cloaca (123) and oropharynx (123)  Typing method: PFGE, MLST,	Free-living birds, treated at the Wildlife Veterinary Hospital (n=123) - Liver and intestinal samples from birds that died.  Coscorba swan Golden Parakeet Rufous-bellied Thrush	O	Brazil	9 <i>E. coli</i> isolates with MDR were isolated from psittacine species (n=4), birds of prey (n=3), passerine species (n=1), and waterfowl (n=1).  Although, MDR <i>E. coli</i> isolates were	(Borges et al., 2017a)



<p>norfloxacin</p> <p>sulphamethaxazole+ tri-metprim</p> <p>tetracycline</p>		<p>PCR identification of virulence and resistance genes</p> <p>Susceptibility test: disc diffusion (CLSI)</p>	<p>American Kestrel</p> <p>White eyed parakeet</p> <p>Crested caracara</p> <p>Blue-fronted parrot</p> <p>Ruddy ground-dove</p>	<p>O</p> <p>O</p> <p>C</p> <p>O</p> <p>C/O</p> <p>O</p> <p>O</p>		<p>detected, none were ESBL-producers.</p>	
<p>amikacin</p> <p>ampicillin</p> <p>cefotaxime</p> <p>cefoxitin</p> <p>ceftiofur</p> <p>ertapenem</p> <p>imipenem</p> <p>phosphomycin</p> <p>gentamicin</p> <p>kanamycin</p> <p>nalidixic acid</p> <p>nitrofurantoin</p> <p>norfloxacin</p> <p>sulphamethoxazole</p>	<p><i>E. coli</i> (STEC and EPEC)</p> <p>(n=not stated)</p>	<p>Culture method: cloaca and oropharynx samples</p> <p>Wild birds (n=123+123), pigeons (n=100+100)</p> <p>PCR: for detection of STEC and EPEC, virulence genes, and typing</p> <p>Susceptibility test:</p>	<p>Wild birds (123) and free-living urban pigeons (n=100)</p> <p>Samples were collected from 123 free-living wild birds that were treated at the Wildlife Veterinary Hospital.</p> <p>For information regarding orders and species of animals, see the reference.</p>	<p>O</p>	<p>Brazil</p>	<p>MDR in 25.0% of the pigeon strains and in 57.0% of the wild bird strains;</p> <p>wild birds also yielded one isolate carrying (ESBLs) gene bla<sub>CTX-M-8</sub>.</p> <p>Wild birds and pigeons could act as carriers of MDR STEC and EPEC</p>	<p>(Borges et al., 2017b)</p>

+ trimethoprim tetracycline		CLSI, PCR for ESBL- genes  Typing methods: PFGE, MLST, antisera-typing and PCR					
clindamycin tetracycline  ampicillin cefuroxime enrofloxacin  trimethoprim- sulphamethoxazole	Resistance profiles in bacteria (n=108) belong to the families including <i>Enterobacteriaceae</i> <i>Pseudomonas</i> <i>Staphylococci</i> <i>Streptococci</i> <i>Burkholderia</i> (n=108)	Culture method: faecal samples from 1996-2014 (n: 663 records)  Susceptibility test: disc diffusion	Raptors (free-living) (n=663)  For species names, see the reference	O	Spain	457 (69%) positive for bacterial species.  Resistance profiles determined for 108 isolates.  More than 50% (34 different bacterial species) resistant to clindamycin,  tetracycline, ampicillin, cefuroxime, enrofloxacin,  Trimethoprim- sulphamethoxazole.  MDR in 71% (27 of 38) <i>E. coli</i> isolates	(Vidal et al., 2017)

<p>ampicillin chloramphenicol</p> <p>Ciprofloxacin</p> <p>quinupristin– dalfopristin</p> <p>streptomycin</p> <p>tetracycline teicoplanin vancomycin</p> <p>erythromycin gentamycin and kanamycin</p>	<p>VRE <i>E. faecium</i> (n=6)</p>	<p>Culture method: faecal samples</p> <p>Susceptibility methods: disc diffusion (EUCAST)</p> <p>Typing method: MLST</p>	<p>Wild Red-Legged Partridges (<i>Alectoris rufa</i>) (n=305).</p> <p>The partridges were hunted in their native lands, where they live and reproduce freely in the wild.</p>	<p>O</p>	<p>Portugal</p>	<p>six vanA-<i>E. faecium</i> were recovered (of 305 samples). Isolates were tested for antibiotic resistance  and virulence genes. six isolates showed erythromycin resistance and harboured the erm(B) gene and four that were tetracycline resistant showed the tet(M) gene.</p>	<p>(Silva et al., 2017)</p>
<p>penicillin G</p> <p>oxacillin</p> <p>ceftazidime</p> <p>cefotaxime</p> <p>tetracycline sulphamethoxazole- trimethoprim</p> <p>streptomycin kanamycin</p> <p>gentamicin erythromycin lincomycin</p> <p>ciprofloxacin chloramphenicol</p>	<p>Cultivable microbiota:  In total, 697 different operational taxonomic units at genus level.  63 taxonomic units were detected:  <i>Catelliboccus marimammalium</i> had the highest prevalence.</p>	<p>Culture method: faecal samples</p> <p>Susceptibility methods:  Disc diffusion (CLSI)</p> <p>Typing method:  Metagenomics and microbial</p>	<p>herring gulls (<i>Larus argentatus</i>), black- headed gull (<i>L. ridibundus</i>), Caspian gull (<i>L. cachinnans</i>), and great black-backed gull (<i>L. marinus</i>).</p> <p>N for each species is lacking.</p>	<p>O</p>	<p>Italy</p>	<p><i>C. marimammalium</i> are predominant microbiota in the cloacal samples of <i>Larus argentatus</i>. In this study, this species of gull is a reservoir of bacteria carrying a wide- spectrum of genes encoding AMR. The same genes were detected in both cultivable microbiota and in the total DNA of the samples.</p>	<p>(Merkeviciene et al., 2017)</p>

	The bacterial amount of other genera was up to 5% with the most highly prevalent being <i>Psychrobacter</i> (4.7%), <i>Helicobacter</i> (4.5%), unclassified <i>Enterococcaceae</i> (3.2%), <i>Pseudomonas</i> (2.9%), and <i>Brachyspira</i> (2.6%). Many other species also detected.	profiling analysis, PCR-detection of resistance genes					
ampicillin amoxicillin + clavulanic acid chloromphenicol nalidixic acid tetracycline gentamicin ciprofloxacin ceftazidime streptomycin cefoxitin cefotaxime kanamycin tobramycin	<i>E. coli</i> (n=148)	Culture method: Faecal swabs  Typing methods: PFGE, DNA sequencing, PCR: $\beta$ -lactam resistance genes (blaCTX-M, blaSHV, blaTEM, and blaOXA)	Healthy cage birds from 15 different pet shops (n=148 cage birds):  <i>Serinus canaria</i> (n=44) <i>Melopsittacus undulatus</i> (n=34) <i>Taeniopygia guttata</i> (n=33) <i>Agapornis roseicollis</i> (n=7) <i>Nymphicus hollandicus</i> (n=6) <i>Amadina fasciata</i> (n=6)	H H H H	Turkey	4 (2.7%) of <i>E. coli</i> isolates ESBL producers. <i>E. coli</i> isolates were isolated from <i>Poephila guttata</i> (n:1) and <i>Melopsittacus undulatus</i> (n=3).  Pulsed-field gel electrophoresis used for molecular typing of the $\beta$ -lactam resistance gene (blaCTX-M); 2 different pulsotypes from the same pet	(Yilmaz and Dolar, 2017)

cephalothin aztreonam  sulphamethoxazole/trimethoprim  imipenem cefotetan  amikacin cefepodoxim  ceftazidime  cefotaxime + clavulanic acid  cefepodoxime and cefepime		Susceptibility test: disc diffusion (CLSI), ESBL- production test	<i>Carduelis carduelis</i> (n=6)  <i>Regulus regulus</i> (n=2)  <i>Psephotus pulcherrimus</i> (n=2)  <i>Erythrura gouldiae</i> (n=2)  <i>Cyanoramphus novaezelandiae</i> (n=1)  <i>Psittacus erithacus</i> (n=1)  <i>Psittacula krameri</i> (n=1)  <i>Neochmia ruficauda</i> (n=1)  <i>Poephila acuticauda</i> (n=1)  <i>Vidua chalybeata</i> (n=1)	H H H H H H H H O H H		shop. All CTX-M- producing <i>E. coli</i> isolates had almost identical genotypes.	
ampicillin/sulbactam cefazolin cefuroxime gentamicin trimethoprim + sulfamethoxazole  colistin  oxolinic acid ofloxacin	<i>E. coli</i> (n=1050)	Culture method: Faecal samples (n=595)  collected from roosting places used by rook flocks.	Hospitalized patients (n=303), and outpatients, chicken farms (=156), retailed turkeys (n=105), <b>rooks</b> ( <i>Corvus frugilegus</i> ) wintering (n=114) in the area, and wastewaters (375)	O	Czech Republic	PMQR genes in <i>E.coli</i> isolates (n=256, <b>20 from Rooks</b> ) in all areas studied, including highly virulent multiresistant clones such as ST131 producing CTX-M-15 beta-lactamases, including from rooks.	(Roderova et al., 2017)

<p>tetracycline aztreonam piperacillin piperacillin/tazobactam cefoperazone cefotaxime ceftazidime cefepime</p> <p>cefoperazone +sulbactam</p> <p>meropenem ciprofloxacin</p> <p>tigecycline</p> <p>tobramycin</p> <p>amikacin</p>		<p>Typing methods: PFGE, MLST,</p> <p>PCR: plasmid-mediated quinolone resistance (PMQR) and ESBL genes tested by PCR and sequencing. Specific mutations in <i>gyrA</i>, <i>gyrB</i>, <i>parC</i>, and <i>parE</i>.</p> <p>Susceptibility test: microdilution (EUCAST)</p>					
<p>Clindamycin Erythromycin Moxifloxacin Tetracycline</p>	<p><i>C. difficile</i> (n=34)</p>	<p>Culture method: Twenty-seven farms were sampled. Pools of pig faecal</p>	<p>Pig farms and pests like rats (n=26) (<i>Rattus</i> sp.), mice (<i>Mus musculus</i>) (n=53)</p>	<p>O C C</p>	<p>Canada</p>	<p>Resistance to clindamycin, erythromycin, moxifloxacin and tetracycline was variable (41.2, 52.9, 5.9 and 76.5% respectively). MDR</p>	<p>(Andres-Lasheras et al., 2017)</p>

<p>Metronidazole Vancomycin</p>		<p>samples (n = 210), samples of intestinal content from common farm pest</p> <p>species (n = 95) and environment-related samples (n = 93) were collected.</p> <p>Typing method:</p> <p>Ribotyping: PCR and PCR-RFLP toxinotyping</p> <p>Susceptibility test: E-test (CLSI)</p>	<p><b>Pigeons</b> (<i>Columba livia</i>) (n=16)</p>	<p>0</p>		<p>observed in 12 of 34 (35.3%) of the isolates; all resistant to clindamycin, erythromycin and tetracycline.</p> <p>Specification regarding bacterial isolates from different animal species was lacking, however most of the positive samples came from pest species or were pest-related environmental samples.</p> <p>Rodents and pigeons had <i>C. difficile</i> strains of the same ribotypes as those in humans.</p>	
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<p>Carbapenem Colistin (<i>mcr-1</i> gene detection)</p>	<p><i>E. coli</i>  (N, not stated)</p>	<p>Culture method:  From chicken cloaca (120), cloacal swabs from 4 commercial farms (330), faecal swabs or nests from <b>swallows or sparrows</b> (10), faecal swabs from farmers (6), swabs from faecal/anal from dogs (17), <b>flies</b> (150), sewage (5), slaughterhouse (chicken caeca (50), sewage (3)), 16 supermarkets (chicken legs and breast (48).  Typing methods:</p>	<p>739 samples  Poultry Human Dogs Sewage Flies (n=150)- species: not specified.  Wild birds; sparrows ( Swallows (n= not specified)</p>	<p>O</p>	<p>China</p>	<p><i>mcr-1</i> but not <i>bla<sub>NDM-1</sub></i> genes prevalent in hatcheries but <i>bla<sub>NDM-1</sub></i> contaminates flocks through dogs, flies, and wild birds.  Whole genome sequencing indicate  <i>bla<sub>NDM</sub></i> <i>E. coli</i> from different niches provides direct evidence of transmission and contamination.</p>	<p>(Wang et al., 2017b)</p>
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		<p>MLST, PCR-sequencing, BLAST-analysis, 16s rRNA analysis,</p> <p>Whole genome sequencing,</p> <p>PCR-sequencing for detection of carbapenemase (NDM-1) and mcr-1 genes</p> <p>Susceptibility test: Agar dilution method (CLSI and EUCAST)</p>					
<p>Different classes of antimicrobial agents:</p> <p>Aminopenicillin-s</p> <p>Phenicoles</p> <p>Aminoglycosid-e</p> <p>Sulfonamides</p> <p>Dihydrofolate-reductase inhibitor</p>	<p><i>S. enterica</i> (serovar Typhimurium) (N=117)</p> <p>and <i>S. Typhimurium</i> (<i>S. enterica</i> serovar 4,5,12:i:- (N=59)</p>	<p>Culture method:</p> <p>faecal samples</p> <p>Susceptibility test:</p> <p>Disc diffusion (CLSI)</p>	<p>Pig (n=21)</p> <p>Pig farm environment (n=15)</p> <p>Wild bird (n=22)</p> <p>Species: not specified</p> <p>Rodent (n=5)</p> <p>Species: not specified</p>	Most likely O, C, H	Spain	<p>Antimicrobial resistance in 85.8% of the isolates, with 94.1% of them displaying MDR.</p> <p>Number and % of the MDR isolates from wild birds and rodents not specified.</p> <p>Multilocus variable variable Tandem</p>	(Andres-Barranco et al., 2016)

Tetracycline Cephalosporins quinolones		Typing methods:  Multilocus variable Tandem repeat analysis  Phage typing				repeat analysis identified 92 different profiles.	
Gentamicin Cefotaxime Enrofloxacin	<i>E. coli</i>  <i>Salmonella</i> spp.  n= not specified	Sampling: cloacal swabs for detection of bacteria  Typing method: PCR  Susceptibility test: agar diffusion	White storks ( <i>Ciconia ciconia</i> ) (nestlings and adults) (n=90)	C	Spain (wildlife rehabilitatio n centres)	<i>E. coli</i> showing resistance to cefotaxime (37.9%) and against two antimicrobials at once (41.4%) more similar to the prevalence in stork nestlings from landfill-associated colonies (7.9%, 37.1% and 48.6%, respectively for prevalence of <i>Salmonella</i> spp. and <i>E. coli</i> displaying, cefotaxime resistance and resistance against two antimicrobials), and significantly higher than in colonies located in natural habitats (0%; 10.5% and 15.8%, respectively).	(Camacho et al., 2016)

<p>Ciprofloxacin</p> <p>Amoxicillin</p> <p>Ampicillin</p> <p>Gentamicin</p> <p>Streptomycin</p> <p>Erythromycin</p> <p>Chloramphenicol</p> <p>Tetracycline</p>	<p><i>Campylobacter</i> spp (n=35)</p>	<p>Culture method: fresh faeces</p> <p>Typing method: Bacterial species identification: Multiplex PCR, MALDI TOF. 16s rRNA</p> <p>Susceptibility test: microdilution</p>	<p>Domestic (108) and free-living pigeons (n=72)</p>	<p>0</p>	<p>Poland</p>	<p>35 <i>Campylobacter</i> isolates were isolated: <i>C. jejuni</i> (27), <i>C. coli</i> (8). Over 50% were resistant against erythromycin, streptomycin, 40% were resistant against tetracycline and amoxicillin, and 37% against amoxicillin. Resistance against two or three antimicrobial agents were in all isolates.</p> <p>No information was provided regarding division by habitat (domestic vs free-living)</p>	<p>(Dudzic et al., 2016)</p>
<p>ampicillin</p> <p>amoxicillin/clavulanic acid</p> <p>cefotaxime</p> <p>ceftazidime</p> <p>ceftriaxone</p> <p>nalidixic acid</p> <p>ciprofloxacin</p> <p>norfloxacin</p> <p>gentamicin</p> <p>amikacin</p> <p>streptomycin</p> <p>tobramycin</p>	<p><i>Enterobacteriaceae</i> (n=83)</p>	<p>Culture method: n=55 cloacal swabs</p> <p>Typing method: serotyping</p> <p>Susceptibility test: disc</p>	<p>Falconiformes</p> <p>Accipitriformes</p> <p>Strigiformes</p> <p>Charadriiformes</p> <p>Ciconiiformes</p> <p>Passeriformes</p> <p>(n=55)</p>	<p>0</p> <p>0</p> <p>0</p> <p>0</p> <p>0</p> <p>0</p>	<p>Italy (wildlife rescue centre)</p>	<p>Eighty-three bacterial isolates, representing 7 genera, were cultured from 55 cloacal swabs.</p> <p>Multiresistance to three or more groups of antibiotics also occurred. Many of the isolates were <b>geotypic</b> positive</p>	<p>(Giacopello et al., 2016)</p>

tetracycline trimethoprim/sulfamethoxazole  imipenem meropenem		diffusion (CLSI)	For information regarding species, see the reference.			but none of them have shown a <b>phenotypic ESBL</b> profile.  Resistance to <b>imipenem</b> occurred in 9/49 strains  (18.4%) from raptors, 6/18 strains from water birds  (33.3%), and 5/16 (31.2%) strains from passerines.  No bacteria were resistant to meropenem.	
amoxicillin/clavulanic acid ampicillin azithromycin cefoxitin ceftiofur ceftriaxone chloramphenicol  ciprofloxacin gentamicin nalidixic acid streptomycin sulfisoxazole tetracycline trimethoprim/sulfamethoxazole	<i>S. Typhimurium</i> (N=2)	Culture method: faecal samples (114)  Typing method:  serotyping, multiplex PCR  Susceptibility test:	Great-tailed grackles ( <i>Quiscalus mexicanus</i> ) (n= not specified)  And other cohabitant urban birds (n=117)	O	USA	Both isolates were pan-susceptible to the antimicrobial agents	(Grigar et al., 2016)

		microdilution (CLSI)					
cefepodoxime/ cefepodoxime - clavulanic acid	<i>E. coli</i> <i>K. pneumoniae</i> (n, not specified)	Culture method: 300 faecal samples (n=100 human, n=100 poultry and n=100 wild bird samples.  Susceptibility test:  Disc diffusion  PCR: confirmation for the presence of bla CTX-M-I-IV groups  Typing method: MLST, ERIC-2 PCR	Humans Poultry <b>Wild birds:</b> <i>Cattle Egret</i> <i>Black Vulture</i> <i>Pigeon</i> (n=100)	O O O	Nicaragua	The samples were examined for <b>ESBL-producing <i>E. coli</i></b> and <i>K. pneumoniae</i> , revealing the prevalence of 27% in humans, 13% in poultry, and 8% in wild birds.  ESBL producers harboured bla <sub>CTX-M-2</sub> , bla <sub>CTX-M-15</sub> , bla <sub>CTX-M-22</sub> , and bla <sub>CTX-M-3</sub> genotypes. The bla <sub>CTX-M-15</sub> constituted the absolute majority of ESBL genes among all samples.  Highly related <i>E. coli</i> clones among humans, poultry, and wild birds.	(Hasan et al., 2016)

Ampicillin Amoxicillin-clavulanic acid Chloramphenicol Phlorphenicol Gentamicin Amikacin Cephalexin Ceftiofur Cefotaxime Ceftazidime Sulphomethoxazole-trimethprime	<i>Salmonella</i> spp. (n=23)	Culture method: faecal samples  Susceptibility test: disc diffusion  Typing method: PFGE, PCR	Wild birds (n=237): <i>Raptors</i> (n=113) <i>Otus bakkamoena</i> <i>Milvus migrans</i> <i>Ninox scutulata</i> <i>Accipiter trivigatus</i> Others  Non-raptors (n=124) <i>Centropus bengalensis</i> <i>Cuculus saturatus</i> <i>Gorsachius melanolophus</i> <i>Dicrurus macrocercus</i> <i>Caprimulgus affinis</i> <i>Gallinula chlaopus</i> Others  Number of species: not specified)	O H C O ?  C O O O O O O ?	Taiwan  (Wildlife First Aid Station of the Endemic Species Research)	Multiple resistance detected in 62.5% of isolates; 20.8% of isolates susceptible to five classes of all tested antimicrobial agents.	(Huang et al., 2016)
vancomycin teicoplanin ampicillin	<i>Enterococcus</i> spp. (n=not stated)	Culture method: 348 cloacal/ rectal samples	Red-legged partridges (n=127),	O  O	Spain	One VRE-a isolate identified in one wild boar. This isolate was identified as <i>E.</i>	(Lozano et al., 2016)

<p>streptomycin gentamicin</p> <p>kanamycin chloramphenicol tetracycline erythromycin ciprofloxacin trimethoprim– sulfamethoxazole linezolid</p> <p>MICs of vancomycin and teicoplanin were determined by agar dilution technique.</p>		<p>For identification of bacteria biochemical conventional methods (Gram staining,  catalase, and bile esculin test) and PCRs with specific primers  for different enterococcal species (<i>E. faecalis</i>, <i>E. faecium</i>, <i>E. hiraе</i>, <i>E. durans</i>, <i>E. casseliflavus</i>, and <i>E. gallinarum</i>) were performed.</p> <p>Susceptibility test: agar disc diffusion (CLSI)</p>	<p>White storks (n=81),  Red kites (n=59),  Species name for birds: not specified.</p> <p>Wild boars (n=81)</p>	<p>C</p> <p>O</p>		<p><i>faecium</i>, harboured <i>vanA</i> gene included into Tn1546 (truncated  with IS1542/IS1216), and belonged to the new ST993. This isolate contained the <i>erm(A)</i>, <i>erm(B)</i>, <i>tet(M)</i>, <i>dfrG</i>, and <i>dfrK</i> genes.</p> <p>Ninety-six VRE-i isolates were identified (89  <i>E. gallinarum</i> and seven <i>E. casseliflavus</i>), with the following prevalence: red kites (71.2 %), white storks (46.9 %), red- legged partridges (7.9 %), and wild boars (4.9 %).</p> <p>Most <i>E. gallinarum</i> isolates  showed resistance to tetracycline (66.3 %) and/or erythromycin</p>	
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		<p>PCR for detection of acquired vancomycin resistance genes (<i>vanA</i>, <i>vanB</i>, <i>vanD</i>, <i>vanE</i>, and <i>vanG</i>), and intrinsic vancomycin resistance genes (<i>vanC</i>-1 and <i>vanC</i>-2/3, linked to the species <i>E. gallinarum</i> and <i>E. casseliflavus/flavescens</i>, respectively).</p> <p>Typing method: MLST</p>				<p>(46.1 %). High-level resistance to aminoglycosides was present among our VRE-i isolates: kanamycin (22.9 %), streptomycin (11.5 %), and gentamicin (9.4 %).</p>	
<p>ampicillin amoxicillin + clavulanic acid amikacin</p>	<p><i>S. Typhimurium</i> (n=11)</p>	<p>Culture method: Faecal samples</p>	<p>Passerine: greenfinch <i>Chloris chloris</i> (n = 6)</p>	<p>0</p>	<p>England and Wales</p>	<p>Analysis of acquired resistance genes found that all possessed <i>aac(6)-Iaa</i>, although able to confer resistance to certain</p>	<p>(Mather et al., 2016)</p>



aztreonam ceftazidime cefalotin ciprofloxacin cefotaxime cefuroxime cefuroxime axetil ertapenem cefepime cefoxitin gentamicin meropenem tigecycline tobramycin trimethoprim piperacillin-tazobactam		Typing methods: biotype (serotype nad phage typ), PFGE, MLST, whole genome sequencing  Suseceptibility test: agar diffusion	House sparrow <i>Passer domesticus</i> (n =4)  Goldfinch: <i>Carduelis carduelis</i> (n=1)	O  O		aminoglycosides, it has been shown to be a cryptic resistance  gene that is not expressed. No SNPs in <i>gyrA</i> , <i>gyrB</i> , <i>parC</i> , or <i>parE</i> , known to confer resistance to quinolones, were identified in these isolates. Thus, the phenotypic susceptibility profile of the isolates is in congruence with the absence of AMR determinants in the genomes.	
ampicillin ceftriaxone ceftiofur tetracycline sulfamethoxazole +	<i>E. coli</i> , <i>Enterobacter</i> spp. <i>K. pneumoniae</i> <i>S. Typhimarium</i> (n=1 from	Culture method: cloacal swab samples (109)	Illegally trated wild bird (109): Emberizidae (n=60) Thraupidae (n=16) Icteridae (n=12)	O O O	Brazil	Antibiotic resistance in 60 of the 70 selected bacterial isolates. The resistance patterns varied from one to nine of the antibiotics	(Matias et al., 2016)

<p>trimethoprim 19:1 chloramphenicol</p> <p>gentamicin</p> <p>nalidixic acid ciprofloxacin enrofloxacin and nitrofurantoin</p>	<p>Temminck's seedeater (<i>Sporophila falcirostris</i>) and two <i>S. panama</i> from chestnut-capped blackbird (<i>Chrysomus ruficapillus</i>)</p>	<p>PCR: the presence of toxin genes with the multiplex PCR</p> <p>Susceptibility test: microdilution (CLSI)</p>	<p>Cardinalidae (n=8)</p> <p>Turdidae (n=6)</p> <p>Psittacidae (n=3)</p> <p>Misclassified (n=4)</p>	<p>O</p> <p>O</p> <p>O</p> <p>?</p>		<p>tested. The resistance to ceftiofur (71.67%) was the most frequent, followed by ampicillin (46.67%) and ceftriaxone (35%).</p>	
<p>oxacillin</p> <p>Florfenicol</p> <p>Chloramphenicol</p> <p>(PCR-detection of genes against many other antimicrobial agents)</p>	<p><i>S. aureus</i> (155) isolates recovered by culture, 124 of which were available for genotyping.</p>	<p>Culture method: samples from nasal swabs (n=54), skin, wound or abscess swabs (n=18), swabs from pharynx, eyes or ears (n=8), various post-mortem tissue samples (n=29) or faecal samples (n=15).</p> <p>PCR: SCC<i>mec</i> XI, detection of different</p>	<p>A total of 2855 animals as well as a number of faecal samples:</p> <p>16 bird species</p> <p>28 mammal species</p> <p>For information regarding species, see the reference</p>	<p>O</p> <p>H/C/O</p>	<p>Germany</p> <p>Austria</p> <p>Sweden</p>	<p>European wildlife harbours diverse lineages of <i>S. aureus</i>. Some are of public health or animal health interest; others appear to be rare and unique.</p> <p><i>mecA</i>-MRSA, including livestock- associated MRSA, were uncommon to virtually absent.</p> <p>Conversely, several <i>mecC</i>-MRSA were identified suggesting a wildlife reservoir.</p>	<p>(Monecke et al., 2016)</p>

		<p>resistance genes</p> <p>Susceptibility test: Performed on selected isolates (mecC for methicillin resistance- or florfenicol/chloramphenicol resistance genes cfr/fexA-positives); agar dilution (CLSI)</p> <p>Typing method: MLST, <i>spa</i></p> <p>StaphyType DNA microarray or <i>S. aureus</i> Genotyping Kits 2.0 kit.</p>					
erythromycin tetracycline streptomycin gentamicin ciprofloxacin	<i>C. jejuni</i> (n=805)	Culture method:	broilers (n=459), bovines (n =120), human patients (n = 95),		Finland	The proportions of resistant isolates were 5% (broilers),	(Olkkola et al., 2016)

nalidixic		<p>Faecal samples, water samples</p> <p>Susceptibility test: microdilution (EUCAST)</p> <p>PCR: detection of tet-gene.</p> <p>Typing method: MLST</p>	<p>natural waters (n =80), <b>wild birds</b> (n =35) and zoo animals/ enclosures (n=16)</p> <p>Species of wild birds: not specified.</p>	<p>Not applicable since wild birds species not specified.</p>		<p>6.3% (natural waters), <b>11.4% (wild birds)</b>, 11.6% (human patients), 16.7% (bovines) and 31.3% (zoo).</p> <p>Tetracycline resistance most common in the wild birds, water, and zoo isolates.</p> <p>A rare clonal complex ST-1034 CC previously associated with wild birds showed a high proportion of tetracycline-resistant isolates, most originating from the zoo and broilers with closely associated MLST types from these sources. This could suggest a common ancestor for these isolates, possibly originating from wild birds.</p>	
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<p>tetracycline vancomycin erythromycin gentamicin</p> <p>ampicillin chloramphenicol ciprofloxacin teicoplanin</p> <p>quinupristin + dalfopristin</p> <p>rifampicin</p> <p>streptomycin tigecycline</p> <p>linezolid</p> <p>nitrofurantoin.</p>	<p>VRE <i>E. faecium</i> (n=5)</p>	<p>Culture method: faecal sample</p> <p>Identification: Microflex</p> <p>LT MALDI-TOF MS</p> <p>Susceptibility test: E-test (CLSI).</p> <p>PCR: detection of The presence of <i>vanA</i>, <i>vanB</i>, <i>vanC</i> (encoding glycopeptide resistance), <i>tet(M)</i>, <i>tet(O)</i>, <i>tet(K)</i>, <i>tet(L)</i> (<i>tetracycline</i> <i>resistance</i>), <i>erm(A)</i>, <i>erm(B)</i>, <i>mef(A)</i> (erythromycin resistance), <i>ant(4')</i>-Ia,</p>	<p>common raven (<i>Corvus corax</i>) (n=287)</p> <p>Rooks (<i>Corvus frugilegus</i>) (n=99)</p>	<p>O</p> <p>O</p>	<p>Slovakia</p>	<p>VRE with the <i>vanA</i> gene found in 4 (1.4%) of 287 raven samples and in one (1%) of 99 rook samples.</p> <p>All 5 isolates belonged to <i>E. faecium</i> and were multiresistant, with resistance to erythromycin encoded by the <i>erm(B)</i> gene, tetracycline (<i>tet(M)</i> and <i>tet(L)</i> genes), and ampicillin (mutations in C- terminal region of <i>pbp5</i> gene).</p> <p>Clinically important enterococci with the <i>vanA</i> gene in corvids in Slovakia.</p>	<p>(Oravcova et al., 2016)</p>
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		aac(6'), aph(2''), and aph(3')-IIIa (aminoglycoside resistance)					
		Typing method: MLST, PFGE					
ampicillin ceftriaxone ceftiofur cefoxitin amoxicillin + clavulanic acid tetracycline chloramphenicol sulphisoxazole trimethoprim + sulfamethoxazole naladixic acid ciprofloxacin gentamicin	<i>E. coli</i>	Culture method: cloacal samples  Susceptibility test: (CLSI)  Isolates resistant to ceftriaxone or ceftiofur were screened for ESBLs using previously published primers for the CTX-M-, TEM-	American robins, <i>Turdus migratorius</i> (n=29),  American crows, <i>Corvus brachyrhynchos</i> (n=25)  Great horned owls, <i>Bubo virginianus</i> (n=21)  Injured birds routinely brought to the college for treatment	O  O  C	Canada	88.2% of isolates pan-susceptible. Among isolates resistant to at least one antimicrobial, ampicillin resistance most commonly identified. Three birds carried MDR isolates, and ESBL-producing organisms (CTX-M-15 and SHV2a)  were grown from two. A significant relationship between drug-resistant <i>E. coli</i> and urban (vs rural) origin of the bird.	(Parker et al., 2016)

		and SHV-type b-lactamase  Typing: PCR and sequencing					
Ampicillin Ciprofloxacin Chloramphenicol Erythromycin Gentamycin  Nitrofurantoin Norfloxacin  Streptomycin Tetracycline  Rifampicin  Vancomycin	443 isolates  <i>E. faecalis</i>  <i>E. faecium</i>	Culture method: Faecal samples   Susceptibility test: agar diffuson (CLSI)   PCR for detection of resistance genes	<b>Seabirds</b> (n=12):  <i>Spheniscus magellanicus</i> (n=10),  <i>Sterna trudeaui</i> (n=1),  <i>Himantopus melanurus</i> (n=1)  <b>Turtles</b> (n=8): <i>Eretmochelys imbricata</i> (n=6), <i>Chelonia mydas</i> (n=2)  <b>Mammals</b> (n=3):  <i>Balaenoptera acutorostrata</i> (n=1),  <i>Megaptera novaehangliae</i> (n=1), and <i>Grampus griseus</i> (n=1),  found alive or dead in southern coastal Brazil.	C  O  C  O  O  C  C  C	Brazil	Among the 443 enterococci isolated, 62 distinct antimicrobial patterns were observed. This classification was created for grouping strains with similar antimicrobial susceptibility and resistance patterns. Of the 62 observed patterns, 49 (79.03%) enterococcal strains profiles were resistant to one or more of the tested antibiotics. Single, double, and multiple antibiotic resistance patterns were observed in  37.09%, 25.80%, and 16.12% of the isolates, respectively. Resistance to	(Prichula et al., 2016)

						rifampicin occurred most frequently.	
ampicillin amoxicillin + clavulanic acid cefotaxime gentamicin trimethoprim + sulphamethoxazol tetracycline ciprofloxacin cefradine ceftiofur enrofloxacin	<i>S. enterica</i> serotype Enteritidis (90, n = 30 each humans, poultry, seabirds)	Culture method:  Bacteria were grown routinely in liquid culture  Susceptibility test: disk diffusion (CLSI)  Typing method: PCR-based virulotyping, PFGE, MLST	Poultry Humans Seabirds : Franklin gull <i>Leucophaeus pipixcan</i> Kelp gull <i>Larus dominicanus</i> (n= not stated) Penguin <u>order</u> <i>Sphenisciformes</i> , <u>family</u> <i>Spheniscidae</i> species: not specified.	O  O C	Chile	Very close genetic and phenotypic traits shown by isolates suggest inter-species transmission of <i>S. Enteritidis</i> between hosts, likely through anthropogenic environmental contamination	(Retamal et al., 2015)
Sulpha-trimethoprim Penicillin Ampicillin Amoxicillin Amoxicillin/Cefadroxil Cefalexin Cefazolin Cefovecin Oxytetracycline Doxycycline Streptomycin Kanamycin Gentamycin	<i>E. coli</i> (n = 14)  <i>Klebsiella spp.</i> (n=7)  <i>Proteus</i>	Culture method: Conjunctival samples from the conjunctival sac of each eye by sterile swabs.  The enteric flora was	14 birds of prey, 3 Falconiformes: 3 mountain caracaras ( <i>Phalco boenus megalopterus</i> ), 6 Accipitriformes: 4 Egyptian vultures ( <i>Neophron percnopterus</i> ) and 2	O	Italy	All isolates were multidrug resistant (MDR). To the author's knowledge, this is the first report regarding the presence of MDR strains within raptors housed in a zoological garden. Since resistance genes can be	(Sala et al., 2016)



Amikacin Erythromycin Tylosin Lincomycin Clindamycin Chloramphenico-l  Enrofloxacin Ciprofloxacin Marbofloxacin	spp.  (n =6)  <i>Staphylococcus</i> spp.  (n =6)	assessed by cloacal swabs with Amies transport medium.  Typing method:  Serological- API Staph and API 20 E biochemical test systems  Susceptibility test: Antimicrobial susceptibility  tests were performed by Kirby-Bauer disk diffusion method (ECDC 2012, CDC 2013)	red-headed vultures ( <i>Sarcogyps calvus</i> ),  5 Strigiformes: 2 Eurasian eagle-owls ( <i>Bubo bubo</i> ) and 3 snowy owls ( <i>Bubo scandiacus</i> )			transferred to other pathogenic bacteria, this represents a potential hazard for the emergence of new MDR pathogens.  It is not clear the birds harboured MDR-isolates before entering the garden.	
cefotaxime	ESBLproducing <i>E. coli</i> isolates (24 from wild birds and 40 clinical isolates from humans)	Culture method:  cloacal swabs n= 320	Different wild avian species (n=40): sampling during entery examination of rescued	Not possible to determine	Germany	Ten ESBL-producing <i>E. coli</i> isolates with ST410 from wild birds, humans and dogs (n=1) showed identically PFGE-	(Schaufler et al., 2016)

		<p>Susceptibility test: agar method (CLSI)</p> <p>PCR for amplification of different resistance genes: bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, bla<sub>OXA</sub>, bla<sub>TEM</sub>, tetA/B/C, sul1, sul2, sul3, strA/B, aadA1-like and aaC4, gyrA, AmpC beta-lactamase</p> <p>Plasmid profile analysis</p> <p>Typing method: PFGE, MLST, Whole genome sequencing</p>	<p>wild birds in the small animal clinic in Berlin.</p> <p>(No information is available at species level)</p>			<p>patterns, indicating transmission of this clone between wild birds, humans, companion animals, and the environment.</p>	
<p>oxacillin penicillin G cefoxitin</p>	<p>Coagulase-negative staphylococci: <i>S. sciuri</i></p>	<p>Culture method: Samples from the</p>	<p>Birds of Prey: n=16 <i>Buteo buteo</i>,</p>	C	Portugal	<p>CoNS frequently colonize the nasal tract of birds of prey and these isolates can carry unusual</p>	(Sousa et al., 2016)

erythromycin clindamycin gentamicin tobramycin kanamycin streptomycin fusidic acid mupirocin tetracycline trimethoprim + sulphamethoxazole linezolid chloramphenicol ciprofloxacin	<i>S. xyloso</i> <i>S. kloosii</i>  (n=12)	nasopharynx of 16 birds of prey recovering centre from the  Veterinary Medical Hospital.  Susceptibility test:  Disc diffusion (EUCAST)  Typing method:  PFGE, PCR for detection of the following resistance genes: <i>vga(A)</i> for clindamycin, and <i>erm(T)</i> for erythromycin	<i>Athene noctua</i> , <i>Hieraaetus pennatus</i> , <i>Strix aluco</i> <i>Otus scops</i>  <i>Milvus migrans</i>	C (opportunistic)  O  C  C  C		resistance genes, such as <i>vga(A)</i> and <i>erm(T)</i> , detected in <i>S. sciuri</i> and <i>S.</i> <i>xyloso</i> species. The detection of similar clones of <i>S. xyloso</i> and <i>S. sciuri</i> in different species of birds indicates the possibility of dissemination, not only of resistance  genes but also of clones in natural environments.	
cefoperazone vancomycin amphotericin B	<i>Campylobacter</i>	Culture method: Faecal or	Non-human primates,	H  O	USA	Whole-genome analysis uncovered two distinct clades of	(Weis et al., 2016)

	(n = 97)	<p>cloacal swab samples were collected.</p> <p>Typing method:</p> <p>Genomic analysis. These genomes were part of the 100K Pathogen Genome Project using previously published methods.</p> <p>DNA extraction, library preparation, and next-generation sequencing.</p> <p>Susceptibility method:</p>	<p>Crows (American crows)</p> <p>(n=not stated)</p> <p>Chickens, Sheep, and goats</p>		<p><i>C. jejuni</i> genotypes; the first contained genotypes found only in crows, while a second genotype contained "generalist" genomes that were isolated from multiple host species, including isolates implicated in human disease, primate gastroenteritis, and livestock abortion. Two major-lactamase genes were observed frequently in these genomes (<i>oxa-184</i>, 55%, and <i>oxa-61</i>, 29%), where <i>oxa-184</i> was associated only with crows and <i>oxa-61</i> was associated with generalists. Mutations in <i>gyrA</i>, indicative of fluoroquinolone resistance, were observed in 14% of the isolates. Tetracycline resistance (<i>tetO</i>) was present in 22% of</p>	
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		<p>Antibiotic resistance genes were analyzed in every genome using the Resistance Gene Identifier software and the Comprehensive Antibiotic Resistance database (CARD)</p>				<p>the isolates, yet it occurred in 91% of the abortion isolates. Virulence genes were distributed throughout the genomes; however, <i>cdtC</i> alleles recapitulated the crow-only and generalist clades. A specific <i>cdtC</i> allele was associated with abortion in livestock and was concomitant with <i>tetO</i>. These findings indicate that crows harboring a generalist <i>C. jejuni</i> genotype may act as a vector for the zoonotic transmission of <i>Campylobacter</i>.</p>	
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<p>amikacin amoxicillin +clavulanic acid ampicillin cephalexin cephalothin cefoxitin chloramphenicol enrofloxacin gentamicin neomycin norfloxacin sulphamethoxazole- trimethoprim tetracycline</p>	<p>There was growth of microorganism in 62.5% of 253 samples.</p> <p><i>Staphylococcus</i> spp. (15.0%), <i>Micrococcus</i> spp. (11.5%), <i>E. coli</i> (10.7%) and <i>Klebsiella</i> spp. (10.7%)</p>	<p>Culture method: Cloacal swabs (n=253)</p> <p>Susceptibility test: Disc diffusion (CLSI)</p> <p>Typing methods: Not stated</p>	<p>Passerine (confiscated) (n=253) included 34 different species.</p>	<p>A passerine is any bird of the order Passeriformes, which includes more than half of all birds ... Most passerines are omnivorous, while shrikes are carnivorous.</p>	<p>Brazil</p>	<p>Fifteen bacteria genera and seven fungi genera were isolated. MDR observed in <i>Staphylococcus</i> spp., <i>Micrococcus</i> spp., <i>E. coli</i> and <i>Klebsiella</i> spp.</p>	<p>(Braconaro et al., 2015)</p>
<p>ampicillin cefuroxime cefotaxime ceftazidime gentamicin cefadroxil aztreonam ertapenem</p>	<p>ESBL-producing <i>E. coli</i></p>	<p>Culture method: Faecal samples (n=124)</p> <p>Susceptibility test: Disc diffusion (CLSI),</p>	<p>Migratory Franklin's Gulls (<i>Leucophaeus pipixcan</i>) (n=124) migrating from USA and Canada</p>	<p>O</p>	<p>Chile</p>	<p>A total of 91 <i>E. coli</i> isolates with high rates of antibiotic resistance identified. Carbapenemase production not detected, whereas 67 (54%) isolates exhibited an ESBL phenotype due to the presence of CTX-M-15 (61.3%), CTX-M-2</p>	<p>(Baez et al., 2015)</p>

<p>norfloxacin nalidixic acid nitrofurantoin chloramphenicol tetracycline</p> <p>pipemidic acid amikacin</p> <p>streptomycin</p> <p>trimethoprim– sulfamethoxazole</p>		<p>The double-disk diffusion technique was performed for phenotypic ESBL detection.</p> <p>Typing methods: PFGE, MLST</p>				<p>(19.3%), CTX-M-22 (16.1%), and CTX-M-3 (1.6%) coding genes. High genetic diversity</p> <p>observed, with 30 PFGE patterns and 23 sequence types (STs), including ST131 (18%), ST44 (15%), ST617 (9%), and ST10 (9%).</p>	
<p>Cefazolin</p> <p>Cefotaxime</p> <p>Ceftazidime</p> <p>ampicillin</p> <p>amoxicillin + clavulanic acid</p> <p>ciprofloxacin</p> <p>nalidixic acid</p>	<p><i>E. coli</i></p> <p><i>Cryptic clades</i> (monophyletic <i>Escherichia</i> clades were described and referred to as "cryptic" based on the inability to distinguish them from representative <i>E. coli</i> isolates using diagnostic biochemical reactions)</p> <p>Difficult to determine how many isolates were from wild birds</p>	<p>Culture method: Faecal sample</p> <p>Susceptibility test: Disc diffusion (EUCAST)</p> <p>Typing method: PCR</p>	<p>Wild birds (n=594): belonged to 115 species, 81 genera, 42 families, and 17 orders.</p>	<p>N. A. since wild birds species were not stated.</p>	<p>Australia</p>	<p>The similarity of antimicrobial resistance profiles and genetic attributes between <i>Escherichia</i> spp. from suburban and wild birds is reassuring, in that it suggests a low rate of transmission of <i>Escherichia</i> spp. between humans and birds in Australian urban environments. However, the relatively high prevalence of AMR and MDR detected among <i>Escherichia</i> spp. from in-care</p>	<p>(Blyton et al., 2015)</p>

						birds and poultry is concerning.	
amoxicillin-clavulanic acid ampicillin ciprofloxacin penicillin G streptomycin tetracycline	<i>E. coli</i> n=115 isolated from 81 of 146 samples	Culture method: faecal sample (n=146)  Susceptibility test: Disc diffusion (EUCAST)  Typing method: PCR confirmation of resistance genes	Faecal samples (n=30) were collected from: herring gulls ( <i>Larus argentatus</i> ), black-headed gulls ( <i>Larus ridibundus</i> ), lesser black-backed gulls ( <i>Larus fuscus</i> ), hybrid deer species ( <i>Cervus elaphus</i> x <i>Cervus nippon</i> ) and twenty-six from starlings ( <i>Sturnus vulgaris</i> ).  (n of birds not stated)	All bird species are omnivores (O)	Ireland	In total, 5.4% (8/146) of samples exhibited MDR phenotypes. Tetracycline-, ampicillin- and streptomycin-resistant isolates were the most common. The following genes were identified in <i>E. coli</i> . blaTEM, strA, tet(A) and tet(B). Plasmids were identified in all samples that exhibited MDR phenotypes.	(Carroll et al., 2015)
ceftiofur ciprofloxacin enrofloxacin streptomycin gentamycin sulphonamide sulfamethoxazole-trimethoprim nalidixic acid	164 isolates: <i>E. coli</i> (46.5%), followed by <i>Pantoea agglomerans</i> (13.2%) and <i>Enterobacter cloacae</i> (12%).  Other enterobacteria were	Culture method: Faecal sample (n=167)  Susceptibility test: Disk	Healthy <i>Psittacines</i> (n=167) from Wildlife Rehabilitation Center:  three hyacinth macaws ( <i>Anodorhynchus hyacinthinus</i> ), two scarlet macaws ( <i>Ara macao</i> ), seven blue-and-yellow macaws ( <i>Ara ararauna</i> ), one	Most psittacines are omnivores. (O)	Brazil	The antimicrobial susceptibility tests revealed that the enterobacteria found in the intestinal microbiota of the studied birds presented high multidrug resistance rates, which the most frequent resistance	(Lopes et al., 2015)



ampicillin polymyxin B tetracycline azithromycin	isolated less frequently: <i>Salmonella Saintpaul</i> (0.6%), <i>Escherichia hermanii</i> (0.6%), <i>Enterobacter aerogenes</i> (0.6%), <i>Citrobacter diversus</i> (0.6%), <i>Citrobacter freundii</i> (1.8%), <i>Proteus mirabilis</i> (1.8%), <i>Klebsiella pneumoniae</i> (1.8%), <i>Hafnia alvei</i> (1.8%), <i>Citrobacter amalonaticus</i> (2.4%), <i>Enterobacter gergoviae</i> (2.4%), <i>Serratia rubidaea</i> (2.4%), <i>Enterobacter sakazakii</i> (3.6%), and <i>Serratia liquefaciens</i> (4.2%).	diffusion (CLSI)  Typing method:  Serotyping	red-and-green macaw ( <i>Ara chloropterus</i> ), one chestnut-fronted macaw ( <i>Ara severus</i> ), sixty seven cactus parakeets ( <i>Eupsittula cactorum</i> ), three peach-fronted parakeets ( <i>Eupsittula aurea</i> ), sixty six blue-fronted amazon parrots ( <i>Amazona aestiva</i> ), thirteen orange-winged amazon parrots ( <i>Amazona amazonica</i> ), one mealy amazon parrot ( <i>Amazona farinosa</i> ), two yellow-chevroned parakeets ( <i>Brotogeris chiriri</i> ) and one white-eyed parakeet ( <i>Psittacara leucophthalma</i> ).			was to azithromycin among the various isolated strains.	
amoxicillin + clavulanic acid ampicillin  cephalothin ceftazidime chloramphenicol ciprofloxacin	<i>Salmonella</i> (n=39)	Culture method:  Fecal samples (n=2778)	Corvid species:  Rooks (Europe)  North American crows ( <i>Corvus</i> )	O	Canada  European countries	European sites were significantly more likely to yield <i>Salmonella</i> resistant to more than one antibiotic than North American sites, where no resistance	(Janecko et al., 2015)

gentamicin nalidixic acid streptomycin sulfamethoxazole +trimethoprim sulphonamide- compounds tetracycline		Susceptibility test:  Disk diffusion (CLSI)  Typing methods:  Serotyping	<i>brachyrhynchos</i> ) were sampled in USA and Canada.  (n=not stated)			was found. Resistance to nalidixic acid, a quinolone, was recovered in nine isolates from four serovars in four different sites across Europe.	
Amikacin Amoxicillin + clavulanic acid Ampicillin Azetronam Cefotaxime Cefoxitin Ceftazidime Cephalotin Chloramphenicol Gentamicin Imipenem Ciprofloxacin kanamycin	<i>E. coli</i>  N=166 isolates obtained from the 29 cloacal swabs (n=129) and from 8 raw feed samples (n=37)	Culture method:  Fecal samples n= 29  Food n=8  feed n=8   Susceptibility test: Disk diffusion (CLSI)  Typing methods: PFGE	Wild birds from rehabilitation cente:  Goshawk ( <i>Accipiter gentilis</i> n=3),  Eurasian sparrowhawk ( <i>Accipiter nisus</i> n=1),  Grey heron ( <i>Ardea cinerea</i> n=3),  Purple heron ( <i>Ardea purpurea</i> n=1),  Little owl ( <i>Athene noctua</i> n=1),  Eurasian eagle owl  ( <i>Bubo bubo</i> n=4),  Common buzzard ( <i>Buteo buteo</i> n=7),	H C  C (fish) O O O O C	Portugal	The antimicrobial susceptibility from the wild birds, only two isolates from birds showed resistance toward one antimicrobial agent, one against cefoxitin and one against kanamycin and all other isolates presented resistance against to two or more agents.  Results from this study indicate that MDR may be transferred from feed to wild birds kept in a rehabilitation center.	(Pinto et al., 2015)

<p>Nalidixic acid Nitrofurantoin Streptomycin</p> <p>Tetracycline Tobramycin Trimethoprim- Sulfamethoxazol</p>			<p>European nightjar (<i>Caprimulgus europaeus</i> n=1),</p> <p>Western maesh harrier (<i>Circus aeruginosus</i> n=1),</p> <p>Egyptian vulture (<i>Neopheron perconpetrus</i> n=1),</p> <p>Euroasian tawny owl (<i>Strix alucon</i> n=4),</p> <p>Common barn oel (<i>Tyto alba</i> n=2).</p>	<p>O</p> <p>C and scavanger</p> <p>C</p> <p>C</p>			
<p>26 different antimicrobial agents:</p> <p>Not all class and antimicrobial type were stated.</p>	<p>One <i>E. coli</i> (n=400) population of wild mallard ducks in their natural environment over four winter seasons, following the characterization of 100 isolates each consecutive season. (n=400)</p>	<p>Culture method: Fecal samples</p> <p>Susceptibility test: Disk diffusion (CLSI)</p> <p>Typing methods: PFGE</p>	<p>Wild mallard duck (n=unspecified)</p> <p>The 500-meter long river sampling section was highly attended by an estimated 300–1000 mallards. To guarantee collection of fresh and not frozen samples, faeces were collected on freshly fallen snow and at air temperatures not below –3°C.</p>	<p>O</p>	<p>Germany</p>	<p>Only one out of 150 multi-year isolates was resistant, in contrast to 38 out of 243 1-year isolates. Additionally, PFGE types with susceptible isolates were isolated with averagely higher numbers of isolates per PFGE type and winter season compared with resistant isolates. Though almost all resistant isolates</p>	<p>(Rodiger et al., 2015)</p>

						were 1-year, and though there were dramatic changes in PFGE types over the years, it is astonishing that antimicrobial resistance rather increased than disappeared over the 4-year period. It seems that within such a highly dynamic <i>E. coli</i> population, resistances are easily and continuously transferred.	
<p>Identification of resistance genes:</p> <p><i>bla</i>NDM-1, <i>bla</i>CMY-16, <i>fosA3</i>, <i>sul1</i>, <i>sul2</i>, <i>strA</i>,</p> <p><i>strB</i>, <i>aac(6=)-Ib</i>, <i>aadA5</i>, <i>aphA6</i>, <i>tetA(A)</i>, <i>mphA</i>, <i>floR</i>, <i>dfrA7</i>, and <i>merA</i> genes</p>	<i>S. enterica</i> serovar Corvallis strain n=1	<p>Culture method: -</p> <p>Susceptibility test:</p> <p>PCR for verification of resistance genes</p>	wild bird ( <i>Milvus migrans</i> ) (n= not specified)	O (Scavanger)	Germany	<p>This strain carried the IncA/C2 pRH- 1238 plasmid. Complete sequencing of the plasmid was performed, identifying the <i>bla</i>NDM-1, <i>bla</i>CMY-16, <i>fosA3</i>, <i>sul1</i>, <i>sul2</i>, <i>strA</i>, <i>strB</i>, <i>aac(6=)-Ib</i>, <i>aadA5</i>, <i>aphA6</i>, <i>tetA(A)</i>, <i>mphA</i>, <i>floR</i>, <i>dfrA7</i>, and <i>merA</i> genes, which confer clinically relevant resistance to most of the antimicrobial</p>	(Villa et al., 2015)

						classes, including $\beta$ -lactams with carbapenems, fosfomycin, aminoglycosides, cotrimoxazole, tetracyclines, and macrolides. The strain likely originated from the Asiatic region and was transferred to Germany through the <i>Milvus migrans</i> migratory route.	
aminopenicillins phenicols aminoglycosides sulphonamides dihydrofolate reductase inhibitors tetracyclines cephalosporins quinolones	<i>Salmonella</i>  A convenience sample of 41 fattening-pig farms from farmers.  Four nets (2.5 · 9m each) were set up in locations where birds were usually spotted, and these nets were used during the entire morning.  Birds shedding <i>Salmonella</i> spp.	Culture method:  Fecal sample (n=150)  Susceptibility test:  Not stated  PCR for detection of resistance genes	Wild birds (n=not specified)  Pigs,  Rodents	Not relevant  O	Spain	<i>Salmonella</i> -positive pig fecal samples were identified in 56.1% of the 41 farms investigated. Birds shedding <i>Salmonella</i> spp. were detected in 21.4% of the farms despite the low numbers of birds captured in many farms. Most <i>Salmonella</i> isolates from birds (74%) did not show any antimicrobial resistance (AR) pattern and belonged	(Andres-Barranco et al., 2014)

	were detected in 9 (21.9%) farms.	Typing method: serotyping				to phage types rarely seen in the pig population (U310, DT56, DT137, DT164), supporting the likely avian source of infection for most birds.	
10 antimicrobial agents  Not all agents were stated	<i>Enterococcus</i> spp. <i>E. coli</i> <i>K. pneumoniae</i>	Culture method:  Fecal sample (n=150)  Susceptibility test:  Not stated  PCR for detection of resistance genes  Typing method:  MLST	Gulls (n=260)	O	Alaska, USA	Seven (4.7%) <i>E. faecium</i> isolates were found, all of which harbored both the <i>vanA</i> and the <i>esp</i> genes (found in isolates of the CC17 lineage). No other VRE were found.  To investigate the presence of ESBL-producing bacteria, we conducted a selective screen as described. ESBL-producing bacteria were found ( <i>E. coli</i> and <i>K. pneumoniae</i> ), and ESBL genes ( <i>bla</i> <sub>CTX-M</sub> , <i>bla</i> <sub>SHV</sub> , and <i>bla</i> <sub>TEM</sub> ) in ESBL-positive isolates	(Bonnedahl et al., 2014)

						<p>were analyzed (6). We found 33 <i>E. coli</i> and 35 <i>K. pneumoniae</i> ESBL-producing isolates in 55 samples (12 samples had &gt;1 unique isolate), a total of 37% of ESBL-harboring samples.</p>	
<p>ampicillin cefadroxil cefuroxime mecillinam, trimethoprim- sulfamethoxazol nalidixic acid ciprofloxacin chloramphenicol gentamicin nitrofurantoin tigecycline</p>	<i>E. coli</i>	<p>Culture method:  Fecal sample (n=150) and 8 water samples</p> <p>Susceptibility test:  Disk diffusion (EUCAST)</p> <p>Typing method:  repetitive element PCR</p>	Gull ( <i>Chroicocephalus brunnicephalus</i> ) (n=150)	O	Coastlines of the Bay of Bengal-bangladesh	<p>Antibiotic resistance was found in 42.3% (36/85) of the <i>E. coli</i> isolates and multidrug resistance in 11.8%.</p> <p>Isolates from the area with a higher human activity were more resistant than those from an area with a lower level of activity. Most frequent was resistance to ampicillin (29.4%), followed by trimethoprim-sulfamethoxazole (24.7%) and quinolones (22.4%). Carriage of ESBL-producing <i>E. coli</i> was</p>	(Hasan et al., 2014)

		(rep-PCR), MLST				relatively high (17.3%) in the gulls, whereas no ESBL producers were found in the water. All ESBL-producing <i>E. coli</i> isolates, but one, carried bla <sub>CTX-M-15</sub> or bla <sub>CTX-M-15</sub> -like genes. A bla <sub>CTX-M-14</sub> -like enzyme was found as an exception.	
ampicillin ampicillin/sulbactam , cefazolin  cefuroxime,  cefoxitin  gentamicin trimethoprim +  sulfamethoxazole, colistin  oxolinic acid  ofloxacin  tetracycline  aztreonam  piperacillin  piperacillin + tazobactam	<i>E. cloacae</i>  <i>E. coli</i>  (n= not stated)	Culture method:  Cloacal swabs from Little auks (n=215),  faeces of glaucous gulls ( <i>Larus hyperboreus</i> , n=15)    Susceptibility test: microdilution (EUCAST)    Typing method:	Little auks ( <i>Alle alle</i> , <i>Lariformes</i> , <i>Alcidae</i> ) (n=215)  Glaucous gull ( <i>Larus hyperboreus</i> ) (n=15)	O  O	Svalbard, Norway   (The study performed in Czech Republic)	Two <i>E. cloacae</i> (13%) isolates from 15 samples of glaucous gull faeces in Magdalene fjorden grown on MacConkey agar with cefotaxime and were resistant to ampicillin, cefazolin and cefoxitin. None of them were positive in DDST. No <i>E. coli</i> isolates resistant to cephalosporins and fluoroquinolones and no <i>Salmonellae</i> were found	(Literak et al., 2014)



cefoperazone cefotaxime ceftazidime cefepime cefoperazone + sulbactam meropenem ciprofloxacin tigecycline tobramycin amikacin		PFGE, SNP					
Cefaclor Oxacillin Ampicillin Chloramphenicol Cephalexin Neomycin Colistin Ciprofloxacin Oxytetracycline Norfloxacin Lincomycin Gentamycin	<i>Escherichia vulneris</i> (n=82)	Culture method:  Cloacae swab sample  Susceptibility test:  Disk diffusion (CLSI)  Typing methods:  16s rRNA, PCR-squencing	<b>Non-migrating birds:</b> Sand Partridge (n=3) Arabian Babbler (n=3) White-spectacled Bulbul (n=3) Rüppell's Weaver (n=6) Black Scrub Robin (n=3) Arabian Serin (n=3) Philby's Partridge (n=3) Lappet-faced Vulture (n=3)	 O O O O O O O O	Saudi Arabia	All isolates recovered from non-migrating birds were found resistant to Oxacillin while all isolates recovered from migrating  birds demonstrated resistance to Oxacillin, Chloramphenicol, Oxytetracycline and Lincomycin. Some bacterial isolates recovered from non- migrating birds and migrating birds	(Shobrak and Abo- Amer, 2014)

Amoxicillin Enrofloxacin Piperacillin			<b>Migrating birds:</b> Isabelline Shrike (n=3) Barn Swallow (n=3) Tawny Pipit (n=3) Willow Warbler (n=3) Sand Martin (n=3) Isabelline Shrike (n=3)	O C C C C C		exhibited MDR phenotype.	
Ciprofloxacin Tetracycline Oxacillin Penicillin Rifampicin	<i>E. coli</i> (n=92) 41 isolates were recovered from the 30 herring gull faecal samples and 51 isolates	Culture method: Feces sample (n=not stated) Susceptibility test: Disc diffusion. Phylogenetic grouping of <i>E. coli</i> isolates was determined by PCR. Nitrocefin disc tests were performed on each confirmed	Herring gulls ( <i>Larus argentatus</i> ) (n=30) Hybrid deer ( <i>Cervus elaphus</i> x <i>Cervus nippon</i> ) (n=30)	O H	Ireland	The prevalence of resistant isolates was higher in herring gulls (87%) compared to deer (31%). Resistance to this class of antibiotic was found only in non-pathogenic <i>E. coli</i> in herring gulls and in both pathogenic and non-pathogenic <i>E. coli</i> strains in deer. All isolates were susceptible to ciprofloxacin and only one isolate was resistant to tetracycline. In contrast, all of these isolates were	(Smith et al., 2014b)

		<p><i>E. coli</i> isolate that was phenotypically resistant to oxacillin/penicillin to check for <math>\beta</math>-lactamase production. The development of a red colour on the disc was indicative of the production of a <math>\beta</math>-lactamase enzyme.</p> <p>Typing methods: Not stated</p>				resistant to rifampicin, oxacillin and penicillin.	
mupirocinte tracycline trimethoprim +sulfamethoxazole vancomycin teicoplanin erythromycin clindamycin	<i>Staphylococcus</i> spp (n= not specified):  <i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. saprophyticus</i> , <i>S. sciuri rodentium</i> , <i>S. cohnii</i> <i>urealitycum</i> ,	Culture method: Nasal samples  Susceptibility test: Disc diffusion (CLSI)	Birds of prey (n=16) <i>Buteo buteo</i> , <i>Strix aluco</i> , <i>Corvus corone</i> when admitted into CRAS (wild birds' recovering center from	 O C O	Portugal	Six of the 16 tested animals carried staphylococci (37.5%). The <i>S. aureus</i> isolate was penicillin-resistant (with blaZ gene) but methicillin-susceptible and was ascribed to spa-type t012, sequence-type ST30	(Sousa et al., 2014)

tobramycin penicillin G linezolid cefoxitin oxacillin chloramphenicol ciprofloxacin kanamycin	<i>S. gallinarum</i> .	Typing methods:  16s rRNA  MLST	the Veterinary Medical Hospital.			and agr-type III. The <i>S. epidermidis</i> isolate carried blaZ, mecA, mrs(A/B), mphC, tet(K), drfA, and fusC genes, ica operon, and was typed as ST35. The genes ant60-Ia, tet(K), tet(L), dfrG, cat221, cat194, and cat223 were detected in <i>S. saprophyticus</i> or <i>S. gallinarum</i> isolates.	
Beta-lactams Aminoglycosides Tetracyclines Phenicol Macrolides Lincosamide Quinolones Sulpha	Microbiota (n=828 bacterial colonies):  4 phyla, 14 orders, 37 families, and 59 or more genera. <i>Firmicutes</i> was the most commonly encountered phylum (57%) followed by <i>Actinobacteria</i> (24%), <i>Proteobacteria</i> (17%) and <i>Bacteroidetes</i> (0.02%).	Culture method:  Cultivable quail tracheal, crop, cecal, and cloacal  Susceptibility test:  Disk diffusion (CLSI)  Typing methods:  16s rRNA	Northern Bobwhite ( <i>Colinus virginianus</i> )	O	USA	Phenotypic characterization of selected bacterial species demonstrated a high prevalence of resistance to the following classes of antimicrobials: phenicol, macrolide, lincosamide, quinolone, and sulphate.  Nearly all <i>E. coli</i> , Enterobacter, and Neisseria isolates were resistant to following classes of antibiotics: phenicol, macrolide,	(Su et al., 2014)

						lincosamide, quinolone, and sulphate. Either intermediate susceptibility or resistance to beta-lactams and aminoglycosides were also found in these Gram-negative bacterial isolates. The Gram positive bacteria, <i>E. faecalis</i> and <i>Staphylococcus</i> isolates, were resistant to beta-lactam and sulphate antibiotics, in addition to aminoglycosides.	
nalidixic acid ciprofloxacin cefotaxime  ampicillin  chloramphenicol streptomycin gentamicin  sulfoxazole  trimethoprim tetracycline	<i>Salmonella</i> spp. (n=not stated)	Culture method: 379 faecal samples from 921 birds trapped in 31 locations nearby pig premises, and 431 samples from 581 birds of 10 natural settings far	European starling White wagtail Rock pigeon Blackcap European starling House sparrow Barn swallow House sparrow European starling	O  O  O  O  O	Spain	The levels of AR were low, with only three isolates (20%) presenting multidrug resistance. They belonged to two bird species well adapted to human environments. namely house sparrow and European starling.  Of these 3, only 1 (33%) comes from a	(Andres et al., 2013)

		from pig farms.  Susceptibility test: Disc diffusion (CLSI)  Typing methods: Serotyping, Phage typing, PFGE	House sparrow  (n=not stated)	O  C  O  O  O		FPPS bird (a house sparrow) and corresponded to the monophasic variant of Typhimurium. The other two were serotypes frequently isolated from pigs and presenting AR patterns commonly observed in this animal species.	
$\beta$ -lactams tetracyclines quinolones aminoglycosides sulphonamides polypeptides phenicols	<i>Salmonella</i> spp. (n=130)	Culture method: Lymph nodes, fecal samples  Susceptibility test: Disc diffusion (88 isolates) (EFSAs guideline)	Wild animals: canids (n=63), mustelids (n=25), birds (n=24);  - Common Pigeons  - Scops owl  - Badger  - Golden eagle	O  O  H  C  O  C  O	Italy	Almost all the analyzed strains (97.7%) showed resistance/intermediate resistance to at least one class of antibiotics and the highest resistance values were observed for the tetracycline class.	(Botti et al., 2013)

		Typing methods: Phage- and serological typing	- short-toed snake-eagle  - peregrine falcon  ungulates (n=5)	C O  O			
Amikacin ampicillin amoxicillin + clavulanic acid apramycin chloramphenicol ceftazidime ciprofloxacin furazolidine gentamicin cefotaxime nalidixic acid neomycin streptomycin sulphonamide compounds sulphamethoxazole + trimethoprim tetracycline	<i>Salmonella</i> spp.  (Total number not stated)	Culture method:  Susceptibility test: Disc diffusion  (Andrews, 2009; Veterinary Laboratories Agency, 2010).  Typing methods: Phage typing PFGE MLVA-PFGE	Wild birds:  greenfinch (n=8),  siskin (n=5),  house sparrow (n=3),  bullfinch (n=2),  chaffinch (n=1),  goldfinch (n=1),  Other isolates from domestic animals and livestock including: cattle (n=3),  cats (n=4),  horses (n=3),  dogs (n =3), chickens (n =3)  and a pig farm (n =1)	O O O O O O  N. A.	UK	The majority of the examined isolates (26/37) were fully susceptible to all 16 antimicrobial drugs.  The single exception was one DT56 isolate from a house sparrow, that was resistant to sulphonamide compounds only, and yet still possessed 100% similarity in terms of PFGE and MLVA profiles  with 4 other fully sensitive isolates.	(Horton et al., 2013)

tiamulin valnemulin doxycycline tylvalosin lincomycin tylosin	Spirochetes: <i>Brachyspira</i> spp (51)	Culture method:  Fecal samples (205)  Susceptibility test:  commercially available system (VetMIC Brachy antibiotic panels; SVA,  Sweden), following the manufacturer's recommendati ons.  VetMIC Brachy antibiotic panels consist of 48-well plates, in  which two-fold serial dilutions of	Waterfowl:  Graylag geese  Mallard  (n=not stated)	0  0	Spain	no antimicrobial resistance was observed in any of the enteropathogenic isolates recovered	(Martinez-Lobo et al., 2013)
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		antimicrobial agents Typing methods: 16s rRNA Sequencing					
ampicillin chloramphenicol ciprofloxacin  nalidixic acid streptomycin sulfamethoxazole tetracycline	<i>S. enterica</i>  (303 isolated from human, 203 isolates from 13 animal species including wildlife, livestock, pet animals)	Culture method:  Bacterial isolates, pure culture, included,  Susceptibility test:  Broth microdilution  (according to the EFSA recommendations/EUCAST)  Typing methods:  PFGE	Human  Domestic animals  Pet animals  Wildlife: white stork, black vulture, hawk, partridge, quail  (n=not stated)	O C (scavenger) C O O	Spain	A <i>S. Enteritidis</i> strain with strong quinolone resistance is spread on three host environments carrying one of the four variants found for the GyrA protein: (1) Asp87Tyr, the major polymorphism found in 39 <i>Salmonella</i> isolates from human origin and six from poultry; (2) Ser83Phe, with four isolates from human origin and one from white stork ( <i>Ciconia ciconia</i> ); and (3) Asp87Asn or (4) Asp87Gly, with two isolates each from human origins. Several <i>S. Typhimurium</i> strains	(Palomo et al., 2013)

						that presented int1 elements and the classically associated pentaresistance (ACSSuT) phenotype were found distributed between two host environments: domestic animals and humans, domestics and wild animals, or wild fauna plus humans.	
The susceptibility of the enterococcal isolates was tested for 11 antibiotics: vancomycin teicoplanin ampicillin streptomycin gentamicin kanamycin chloramphenicol tetracycline erythromycin quinupristine dalfopristin ciprofloxacin	<i>Enterococcus</i> spp. (n=138): - <i>E. faecalis</i> (n=59) - <i>E. faecium</i> (n=40)  - <i>E. durans</i> (n=27)  - <i>E. hirae</i> (n=12 )  <i>E. coli</i> (n=151)	Culture method: Fecal samples  Susceptibility test: Disk diffusion (CLSI)  Typing methods: PCR detection of resistance genes	Passeriformes, included 16 birds species (176 samples):  Galliformes (n=20 samples): <i>Coturnix coturnix</i> <i>Common quail</i>  Charadriiformes (n=20 samples): <i>Gallinago gallinago</i> <i>Common snipe</i>	O  O O  O O	Portugal	The enterococci strains showed high percentages of resistance to tetracycline (32.6%), to ciprofloxacin (19.6%) and to erythromycin (11.6%). Lower level of resistance (<10%) was detected for ampicillin, chloramphenicol and teicoplanin. One vancomycin-resistant <i>E. faecalis</i> isolate was detected and harbored the <i>vanA</i> resistant gene.	(Santos et al., 2013)

<p>Only the category of high-level resistance (HLR) was considered for streptomycin, gentamicin and kanamycin.</p> <p>Additionally, the susceptibility of the <i>E. coli</i> isolates was tested for 16 antimicrobials:</p> <p>ampicillin amoxicillin + clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam imipenem gentamicin amikacin tobramycin streptomycin</p>			(n=not stated)			<p>Resistance genes detected included tet(M) and/or tet(L), ermB in all tetracycline and erythromycin resistant isolates.</p> <p>Resistance in <i>E. coli</i> isolates was detected for ampicillin, tetracycline, sulfamethoxazole/trimethoprim, streptomycin, and tobramycin. The blaTEM, aadA, aadA5, strA, strB, tet(A) and/or tet(B), and the intI genes were found in all ampicillin, streptomycin, tetracycline, and sulfamethoxazole/trimethoprim-resistant isolates respectively.</p>	
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nalidixic acid ciprofloxacin sulfamethoxazole- trimethoprim tetracycline chloramphenicol							
ampicillin carbenicillin tetracycline oxytetracycline erythromycin flumequine florfenicol enrofloxacin ciprofloxacin danofloxacin neomycin streptomycin amikacin gentamicin lincomycin + spectinomycin	<i>E. coli</i> (n=95)	Culture method:  Fresh fecal samples (n=192) cloacal swabs from clinically healthy wild birds and free- range poultry.  Susceptibility test:  Disk diffusion (CLSI)  Typing methods:  Not stated	swans ( <i>Cygnus olor</i> or <i>C. cygnus</i> ) (n=24),  Northern shovelers ( <i>Anas clypeata</i> ) (n=21),  Eurasian coots ( <i>Fulica atra</i> ) (n=18),  ducks (n=48), chickens (n=32), turkeys (28) and geese (n=21)	H  C  O	Iran	<i>E. coli</i> was isolated from 95 (76 free range and 19 wild) samples, with an isolation rate of 59% and 30% from free range poultry and wild birds, respectively. All isolates from free range poultry were susceptible to amikacin, danofloxacin, enrofloxacin, gentamicin,  neomycin, whereas all were resistant to carbenicillin and erythromycin. In strains from wild birds, most common resistance was  detected to be to carbenicillin and tetracycline. Multi- drug resistance was evident in all isolates	(Seifi and Shirzad, 2013)

						from free range poultry and in 26% of those from wild birds.	
ampicillin cefotaxime ceftazidime ciprofloxacin chloramphenicol florfenicol gentamicin kanamycin nalidixic acid streptomycin sulfamethoxazole trimethoprim tetracycline	<i>E. coli</i> (n=not stated)	Culture method:  Cloacal swabs (414) originating from 55 different bird species.  Susceptibility test:  Disk diffusion (EUCAST)  Typing methods:  PFGE	Cloacal samples were predominately (90.1%) colcollected from birds associated with an aquatic environment, which included gulls (n=150), ducks (n=67), sandpipers, (n=35), swans (n=26), geese (n=22), and rails (n=15). Furthermore, samples were collected from grey heron ( <i>Ardea cinerea</i> ; n=11), great cormorant ( <i>Phalacrocorax carbo</i> ; n=7), common tern ( <i>Sterna hirundo</i> ; n=4), great crested grebe ( <i>Podiceps cristatus</i> ; n=2), ruff ( <i>Ptilomachus pugnax</i> ; n=5), northern lapwing ( <i>Vanellus vanellus</i> ; n=2), and Eurasian oystercatcher ( <i>Haematopus ostralegus</i> ; n=2). Also,	Majority of the wild birds included in this study are omnivores.	The Netherlands	Cefotaxime-resistant <i>E. coli</i> isolates were identified in 65 birds (15.7%) from 21 different species. In all, 65 cefotaxime-resistant <i>E. coli</i> ESBL/AmpC genes were detected, mainly comprising variants of <i>bla<sub>CTX-M</sub></i> and <i>bla<sub>CMY-2</sub></i> . Furthermore, PMQR genes [ <i>aac(6)-Ib-cr</i> , <i>qnrB1</i> , and <i>qnrS1</i> ] coincided in seven cefotaxime-resistant <i>E. coli</i> isolates. Overall, replicon typing of the ESBL/AmpC-carrying plasmids demonstrated the predominant presence of IncI1 (n=31) and variants	(Veldman et al., 2013)

		<p>typical seabirds were sampled, including auk (n=19), northern gannet (<i>Morus bassanus</i>; n=3), and northern fulmar (<i>Fulmarus glacialis</i>; n=3).</p> <p>Birds not associated with an aquatic environment represented a relative small proportion (9.9%) in this study and were sent in mainly to examine their cause of death. This third category involved</p> <p>a large number of birds of prey (n=24), including 8 species: common buzzard (<i>Buteo buteo</i>; n=14), northern goshawk (<i>Accipiter gentilis</i>; n=2), Eurasian sparrowhawk (<i>Accipiter nisus</i>; n=1), western marsh harrier (<i>Circus aeruginosus</i>; n=1), common kestrel (<i>Falco tinnunculus</i>; n=1), barn owl (<i>Tyto alba</i>; n=3), tawny owl (<i>Strix</i></p>			<p>of IncF (n=18). Our results indicate a wide dissemination of ESBL and AmpC genes in wild birds from The Netherlands, especially among aquatic-associated species (waterfowl, gulls, and waders). The identified genes and plasmids reflect the genes found predominantly in livestock animals as well as in humans.</p>	
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			<p><i>aluco</i>; n=1), and Eurasian eagle-owl (<i>Bubo bubo</i>; n=1). The latter group also involved feral pigeons (<i>Columbia livia domesticus</i>; n=4), common wood pigeon (<i>Columba palumbus</i>; n=1), stock dove (<i>Columba oenas</i>; n= 1), Eurasian woodcock (<i>Scolopax rusticola</i>; n=8), common starling (<i>Sturnus vulgaris</i>; n=1), and black crow (<i>Corvus corone</i>; n=1).</p> <p>Although carcasses were collected from all over The Netherlands, most birds were sent in from the northwest part of the country.</p> <p>The majority of the birds were wildlife, but a small proportion (3.4%) were free-flying domestic birds, including ducks (<i>Anas platyrhynchos domesticus</i>; n = 6), geese (<i>Anser anser domesticus</i>; n = 3),</p>			
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			feral pigeons ( <i>Columbia livia domesticus</i> , n=4), and one captive black swan ( <i>Cygnus atratus</i> ).				
ampicillin amoxicillin + clavulanic acid chloramphenicol cefamandole ceftazidime ceftriaxone cefotaxime cephalothin ciprofloxacin doxycycline hydrochloride gentamicin kanamycin nalidixic acid nitrofurantoin streptomycin sulphamethoxazole + trimethoprim sulphonamides tetracycline trimethoprim	<i>Salmonella</i> , Verocytotoxigenic <i>E. coli</i> O157 and <i>Campylobacter</i> (n=not detected in wild birds)	Culture method: Eighty-nine composite (five different samples from the same animal species combined) faecal  Susceptibility test: Disc diffusion (CLSI)  Typing methods: PCR-detection of resistance genes	cattle (n=24), pigs (n=14), sheep (n=4), poultry (n=4), horses (n=7), deer (n=4), dogs (n=9), rodents (2) and <b>wild  birds (n=20)</b> samples, 16 composite soil samples plus 35 individual water samples.	Wild birds species were not stated. Diet cannot be specified.	Ireland	<i>Salmonella</i> , Verocytotoxigenic <i>E. coli</i> O157 and <i>Campylobacter</i> were not detected in <b>wild  birds</b> , in this study and hence no resistance data.	(Bolton et al., 2012)



cefotaxime	<i>E. coli</i> (n=171) from Germany and (n=91) from Mongolia	<p>Culture method: cloacal swabs from ringing the nestlings from (n=16) wild avian species</p> <p>Susceptibility test: cefotaxime were confirmed as ESBL producers using the phenotypic confirmatory test for ESBL production (CLSI)</p> <p>Typing methods: MLST, PFGE</p>	Birds of prey included many different species.  (n of individual birds not stated)	Since it is not indicated which bacterial isolates were isolated from which bird species, the information regarding diet is less relevant. However, most of birds of prey are omnivores.	Germany and Mongolia	<p>ESBL production in 13.8% (n =9) of the sixty-five German and in 10.8% (n =4) of the thirty-seven Mongolian <i>E. coli</i> isolates.</p> <p>Whereas bla<sub>CTX-M-1</sub> predominated among German isolates (100%), bla<sub>CTX-M-9</sub> was the most prevalent in Mongolian isolates (75%). We identified sequence types (STs) that are well known in human and veterinary clinical ESBLproducing <i>E. coli</i> (ST12, ST117, ST167, ST648) and observed clonal relatedness between a Mongolian avian ESBL-<i>E. coli</i> (ST167) and a clinical isolate of the same ST that originated in a hospitalised patient in Europe.</p>	(Guenther et al., 2012)
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ampicillin cefazolin chloramphenicol oxytetracycline minocycline kanamycin gentamicin nalidixic acid orbifloxacin acid enrofloxacin colistin fosfomycin	<i>E. coli</i> (n=138)	Culture method: fecal samples (n=192) of wild cranes that migrated for wintering to the Izumi plain, Kagoshima prefecture in Japan Susceptibility test: Agar dilution (CLSI), PCR detection of resistance genes Typing methods: Not performed	Wild Cranes (N=not stated). From the middle of October every year, more than ten thousand cranes migrate from Siberia and north China to the Izumi plain, Kagoshima prefecture in Japan for wintering	O	Japan	The numbers of isolates that were resistant to the antimicrobials used in this study are as follows: oxytetracycline, 22 isolates; minocycline, 7 isolates; ampicillin (ABPC), 4 isolates; nalidixic acid, 4 isolates; enrofloxacin, 2 isolates; kanamycin, one isolate. Multidrug resistant isolates exhibiting 2–4 drug resistances were obtained. All of the OTC-resistant isolates carried either the <i>tet(A)</i> or <i>tet(B)</i> gene. The <i>blaTEM</i> gene was found in all of the ABPC-resistant isolates.	(Kitadai et al., 2012)
ampicillin amoxicillin + clavulanic acid	<i>Enterococcus</i> spp. (n=31) <i>E. coli</i> (36)	Culture method: Fecal seamples from individually	Buzzards ( <i>Buteo buteo</i> ) (n=not stated)	C	Portugal	The <i>E. coli</i> and enterococci isolates showed high levels of resistance to streptomycin and tetracycline. The	(Radhouani et al., 2012)

cefoxitin cefotaxime ceftazidime aztreonam imipenem gentamicin amikacin tobramycin streptomycin  nalidixic acid ciprofloxacin sulfamethoxazole + trimethoprim tetracycline chloramphenicol  <i>Enterococcus</i> : vancomycin teicoplanin ampicillin streptomycin gentamicin kanamycin chloramphenicol		animals (n=42)  Susceptibility test:  Disc diffusion (CLSI)  Detection of resistance genes by PCR   Typing methods:  Not stated				following resistance genes were detected: blaTEM (20 of 22 ampicillin-resistant isolates), tet(A) and/or tet(B) (16 of 27 tetracycline- resistant isolates), aadA1 (eight of 27 streptomycin- resistant isolates), cmIA (three of 15 chloramphenicol- resistant isolates), aac(3)-II with/without aac(3)- IV (all seven gentamicin-resistant isolates) and sul1 and/or sul2 and/or sul3 [all eight sulfamethoxazole/tri methoprim-resistant (SXT) isolates]. intI1 and intI2 genes were detected in four SXT- resistant isolates. Most of the tetracycline-resistant strains carried the tet(M) and/or tet(L) genes. The erm(B) gene was detected in 80% of erythromycin- resistant isolates. The
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tetracycline erythromycin  quinupristin– dalfopristin ciprofloxacin						vat(D) and/or vat(E) genes were found in nine of the 17 quinupristin–dalfopristin-resistant isolates. The enterococcal isolates showing high-level resistance for kanamycin, gentamicin and streptomycin contained the aph(39)-IIIa, aac(69)-aph(20) and ant(6)-Ia genes, respectively.	
azithromycin ciprofloxacin erythromycin gentamicin  florfenicol  nalidixic acid telithromycin clindamycin	<i>Campylobacter</i>  (n=9)	Culture method:  Livestock farms Faecal swabs.  Selective culture,  Typing method:  Species specific PCR, MLST, PFGE, resistance	Wild small animals (n=142)  Birds  19 bird species (n=188)	O	USA	Campylobacter found only in wild birds. Of the 188 birds sampled. 9 positive isolates for Campylobacter (prevalence 4.8%) from 3 birds: originated from House sparrow, rose-breasted grosbeak, white-throated sparrow.  Two isolates from house sparrow resistant to tetracycline,	(Sippy et al., 2012)

		gene location, AR profile.  Susceptibility tests:  Microbroth dilution (CLSI), PCR of tet(O) and gyrA				ciprofloxacin and nalidixic acid. A plasmid location of the <i>tet(O)</i> gene was indicated in both cases. A single point mutation in the <i>gyrA</i> identified (fluoroquinolones resistance).	
ampicillin sulphamethazole- trimethoprim gentamicin  Streptomycin ciprofloxacin chloramphenicol tetracycline	<i>E. coli</i>  (n=114 from herring gulls)	Culture method:  Five fresh fecal samples,  17 cloacal swabs  10 wastewater samples  Susceptibility test:  Disc diffusion (CLSI),  Detection of resistance gene	Herring gulls ( <i>Larus argentatus</i> )  (number not stated, see culture number)  Waste water	O	USA	Higher proportion of AMR bacteria from wastewater (59.2%) compared with herring gulls feces (17.5%).  Similar resistance pattern and shared resistance genes suggest possible wastewater contamination of the local environment.	(Alroy and Ellis, 2011)

		Typing methods: Not stated					
ampicillin amoxicillin + clavulanic acid cefotaxime chloramphenicol sulphamethoxazole + trimethoprim nalidixic acid streptomycin tetracycline	27 bacterial species, 183 isolates belonging to Enterobacteriaceae  Predominated by <i>E. coli</i>	Culture method: faecal swabs (n=218) and the internal organs of 21 subjects (19 <i>E. rubecula</i> , one <i>S. atricapilla</i> and one <i>S. borin</i> ) found dead in the mist-nets were collected. Furthermore, 19 pooled fresh faecal samples were collected from the boxes used to temporarily house the birds and were submitted to mycological examination.  Each pooled sample	Migratory birds: <i>Passeriformes</i> ( <i>Erithacus rubecula</i> , <i>Turdus philomelos</i> , <i>Hirundo rustica</i> , <i>Fringilla coelebs</i> , <i>Monticola solitarius</i> , <i>Alauda arvensis</i> , <i>Carduelis chloris chloris</i> , <i>Sylvia atricapilla</i> , <i>Sylvia cantillans</i> , <i>Phoenicurus phoenicurus</i> , <i>Hippolais icterina</i> , <i>Saxicola rubetra</i> ,  <i>Phylloscopus trochilus</i> , <i>Anthus trivialis</i> , <i>Sylvia borin</i> , <i>Saxicola torquata</i> ,	O  O O O O O H O O O O O (granivore) O C (insectivore) O C (insectivore)	Italy	Almost all of the isolates were susceptible to sulphamethoxazole/trimethoprim (99.4%), cefotaxime (98.9%), nalidixic acid (96.7%), chloramphenicol (95.6%), and tetracycline (93.4%). Alternatively, many strains were resistant to ampicillin (42.6%), amoxicillin-clavulanic acid (42.6%), and streptomycin (43.7%).	(Foti et al., 2011)

		<p>contained one to three faecal samples, which were obtained from each of the 19 boxes.</p> <p>Susceptibility test:</p> <p>Disc diffusion</p> <p>(Not standard method stated)</p> <p>Typing methods:</p> <p>Not stated</p>	<p><i>Sylvia melanocephala</i>),</p> <p><i>Caprimulgiformes</i> (<i>Caprimulgus europaeus</i>).</p> <p>(n=not stated)</p>	<p>O</p> <p>C (insectivore)</p>			
<p>gentamicin</p> <p>kanamycin</p> <p>streptomycin</p> <p>ampicillin</p> <p>chloramphenicol</p> <p>ciprofloxacin</p> <p>erythromycin</p> <p>tetracycline</p> <p>vancomycin</p>	<p><i>Enterococcus</i> spp. isolates (n=1500) obtained from the feces of 48 humans, 209 domestic food animals, and <b>155 wild geese</b></p>	<p>Culture method:</p> <p>Fecal swab samples</p> <p>Susceptibility test:</p> <p>Three aminoglycoside and six non-aminoglycoside (CLSI)</p>	<p><b>Wild geese</b> (n=155)</p> <p>Humans (n=48)</p> <p>Domestic food animals (n=209)</p>	<p>O</p>	<p>Republic of Korea</p>	<p>The 180 <i>Enterococcus</i> isolates that showed high levels of resistance to aminoglycoside antibiotics (HLAR) were screened for virulence genes encoding for aggregation substance (agg), cytolysin activator (cylA), gelatinase (gelE) and surface</p>	<p>(Han et al., 2011)</p>

		<p>All isolates resistant to</p> <p>one or more of the three aminoglycoside antibiotics (GEN, KAN, and STR)</p> <p>were used for high-level aminoglycoside</p> <p>Typing methods:</p> <p>Not stated</p>				<p>protein (esp). Of those, the gelE gene was found most frequently in chickens and ducks of the HLAR isolates, while 56 <i>E. faecalis</i> and 13 <i>E. faecium</i> HLAR were gelatinase positive and showed hemolysin activity.</p>	
<p>tiamulin</p> <p>valnemulin</p> <p>doxycycline</p> <p>lincomycin</p> <p>tylosin</p> <p>ampicillin</p>	<p><i>Brachyspira</i> spp. (n=48)</p> <p>Presumed pathogens (<i>Brachyspira alvinipulli</i>, <i>Brachyspira intermedia</i>, <i>Brachyspira pilosicoli</i>), commensals (<i>Brachyspira murdochii</i>, <i>Brachyspira innocens</i>, "<i>Brachyspira pulli</i>"), and isolates of undetermined</p>	<p>Culture method:</p> <p>Susceptibility test:</p> <p>Broth dilution in VetMICTM Brachy panels (SVA)</p> <p>beta-Lactamase detection. (Nitrocefin disks)</p>	<p>Commercial laying hens (n=30)</p> <p>Free-living wild mallards (<i>Anas platyrhynchos</i>) (n=18).</p>	0	Sweden	<p>Five isolates showed decreased susceptibility to ampicillin (minimum inhibitory concentration</p> <p>16 to &gt;32 mg/ml), including two "<i>B. pulli</i>" and one <i>B. alvinipulli</i> from laying hens, and isolates of <i>B. pilosicoli</i> and "<i>B. pulli</i>" from mallards. Decreased susceptibility to ampicillin was associated with</p>	(Jansson and Pringle, 2011)



	species affiliation were included.	Typing method:  Amplification and sequencing of the b-lactamase and 23S rRNA genes.				b-lactamase activity in four isolates. A new variant of a class D b-lactamase gene designated bla $oxa$ -192 was identified in a <i>B. pilosicoli</i> isolate of mallard origin.	
vancomycin teicoplanin ampicillin  streptomycin gentamicin  kanamycin chloramphenicol tetracycline  erythromycin quinupristin-dalfopristin ciprofloxacin	<i>Enterococcus</i> spp. (n=54)	Culture method:  Fecal samples   Susceptibility test:  Disc diffusion (CLSI),   Typing methods: Detection of antimicrobial resistance genes by PCR	Seagulls ( <i>Larus cachinnans</i> ) (n=57)	O	Portugal	Almost 78% of the recovered enterococci showed resistance against one or more antibiotics and these isolates were identified to the species level. <i>E. faecium</i> was the most prevalent species (52.4%).	(Radhouani et al., 2011)
vancomycin teicoplanin ampicillin	<i>Enterococcus</i>  <i>E. coli</i>	Culture method:  Fecal samples, cloacal swabs (n=220) from	Nine different passerine species  (Number of birds not specied)	O  Most passerines are omnivores	Portugal	vanA-containing enterococcal isolates (four <i>E. faecium</i> and	(Silva et al., 2011)

<p>streptomycin gentamicin</p> <p>kanamycin chloramphenicol tetracycline</p> <p>erythromycin quinupristin- dalfopristin ciprofloxacin</p>	<p>(Total number of bacterial species not specified)</p>	<p>nine passerine species (n=178 samples) and two game bird species (n=42 samples)</p> <p>Susceptibility test:</p> <p>Disc diffusion (CLSI), Detection of ESBL-producing <i>E. coli</i> isolates (Levine-CTX plates)</p> <p>Typing methods:</p> <p>Disc diffusion, PCR detection of resistance genes</p>			<p>two <i>E. durans</i>) and vanC-1 <i>E. gallinarum</i> isolates were detected in six and seven faecal samples, respectively. VRE isolates showed ampicillin (n=11), ciprofloxacin (n=9), tetracycline (n=6), erythromycin (n=5), quinupristin/dalfopristin (n=3) and high-level kanamycin resistance (n=1). The <i>tet(L)</i> and/or <i>tet(M)</i> gene was found in all tetracycline-resistant isolates and the <i>erm(B)</i> gene in all erythromycin-resistant isolates. Three <i>vanA</i> containing <i>E. faecium</i> and two <i>E. gallinarum</i> presented specific sequences of the Tn5397 transposon. Four VRE isolates harboured the <i>ace</i> virulence gene. One faecal sample revealed one ESBL-containing <i>E. coli</i></p>	
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						isolate that belongs to the A phylogenetic group, showed a phenotype of resistance to $\beta$ -lactams and tetracycline, and harboured the bla <sub>CTX-M-14</sub> , bla <sub>SHV-12</sub> and the <i>tet(A)</i> genes.	
tetracycline ampicillin streptomycin chloramphenicol nalidixic acid cefadroxil sulfamethoxazole fosfomycin  tigecycline trimethoprim nitrofurantoin mecillinam	<i>E. coli</i>  (n=83)	Culture method:  faecal samples (n=100) by cloacal swabs from juvenile non-fledged black-headed gulls ( <i>L. ridibundus</i> ). Sampling was performed in one colony of about 500 nests, which was situated on a small island very close (about 50 m)  Susceptibility test:	Black-headed gull ( <i>Larus ridibundus</i> )  (n=not stated)  Human	O	Sweden	Despite a low frequency of antibiotic resistance among the isolates from gulls, ESBL-producing <i>E. coli</i> isolates were found, two with bla <sub>CTX-M-14</sub> and one with bla <sub>CTX-M-15</sub> . The same CTX-M types were dominant among human ESBL isolates. In addition, gull isolates were dispersed among the human samples in the PhenePlate <sup>TM</sup> clustering system, indicating that they neither differ from the human isolates nor form any	(Bonnedahl et al., 2010)

		disc diffusion on Iso-Sensitest agar (Swedish Reference Group for Antibiotics)  Typing methods:  PCR-based replicon typing, MLST				separate clonal clustering.	
ampicillin  streptomycin spectinomycin chloramphenicol gentamicin  tetracycline	<i>E. coli</i>  201 putative <i>E. coli</i> isolates cultured from the faeces (n=160), heart (n=6), liver (n=9), lung (n=13), spleen (n=6) and kidney (n=7).  On the avian host species level we isolated <i>E. coli</i> from 40 of 55 avian species tested.	Culture method:  Faecal <i>E. coli</i> via cloacal swabs taken while ringing the birds in the rural Eichsfeld region. Furthermore cloacal swabs and in case of dissection of animals organ samples were taken during entrance	European wild bird species  (50 different species)  (n=226)	Because of high number of species in this study we assume there are a combination of O; C, H, probably dominated by O.	Germany	Nine of the 187 <i>E. coli</i> isolates (4.8%) exhibited multiresistant phenotypes including resistances against beta-lactams, aminoglycosides, fluoroquinolones, tetracyclines and sulfonamides. By comparing avian <i>E. coli</i> resistance frequencies with frequencies known for <i>E. coli</i> isolated from livestock and companion animals analogous profiles	(Guenther et al., 2010c)

		<p>evaluation in a rescue station</p> <p>for injured wild birds in a veterinary hospital.</p> <p>(275 samples from 226 birds).</p> <p>Susceptibility test:</p> <p>Disc diffusion (CLSI),</p> <p>Typing methods:</p> <p>RAPD-PCR,</p> <p>PCR detection of resistance genes</p>				<p>were identified. Multiresistant <i>E. coli</i> strains were isolated from synanthropic avian species as well as from birds of prey, waterfowl and passerines.</p>	
<p>ampicillin</p> <p>cefadroxil</p> <p>meropenem</p> <p>gentamicin</p> <p>tobramycin</p> <p>streptomycin</p> <p>tetracycline</p> <p>ciprofloxacin</p>	<p><i>E. coli</i></p> <p>(n=145)</p>	<p>Culture method:</p> <p>532 fresh faecal, or cloacal, samples</p> <p>Susceptibility test:</p>	<p>Glaucous-winged gull</p> <p>(n=not stated)</p>	<p>O</p>	<p>Russia, Commander Islands and Kamchatka</p> <p>(Study was performed in Sweden)</p>	<p>Despite overall low resistance levels in randomly selected <i>E. coli</i> (one from each sample), we found multi-resistant ESBL-producing <i>E.</i></p>	<p>(Hernandez et al., 2010)</p>

<p>sulfamethoxazole + trimethoprim trimethoprim chloramphenicol</p> <p>fosfomycin nitrofurantoin</p>		<p>ESBL production was confirmed</p> <p>by disc diffusion synergy test according to Swedish Reference Group for Antibiotics (SRGA)</p> <p>and ESBL-producing isolates analysed by PCR for the presence of CTX-M, TEM and SHV type ESBL enzyme genes (<i>bla</i>CTX-M, <i>bla</i>TEM and <i>bla</i>SHV)</p> <p>Typing methods:</p> <p>MLST, PCR detection of resistance genes</p>				<p><i>coli</i> harbouring <i>bla</i>CTX-M-14 and <i>bla</i>CTX-M-15 using selective screening. Among these multi-resistant ESBLproducing <i>E. coli</i> we found one <i>bla</i>CTX-M-15 harbouring strain belonging to the O25b-ST131 clone, recognized for its clonal disseminated worldwide as a human pathogen.</p>	
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Amoxicillin + clavulanic acid ampicillin cephalothin ceftazidime chloramphenicol ciprofloxacin gentamicin nalidixic acid streptomycin sulfamethoxazole- trimethoprim sulfonamide compounds tetracycline	<i>E. coli</i>  (n= mallards 65+ Herring gulls 27+ Water birds 365)	Culture method:  Fecal samples   Susceptibility test:  Disc diffusion (CLSI)   Typing methods:  PCR detection of resistance genes  PFGE	Mallards ( <i>Anas platyrhynchos</i> ) (n=86)  Herring gulls ( <i>Larus argentatus</i> ) (n=27)  Waterbird (gulls, cormorants, and sometimes also ducks)  (n=244)	O  O  Cannot be specified.	Poland	65 isolates from mallards, 23 of these were resistant.  18 suspected <i>E. coli</i> isolates were detected in 27 samples from herring gulls. Three <i>E. coli</i> isolates were antibiotic resistant (11%)  From 365 waterbird feces, 58 suspected <i>E. coli</i> isolates were obtained by nonselective cultivation. Antibiotic resistance was detected 1.4% (5/365).	(Literak et al., 2010a)
Ampicillin Cefaclor Ciprofloxacin Chloramphenicol Nalidixic acid Amikacin Amoxicillin Cefepim	<i>S. enterica</i> serovar Enteritidis	Culture method:  Fecal sample   Susceptibility test:  Done, but method and reference not stated.	Blue-fronted Amazon parrot ( <i>Amazona aestiva</i> ), Capativit/freely in the wild (n=103)	O	Brazil	All examined <i>Salmonella</i> isolates were susceptible to Ampicillin, cefaclor Ciprofloxacin, chloramphenicol. Three isolated demonstrated to be MDR (resistant against more than 4 antimicrobial agents).	(Marietto-Goncalves et al., 2010)

Josamycin Lincomycin Neomycin Perfloxacin Sulfonamide Ceftiofur Cotrimoxazol Enerofloxacin Streptomycin Gentamicin Tetracycline Trimethopime Vancomycin		PCR-detection of class 1 integron gene  Typing methods:  Serological test (agglutination)					
Vancomycin Teicoplanin Ampicillin Streptomycin Gentamicin Kanamycin Chloramphenicol Tetracycline Erythromycin	<i>Enterococcus:</i> <i>E. faecium</i> was the most prevalent species in seagulls (50%), followed by <i>E. faecalis</i> and <i>E. durans</i> (10.4%), and <i>E. hirae</i> (6.3%).	Culture method:  Fecal samples recovered in the soil along the entire Berlengas Island (n=54)  Susceptibility test:	Sea gulls ( <i>Larus cachinnans</i> ) (n=57)	O	Portugal	VanA-containing enterococcal strains were detected in 10.5% of the 57 seagull faecal samples studied. Four of the vanA-containing enterococci were identified as <i>E. faecium</i> and two as <i>E. durans</i> . The	(Radhouani et al., 2010)



<p>Quinupristin-dalfopristin ciprofloxacin</p>		<p>Disk diffusion (CLSI),</p> <p>Detection of antimicrobial resistance genes by PCR</p> <p>Typing methods:</p> <p>MLST, The whole-cell proteomic profile of vanA-containing <i>Enterococcus</i> strains was applied</p> <p>to evaluate the discriminatory power of this technique for their identification.</p> <p>Protein identification by MALDI-TOF/TOF,</p> <p>One and two-dimensional</p>				<p>tet(M) gene was found in all five tetracycline-resistant vanA strains. The erm(B) gene was demonstrated in all six</p> <p>erythromycin-resistant vanA strains. The hyl virulence gene was detected in all four vanA-containing <i>E. faecium</i></p> <p>isolates in this study, and two of them harboured the purK1 allele. In addition these strains also showed ampicillin and ciprofloxacin resistance.</p>	
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		electrophoresis, and proteomics,  Sequence alignments and construction of the phylogenetic tree					
Tetracycline Ampicillin Streptomycin Chloramphenicol Nalidixic acid Cefadroxil	<i>E. coli</i> (n=153)  ESBL producing bacteria were initially identified by phenotypic tests. 17 isolates (16 <i>E. coli</i> and one <i>Enterobacter cloacae</i> , all from separate samples)	Culture method:  90 fecal samples per colony were collected from juvenile non-fledged birds.  Susceptibility test:  Disc diffusion (the Swedish Reference  Group for Antibiotics (SRGA)	Human  Yellow-Legged Gulls (n=not stated)	O	France	Nearly half the isolates (47,1%) carried resistance to one or more antibiotics (in a panel of six antibiotics), and resistance to tetracycline, ampicillin and streptomycin was most widespread. In an ESBL selective screen, 9,4% of the gulls carried ESBL producing bacteria and notably, 6% of the gulls carried bacteria harboring CTX-M-1 group of ESBL enzymes, a recently introduced and yet the most	(Bonnedahl et al., 2009)

		<p>Typing methods:</p> <p>For detection of ESBL producing bacteria, samples were plated onto on MacConkey agar (Oxoid, Basingstoke, UK) with two antibiotic discs containing cefotaxime (5 mg) and ceftazidime (10 mg), respectively.</p> <p>MLST</p>				<p>common clinical CTX-M group in France.</p> <p>MLST and phylogenetic group designations were established for the ESBL isolates, revealing that birds and humans share <i>E. coli</i> populations.</p>	
<p>amoxicillin</p> <p>ampicillin</p> <p>ticarcillin</p> <p>sulfonamides potentiated-sulfonamides</p> <p>tetracyclines</p> <p>streptomycin</p>	<p><i>E. coli</i></p> <p>(n=142)</p>	<p>Culture method:</p> <p>Fecal samples (n=175)</p> <p>Susceptibility test:</p>	<p>Mallard ducks (n=not stated)</p>	<p>O</p>	<p>Germany</p>	<p>High MIC values against amoxicillin, ampicillin, ticarcillin, sulfonamides, potentiated sulfonamides, tetracyclines and streptomycin. Neither ESBL production nor the presence of the respective genes was</p>	<p>(Ewers et al., 2009)</p>

		Agar diffusion (CLSI)  Typing methods:  Virulence genotyping  MLST, PFGE				confirmed. Although this is just a single finding the potential of wild birds to present a reservoir of multi-resistant strains seems to be of high relevance and should be carefully kept in mind.	
<i>E. coli</i> and <i>Salmonella</i> :  Each plate tested the susceptibility of the strain to 15 antimicrobials:  amikacin  amoxicillin + clavulanic acid  cefoxitin  ceftiofur  ceftriaxone ciprofloxacin  nalidixic acid gentamicin trimethoprim +	<i>E. coli</i> (n=206), <i>Salmonella</i> , <i>M. avium</i> subsp. <i>Paratuberculosis</i> (n=0)	Culture method:  During winter 2008, 200 gut-content swabs were also collected (n=434)  Susceptibility test:  Sisc diffusion  (National Antimicrobial Resistance Monitoring System (NARMS).	Wild European Starlings (n=not specified)	0	USA	Findings in this study suggest that starlings are not a significant source of <i>Salmonella</i> spp., <i>M. avium</i> subsp. <i>paratuberculosis</i> , <i>E. coli</i> O157, or other shiga toxin-producing <i>E. coli</i> in the feedlot assessed. However, they may have the potential to spread pathogenic <i>E. coli</i> (APEC), an important pathogen of poultry and a potential pathogen to human beings. Notably, some isolates displayed antimicrobial resistance.	(Gaukler et al., 2009)

<p> sulphamethoxazole  streptomycin  kanamycin  chloramphenicol  sulfizoxazole  ampicillin  tetracycline </p>		<p> Typing  methods: PCR </p>					
<p> vancomycin  amikacin  cephalosporins  spectinomycin  sulphadimethoxime  chloramphenicol  gentamicin  enrofloxacin  erythromycin  ticarcillin </p>	<p> <i>Enterococcus</i> spp.  (n=54) </p>	<p> Culture  method:  Fecal samples  were obtained  for culture  from the  cloaca with  the use of  culture swabs.    Susceptibility  test:  Microwell  broth dilution  panels,  Vancomycin  MIC values (E-  test)  (CLSI)  PCR-detection  of resistance  genes (vanA, </p>	<p> Free-living and captive  raptors:    Twenty-five birds were  enrolled in this study:  21 free-living raptors  including and 4 captive  raptors.    The rapotors include  different species. </p>	C	USA	<p> The <i>Enterococcus</i>  showed almost  complete    resistance to  amikacin, first-  generation  cephalosporins,  spectinomycin, and  sulphadimethoxime.    Isolates  demonstrated  variable resistance to  chloramphenicol,  gentamicin,  enrofloxacin,  erythromycin, and  ticarcillin. No  phenotypically  vancomycin-resistant  <i>E. faecalis</i> isolates  were recovered from  any of the raptors;  three isolates had  intermediate level </p>	(Marrow et al., 2009)

		vanB, vanC-1, and vanC-2/3)  Typing methods: PCR, ribotyping				susceptibility. A significantly higher number of isolates collected from captive birds demonstrated resistance to chloramphenicol than those obtained from free-living birds.	
ampicillin amoxicillin-clavulanic acid cefotaxime cefoxitin ceftazidime imipenem aztreonam gentamicin tobramycin amikacin streptomycin tetracyclin trimethoprim-sulfamethoxazole nalidixic acid	<i>E. coli</i>  1 isolate from each sample (n=53)	Culture method:  Fecal samples (n=53)  Susceptibility test:  Disc diffusion (CLSI),  PCR-detection of resistance genes  Typing methods:  Not stated	Seagulls ( <i>Larus cachinnans</i> )  (n=not stated)	O	Portugal	The percentages of resistant isolates for each of the drugs were ampicillin (43·4 per cent), tetracycline (39·6 per cent), nalidixic acid (34·0%), streptomycin (32·1%), trimethoprim-sulfamethoxazole (26·4%), ciprofloxacin (18·9%), chloramphenicol (18·9%), gentamicin (7·5%), tobramycin (7·5%) amikacin (5·7%) and amoxicillin-clavulanic acid (1·9 per cent). All the isolates were	(Radhouani et al., 2009)

ciprofloxacin chloramphenicol						susceptible to cefotaxime, ceftazidime, cefotaxime, aztreonam and imipenem	
Ampicillin Amoxiclav Piperacillin/Tazobactam Cephalothin Cefuroxime Ceftazidime Cefotaxime Aztreonam Imipenem Nalidixic acid Ciprofloxacin Streptomycin Tetracycline Chloramphenicol Trimethoprim Sulphamethoxazole	<i>Edwardsiella</i> spp. (n=39)	Culture method:  Fecal samples (n=49)  Susceptibility test:  Agar dilution (CLSI)  Typing methods:  16srRNA	Antarctic gentoo penguins:  <i>Pygoscelis papua</i> (n=49)  Birds were caught with a hand-net, and samples were taken from their cloacae with sterile cotton swabs.	C	Neko Harbour on the Antarctic Peninsula  (64°50-S, 062°33-W).  The study was performed in Sweden.	Of the 42 <i>Enterobacteriaceae</i> isolates found, 39 belonged to the genus <i>Edwardsiella</i> . All isolates were susceptible to the 17 antibiotics tested.	(Bonnedahl et al., 2008)
ampicillin amoxicillin +	<i>E. coli</i> (n=112)	Culture method:	Wild animals (n=72):	Among these animal species we	Different Natural Parks of the	The following percentages of resistance were	(Costa et al., 2008)

<p>clavulanic acid (AMC) cefotaxime</p> <p>cefoxitin</p> <p>ceftazidime</p> <p>imipenem</p> <p>aztreonam</p> <p>gentamicin</p> <p>tobramycin</p> <p>amikacin</p> <p>streptomycin</p> <p>tetracycline</p> <p>trimethoprim-sulfamethoxazole</p> <p>nalidixic acid</p> <p>ciprofloxacin</p> <p>chloramphenicol</p>		<p>Fecal sample (72)</p> <p>Susceptibility test:</p> <p>Agar disk diffusion (CLSI).</p> <p>Screening test for detection of ESBLs was carried out by the double disk diffusion test (using cefotaxime, ceftazidime, and AMC disks) in those isolates that showed resistance or intermediate susceptibility to broad-spectrum cephalosporins (cefotaxime and/or ceftazidime).</p>	<p>14 birds of prey,</p> <p>10 owls,</p> <p>7 foxes,</p> <p>6 wild rabbits,</p> <p>5 genets,</p> <p>4 forest wildcats,</p> <p>3 storks,</p> <p>3 deer,</p> <p>3 otters,</p> <p>2 wolves,</p> <p>2 mouflons,</p> <p>1 badger,</p> <p>1 partridge,</p> <p>1 hedgehog,</p> <p>1 pigeon,</p> <p>1 ferret,</p> <p>1 quail,</p> <p>1 wild boar,</p> <p>1 salamander,</p> <p>1 snake,</p> <p>1 winter wren,</p>	<p>find both O, C , and H.</p> <p>Since it is not specied which resistance the authors found in which animal species, we do not specify the diet of these animal species.</p>	<p>north and center of Portugal</p>	<p>obtained: tetracycline, streptomycin, ampicillin, and trimethoprim-sulfamethoxazole (range 19–35%); nalidixic acid (14%); ciprofloxacin (9%); amoxicillin-clavulanic acid, gentamicin, tobramycin, and chloramphenicol (range 4.5–7%); cefotaxime, and aztreonam(1.8%); ceftazidime (0.9%); and amikacin, cefoxitin, and imipenem (0%). A blaTEM gene was found in 22 of the 25 ampicillin-resistant isolates, and the gene encoding CTX-M-14 b-lactamase was identified in the two cefotaxime-resistant isolates (recovered from a common kestrel and a sparrowhawk), associated with</p>	
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		Typing methods: Not stated	1 jay, 1 magpie,1 Mediterranean turtle			blaTEM-52 gene in one of them.	
ampicillin nalidixic acid streptomycin kanamycin gentamicin chloramphenicol tetracycline amoxicillin/ clavulanic acid ceftiofur sulfoxazole amikacin cephalothin	<i>Enterobacteriaceae:</i> <i>E. coli</i> (n=2)  <i>Pantoea agglomerans</i> (n=31)	Culture method: Fecal samples (n=33)  Susceptibility test: Disk diffusion (CLSI)  Typing methods: PCR- detection of resistance genes	Yellow-headed blackbirds (YHB) ( <i>Xanthocephalus xanthocephalus</i> )  (n= two adult males, four adult females, 12 fledgling male chicks, and three fledgling female chicks)	0	USA  North Dakota	Collectively, 12 of the 33 isolates (36%) exhibited  no resistance to any antimicrobial tested. However, several multidrug-resistant isolates of varying genera were identified. Among the antimicrobial resistances observed, the most common was to ampicillin (60%), followed by cephalothin (33%).	(Gibbs et al., 2007)
Ampicillin amoxicillin- clavulanic acid cefalothin ceftazidime chloramphenicol	<i>Salmonella</i> spp. (n=207)	Culture method: Cloacal swabs were collected from young, not flying, black-headed gulls captured	Black-headed gulls ( <i>Larus ridibundus</i> ) (n=1095) from 1984-2005	0	Czech Republic	Although 95% of <i>Salmonella</i> isolates (197 out of 207) were pansusceptible, the prevalences of resistance increased significantly from 1 (2%) out of 59	(Cizek et al., 2007)

streptomycin gentamicin  tetracycline sulfonamide  sulfamethoxazole + trimethoprim  nalidixic acid ciprofloxacin		in six colonies in the Czech Republic.  Susceptibility test: Disk diffusion (CLSI)  Typing methods:  Serotyping  Phage typing				isolates in 1984–1986 and 3 (3%) out of 100 isolates in 1991– 1994 to 6 (13%) out of 48 isolates in 2005. Furthermore, in 2005, two isolates were nalidixic  acid-resistant and one isolate was multidrug-resistant  <i>S. Typhimurium</i> DT 104.	
nalidixic acid ciprofloxacin erythromycin  ampicillin	<i>C. lari</i>  (n=109) from 49 of 2641 samples, mostly from samples in which  two or three of the selected colonies were <i>C. lari</i> .	Culture method:  Fecal samples, water  Susceptibility test:  the agar incorporation (doubling dilution) (CLSI)	Cattle,  Wildlife,  Water  (n=not specified)	Wildlife birds were not stated.	UK	All isolates were resistant to nalidixic acid and ciprofloxacin. Resistance to erythromycin and ampicillin was uncommon, but was observed in isolates from wild birds, cattle, wild mammals and water  samples. The presence of the same cMRP in multiple hosts provides further evidence of	(Leatherbarrow et al., 2007)

		Typing methods: PFGE				transmission between livestock, wildlife and the environment, or for a common source of infection.	
Genes	Enterococci (n=not stated)  <i>E. coli</i> (n=not stated)	Culture method: Water samples and fecal swabs were collected.  Typing method: PCR  Susceptibility test: Antibiotic resistance analysis; ARA (SAS software version 8; SAS Institute), PCR	Rabbits (n=971)  Dogs (n=872)  Birds (n=922)  Cats (n=48)  Unknown animal (n=224)		USA	The study suggests that bird and wild animal feces, soil amendments, and/or fecal coliform growth in the storm drain are the major contributors to the fecal bacterial pollution in downstream areas.	(Jiang et al., 2007)
Ampicillin Penicillin G Gentamicin Ciprofloxacin	<i>Enterococcus</i> spp.  <i>E. coli</i>	Culture method: Fresh fceces	Migratory Canada geese ( <i>Branta Canadensis</i> ) (n=not stated)	0	USA	>95% of <i>E. coli</i> isolates were resistant to penicillin G, ampicillin, cephalothin, and sulfathiazole; no <i>E.</i>	(Middleton and Ambrose, 2005b)

Cephalothin Sulfathiazole Streptomycin Sulfathiazole Tetracycline Chlorotetracycline	(n=not specified)	Susceptibility test:  Done., but the method has not stated.  Typing methods:				<i>coli</i> were resistant to ciprofloxacin.  Enterococci: showed highest resistance to cephalothin, streptomycin, and sulfathiazole;  no enterococci were resistant to chloramphenicol.  Multiple antibiotic resistance (MAR) profiles were also detected among both bacterial species.	
Vancomycin Teicoplanin Ampicillin Streptomycin Gentamicin, Kanamycin Chloramphenicol Tetracycline Erythromycin Quinupristin-dalfopristin Ciprofloxacin	<i>E. faecium</i> (n=45) <i>E. faecalis</i> (n=73) <i>E. hirae</i> (n=14) <i>E. casseliflavus</i> (n=4) <i>E. gallinarum</i> (n=2) <i>Enterococcus</i> spp. (n=2)	Culture Method: Faecal samples were obtained from animals and cultured. Colonies with typical enterococcal morphology were identified by cultural characteristics and	<b>77 wild animals</b> (14 birds of prey, 10 owls, seven foxes, six wild rabbits, five European genet, four forest wildcats, four salamanders, three storks, three magpies, three deer, three vipers, three otters, two wolves, two mouflon, two badgers, one partridge, one hedgehog, one pigeon, one ferret, one quail and one wild boar)	O, H, C	Portugal	The study reported that 44 of the 140 isolates (31.4%) showed susceptibility to all the antibiotics tested (5.5% of <i>E. faecalis</i> , 62.2% of <i>E. faecium</i> , and 78.6% of <i>E. hirae</i> ). Neither ampicillin-resistance nor acquired vancomycin-resistance was detected. Notably, 1.4% of the isolates showed high-level-resistance for	(Poeta et al., 2005)

		<p>biochemical tests (API ID20 Strep system, BioMe´rieux).</p> <p>Typing Method: PCR</p> <p>Susceptibility test: Disk diffusion method (NCCLS), PCR (antibiotic resistance genes + virulence factors)</p>				<p>gentamicin or streptomycin. Tetracycline and erythromycin resistances were shown in 28.6% and 20.1% of the isolates, respectively. A wide variety of virulence genes were detected in most of <i>E. faecalis</i> isolates but were rarely found in <i>E. faecium</i> and not detected in the other species.</p>	
<p>amikacin amoxicillin + clavulanic acid ampicillin apramycin cefoxitin ceftiofur ceftriaxone cephalothin chloramphenicol ciprofloxacin gentamicin imipenem kanamycin nalidixic acid streptomycin sulfamethoxazole</p>	<p><i>E. coli</i> (n=not stated)</p>	<p>Culture method: Cloacal swabs from 24 birds and guano from 7-8 birds</p> <p>Susceptibility test: Disk diffusion (CLSI)</p> <p>Typing methods: Not done.</p>	<p>Canada geese (n=24)  Surface water</p>	O	USA	<p>The proportion of isolates resistant to antimicrobial agents was significantly greater (<math>p = 0.0004</math>) among <i>E. coli</i> isolates from Craven County geese, where interaction with swine waste lagoons was observed. Antimicrobial resistance patterns in this population matched those most commonly reported for swine <i>Enterobacteriaceae</i></p>	(Cole et al., 2005)

tetracycline trimethoprim + sulfamethoxazole						from the National Antimicrobial Resistance Monitoring System (NARMS) studies (e.g., tetracycline, streptomycin, sulfamethoxazole, and ampicillin resistance). Most <i>E. coli</i> isolates (72%) recovered from Craven County geese exhibited resistance to >1 antimicrobial agent. In contrast, resistant <i>E. coli</i> recovered from agricultural geese in Georgia (Griffin) with no apparent contact with livestock wastes had a lower proportion of resistance (19%) and only exhibited resistance to $\beta$ -lactam antimicrobial agents (cefoxitin-amoxicillin/clavulanic acid-cephalothin).	
<b>Aminoglycosides</b> Neomycin Gentamicin Streptomycin <b>Phinicals</b> Chloramphenicol	<i>E. coli</i> (n=1286)	Culture method: Fecal samples from animals examined in this study.	Fecal samples were obtained from dairy and beef cattle, swine, horses, sheep, goats, chickens, cats, dogs, <b>deer, ducks, and</b>	0	USA	Resistance to at least one antimicrobial agent was demonstrated in isolates from	(Sayah et al., 2005)

<p><b>Quinolones and fluoroquinolones</b> Ofloxacin Nalidixic acid <b>Sulfonamides and potentiated sulfonamides</b> Sulfamethoxazole-trimethoprim Sulfisoxazole <b>Tetracyclines</b> Tetracycline <b>Beta-lactams</b> Ampicillin <b>Nitrofurans</b> Nitrofurantion <b>Cephalosporins</b> Cephalothin</p>		<p>Susceptibility test: Disk diffusion (CLSI)</p> <p>Typing methods:</p>	<p><b>geese</b> (n=not specified).</p> <p>Samples from environment, water</p>			<p>livestock, companion animals, human septage, wildlife, and surface water. In general, <i>E. coli</i> isolates from domestic species showed resistance to the largest number of antimicrobial agents compared to isolates from human septage, wildlife, and surface water.</p> <p>Most MDR isolates were from pigs.</p>	
<p>amikacin augmentin ampicillin carbenicillin ceftazidime ceftiofur cephalothin chloramphenicol ciprofloxacin enrofloxacin gentamicin piperacillin tetracycline ticarcillin tobramycin tribrissen</p>	<p><i>E. coli</i>, <i>Enterobacter cloacae</i>, <i>Citrobacter freundii</i>, and <i>Klebsiella pneumoniae</i> (n=25)</p>	<p>Culture method: Cloacal swabs were obtained by inserting a sterile Cultureswab</p> <p>Susceptibility test: automated system (VITEK) (CLSI)</p> <p>Typing methods: Not stated.</p>	<p>Seabirds: common murre (<i>Uria aalge</i>) (n=24), Gulls (<i>Larus</i> spp.) (n=49), other seabirds (n=14)</p>	<p>C</p> <p>O</p> <p>Not specifies species</p>	<p>USA rehabilitators in California and Washington</p>	<p>At least 25 bacterial species were identified, including multiple strains of <i>E. coli</i>, as well as <i>Enterobacter cloacae</i>, <i>Citrobacter freundii</i>, and <i>Klebsiella pneumoniae</i>.</p> <p>Antibiotic resistance was found in 13 of 19 bacterial isolates tested, including <i>E. coli</i>, <i>K. pneumoniae</i>, <i>Acinetobacter baumannii</i>, and <i>Pseudomonas</i></p>	<p>(Steele et al., 2005)</p>

						<i>aeruginosa</i> . Among the isolates tested, <i>Ps. aeruginosa</i> was resistant to the most antibiotics.	
Amoxicillin Chloramphenicol Ciprofloxacin Doxycycline Erythromycin Gentamicin Metronidazole Nalidixic acid Neomycin Streptomycin	<i>C. jejuni</i> (n=139)	Culture method: Fecal samples  Susceptibility test: Microdilution (CLSI)  Typing methods: Not stated.	Thrushes (n=94) Raptors (n=9) Shorebirds (n=36) Each group included several species.	O C O	Sweden	Observed MICs were generally low, with only low to moderate incidence of resistance to the tested compounds. One isolate, however, was resistant to nalidixic acid and ciprofloxacin, indicating that quinolone-resistant genotypes of <i>C. jejuni</i> have the potential to spread to wild bird hosts.	(Waldenstrom et al., 2005)
tetracycline chloramphenicol ampicillin kanamycin streptomycin	Gram-negative bacteria (n=191 from 19 birds)	Culture method: Cloacal swabs (n=30)  Susceptibility test: Agar diffusion (CLSI)  Typing methods: Not stated.	Wild birds (n=30 were examined) (16 species): Family: <b>Bucconidae</b> Rusty-breasted Nunlet  <b>Picidae</b> White-barred Piculet  <b>Dendrocolaptidae</b> Black-billed Scythebill  <b>Furnariidae</b> Ochre-cheeked Spinetail	O C C (insectivore) O	Brazil	At Salto da Divisa 97% of the isolates exhibited a resistant phenotype, and resistance to more than one antibiotic was frequent (71%). At Jequitinhonha 36% of isolates were resistant, but 94% showed resistance to only one antibiotic. Of the five antibiotics tested, resistance to ampicillin	(Nascimento et al., 2003)



			White-collared Foliage-Gleaner Pale-legged Hornero  <b>Formicariidae</b> Slaty Antshrike  Scalloped Antbird  White-shouldered Fire-Eye Slender Antbird  <b>Conopophagidae</b> Black-cheeked Gnatcatcher  <b>Tyrannidae</b> Ash-throated Casiornis Yellow-breasted Flycatcher  <b>Pipridae</b> White-bearded Manakin  <b>Muscicapidae</b> Creamy-bellied Thrush  <b>Emberizidae</b> Violaceous Euphonia	 O    O   O  O  O		was most frequent (in both areas), whereas kanamycin resistance was found in only one isolate.	
ampicillin chloramphenicol ciprofloxacin kanamycin streptomycin sulphamethoxazole	Enterobacteriaceae (n=61)	Culture method: Rectal swabs cultured.  Typing method:	Magpies ( <i>Pica pica</i> ) (n=37) Rabbits ( <i>Oryctolagus cuniculus</i> ) (n=13)	 O H	UK	All 61 Enterobacteria tested from 13 rabbits were susceptible to the tested antibiotics with the exception of one	(Livermore et al., 2001)

tetracycline trimethoprim		Resistant isolates further characterised by API20E strips.  Suceptibility tests: MICs determined on ISO-Sensitest agar according to the method of the British society for antimicrobial chemotherapy.  PCR of selected resistance genes.				isolate resistant to tetracycline. In contrast, 8 out of 20 magpies yielded antibiotic-resistant Enterobacteria with 25/97 isolates resistant to on or more antibiotic with tetracycline being most common.	
clindamycin chloramphenicol ciprofloxacin erythromycin oxacillin penicillin G rifampin tetracycline, trimethoprim- sulfamethoxazole vancomycin	<i>S. lentus</i> (n=57)	Culture method: Fecal samples  Susceptibility test: Disk diffusion (CLSI)  Typing methods: Not stated	wild turkeys ( <i>Meleagris gallopavo</i> ) (26)	0	USA	Only 3 isolates showed resistance to clindamycin, 3 isolates were resistant to oxacillin, 3 isolates were resistant to penicillin G, and 1 isolate was resistant to erythromycin. Multiple antibiotic resistance was also minimal.	(DeBoer et al., 2001)
Amicacin Penicillin G	Bacterial species (n=449)	Culture method:	Free-living waterbirds:		USA	Multi-drug resistance was observed among	(Fallacara et al., 2001)

Erythromycin Lincomycin Gentamicin Neomycin Vancomycin Trimethoprim Imipenem Ciprofloxacin Tobramycin Trimothoprim-sulfa Cefepim Bacitracin Netilimicin Cefazolin Cephatolin Piperacillin-tazobactam Piperacillin Ampicillin-sulbactam	<i>C. jejuni</i> (n=223) <i>S. java</i> (n=1) <i>E. coli</i> (n=331) <i>Pasturella multocida</i> (n=0)	Fecal samples  Susceptibility test: Disk diffusion (CLSI)  Typing methods: Not stated	Canada geese  Mallard ducks  (n=not stated)	O  O		several bacterial species against penicillin G, lincomycin, bacitracin, vancomycin, and erythromycin.	
Ampicillin Chloramphenicol Tetracycline Erythromycin Norofloxacin Nalidixic-acid Norfloxacin Ofloxacin	<i>Campylobacter</i> spp. <i>C. jejuni</i> (n=30) <i>C. coli</i> (n=20) <i>C. lari</i> (n=4)	Culture method: Fecal samples from sparrow Susceptibility test: Agar diffusion, PCR-detection of resistance genes  Typing method: Not stated	Sparrow (n= not specified)	O	Japan	3 (of 10) <i>C. jejuni</i> isolates were resistant against quinolone. Sparrow that contained quinolone resistant isolates seemed to have contact with industrial animals or their feed.	(Chuma et al., 2000)
fosfomycin tetracycline minocycline streptomycin	Shiga toxin-producing <i>E. coli</i> Two types isolates were identified.	Culture method: Fresh fecal samples (n=50)	Seagulls (n=1)	O	Japan	Phylogenic analysis of the deduced Stx amino acid sequences	(Makino et al., 2000)

chloramphenicol kanamycin nalidixic acid norxacin cefdinir sulphonamide spectinomycin streptomycin rifampicin trimethoprim	Both were identified as <i>E. coli</i> serotypes O136:H16 and O153:Hw and were designated K-7 and K-10	Susceptibility test: disk diffusion  Typing methods: PCR, Plasmid profiles PFGE <i>Stx</i> gene sequence Isolation of phage and its genetic Study				demonstrated that the <i>Stx</i> toxins of seagull-origin STEC were closely associated with those of the human-origin, but not those of other animal-origin STEC. K-7 was resistant to spectinomycin, sulphonamide, ampicillin and penicillin G, while K-10 was resistant to spectinomycin, sulphonamide, ampicillin, penicillin G and tetracycline.	
Amikacin Amoxicillin + clavulanic acid ampicillin ceftiofur cephalothin chloramphenicol ciprofloxacin gentamicin kanamycin nalidixic acid streptomycin sulfamethoxazole tetracycline trimethoprim + sulfamethoxazole	<i>Salmonella</i> (n=22)	Culture method: Gross and histologic lesions  Susceptibility test: Microdilution (CLSI)  Typing methods: RAPD, PFGE, Detection of antimicrobial resistance gene by PCR	Nondomestic birds:  Passerine birds (n=7)  Gallinaceous birds (n=4)  Psittacine birds (n=4)  Other species: Owl (n=1)  Heron (n=1)  Emu (n=2)  Laughing gull (n=1)	O  O  O  C  C  O  O	Southeaster n USA	Despite the general susceptibility of these <i>Salmonella</i> isolates to most antimicrobial agents, antibiotic resistance-associated genes <i>int11</i> , <i>merA</i> , and <i>aadA1</i> were identified in a number of these isolates.	(Hudson et al., 2000)

			Pigeon (n=1) Hawk (n=1)	O O			
ampicillin chloramphenicol kanamycin neomycin streptomycin tetracycline	<i>E. coli</i> (n=202)	Culture method: Fecal samples  Susceptibility test: Agar diffusion (not standardised method from EUCAST/CLSI)  Typing methods: multilocus enzyme electrophoresis (MLEE), plasmid profile, Biotype analysis	Mamalian species (n=81) representing 39 families and 14 orders. Also a strain from a reptile and 10 from different families of birds collected in Mexico.	The species included all variants; H, C, and O.	Australia  Mexico	Strains from Mexican wild mammals were, on average, as resistant to antibiotics as strains from humans in cities. On average, the Australian strains presented a lower antibiotic resistance than the Mexican strains. Of the antibiotics tested, resistance to streptomycin was most frequent while resistance to chloramphenicol was rare	(Souza et al., 1999)
<i>Salmonella</i> isolates: sulphisoxazole streptomycin ciprofloxacin gentamicin ampicillin trimethoprim chloramphenicol tetracycline  <i>C. jejuni</i> isolates: erythromycin	Enteropathogenic bacteria:  <i>Salmonella</i> spp., (n=2)  <i>Campylobacter</i> spp. (n=3)  EHEC 0157H7 (n=0)	Culture method: Rectal swabs  Susceptibility test: Disk diffusion (Swedish Reference Group for Antibiotics.)	Gulls (n=50)  Passerines (n=101)	O  O	Sweden	Among the 50 gulls examined, we found 2 isolates of <i>S. typhimurium</i> with multiple antibiotic resistance. Three isolates of <i>C. jejuni</i> were found in the 101 stool samples from passerines. All of <i>C. jejuni</i> isolates were resistant to ampicillin	(Palmgren et al., 1997)

nalidixic acid norfloxacin ciprofloxacin chloramphenicol ampicillin tetracycline clindamycin		Typing methods: Not stated				and one isolate was resistant to nalidixic acid.  We did not isolate EHEC 0152H7 in any of the bird stools examined.	
Ampicillin tetracycline erythromycin gentamicin streptomycin and kanamycin	<i>C. jejuni</i> and <i>C. coli</i> (n=254 isolates)	Culture method: Rectal swabs and stool specimens  Susceptibility test: Disk diffusion (Not standard method from CLSI or EUCAST)  Typing methods: Biotype and plasmid profiles	wild and domestic animals  The only wild animal was sparrow: These organisms were isolated from: 59 chicken (60.2%), 65 swine (59.1%), 31 black rats (57.4%), <b>61 sparrows (45.5%)</b> , 21 ducks (40.5%), 32 cows (19.5%) and 27 sheep (15.3%).	0	Portugal	Antimicrobial susceptibility testing revealed that <b>5.5%</b> of the strains were resistant to ampicillin, <b>5.5%</b> to tetracycline, <b>12.6%</b> to erythromycin and <b>23.5%</b> to streptomycin. Resistance to erythromycin ( <b>26.2%</b> ) and to streptomycin ( <b>58.4%</b> ) was particularly high in isolates from swine. No information regarding resistance in <i>Campylobacter</i> isolated from sparrow.	(Cabrita et al., 1992)

### 11.1.3 - Table 3. Antimicrobial resistance in water-living animals and fishes (vertebrates and invertebrate)

Antimicrobial agents	Bacterial species	Methodology for sampling and determination of resistance	Animal species	Omnivorous (O) Carnivorous (C) Herbivorous (H) Filter feeders (F)	Country	Results	Reference
Ampicillin Gentamicin Streptomycin Levofloxacin Erythromycin Quinupristin/dalfopristin Linezolid Teicoplanin Vancomycin Daptomycin Tetracycline	<i>E. faecalis</i> <i>E. faecium</i> <i>E. durans</i> <i>E. hirae</i> <i>E. casseliflavus</i> (n=46)	Broth micro-dilution (EUCAST) and PCR for resistance genes	<b>Venus clam</b> (n=37 batches), wild <i>Chamelea gallina</i>	F	Italy	13 (28.3%) were susceptible to all antibiotics, whereas 33 (71.7%) clam were resistant to at least one antibiotic.  All isolates were fully susceptible to linezolid, teicoplanin and vancomycin.  <i>E. faecium</i> and <i>E. hirae</i> possessed resistance to the largest number of drugs.  The resistance associations detected more frequently were AMP-STR-ERY and AMP-CN-LEV.  Genes detected: <i>bla</i> (Z), <i>tet</i> (M) and (L), <i>erm</i> (B), <i>aac</i> (6'')-Ie- <i>aph</i> (2'')-Ia and <i>ant</i> (6')-Ia	(Citterio et al., 2017)
Ampicillin Amoxicillin Amoxicillin/clavulanic acid Mecillinam Piperacillin/tazobactam Chloramphenicol	Enterobacteriaceae (n=199)	Susceptibility test: agar diffusion (EUCAST)  WGS of selected strains	N=549 bivalves examined, Blue mussels ( <i>Mytilus edulis</i> ) Oysters ( <i>Ostrea edulis</i> ) Scallops ( <i>Pecten maximus</i> )	F	Norway	38% of isolates showed resistance to at least one antibacterial agent, while multidrug-resistance were seen in 9 (5%) isolates. One isolate had resistance towards 15 agents.	(Grevskott et al., 2017)

<p>Ciprofloxacin Levofloxacin Nalidixicacid Norfloxacin Nitrofurantoin Gentamicin Tobramycin Streptomycin Kanamycin Trimethoprim Trimethoprim/sulf Amethoxazole Cefotaxime Ceftazidime Doxycycline Tetracycline Colistin sulfate Imipenem Meropenem</p>			<p>Horse mussels (<i>Modiolus modiolus</i>) Banded carpet shell (<i>Politapes rhombiodes</i>)</p>			<p>75 of 199 isolats had AMR. Of these resistance towards extended-spectrum penicillins (83%), aminoglycosides (16%), trimethoprim (13%), sulfonamides (11%), tetracyclines (8%), third-generation cephalosporins (7%), amphenicols (5%), nitrofurans (5%), and quinolones (5%), were observed.</p> <p>Genes extended-spectrum penicillins (<i>bla</i>TEM-1), third-generation cephalosporins(<i>bla</i>CTX-M-14, <i>bla</i>CTX-M-15), aminoglycosides [<i>strA</i>-<i>strB</i>, <i>aacA5</i>, <i>aac</i>(3)-IIId, <i>aph</i>(3)-Ia], trimethoprim (<i>dhfrA17</i>, <i>dhfrA5</i>, <i>dhfrA14</i>), sulfonamides (<i>su1</i>, <i>su2</i>), tetracyclines [<i>tet</i>(A), <i>tet</i>(B), <i>tet</i>(D)], amphenicols (<i>catA1</i>), quinolones (<i>qnrS1</i>), and macrolides(<i>mphA</i>).</p> <p><i>In vitro</i> conjugal transfer to <i>E. coli</i> shown for ampicillin, trimethoprim, sulfamethoxazole, tetracycline, cefotaxime and ceftazidime.</p>	
<p>Ampicillin Ciprofloxacin Chloramphenicol</p>	<p><i>E. faecalis</i> <i>E. faecium</i> (n=443)</p>	<p>Culture method: Fecal samples</p>	<p><b>Seabirds</b> (n=12):</p>	<p>C</p>	<p>Brazil</p>	<p>Among the 443 enterococci isolated, 62 distinct antimicrobial patterns were</p>	<p>(Prichula et al., 2016)</p>



<p>Erythromycin Gentamycin Nitrofurantoin Norfloxacin Streptomycin Tetracycline Rifampicin Vancomycin</p>		<p>Susceptibility test: agar duffuson (CLSI)</p> <p>PCR for detection of resistance genes</p>	<p><i>Spheniscus magellanicus</i> (n=10), <i>Sterna trudeaui</i> (n=1), <i>Himantopus melanurus</i> (n=1)</p> <p><b>Turtles</b> (n=8): <i>Eretmochelys imbricata</i> (n=6), <i>Chelonia mydas</i> (n=2)</p> <p><b>Mammals</b> (n=3): <i>Balaenoptera acutorostrata</i> (n=1), <i>Megaptera novaeangliae</i> (n=1), and <i>Grampus griseus</i> (n=1), found alive or dead in southern coastal Brazil.</p>	<p>O</p> <p>C</p> <p>O</p> <p>O</p> <p>C</p> <p>C</p> <p>C</p>		<p>observed. This classification was created for grouping strains with similar antimicrobial susceptibility and resistance patterns. Of the 62 observed patterns, 49 (79.03%) enterococcal strains profiles were resistant to one or more of the tested antibiotics. Single, double, and multiple antibiotic resistance patterns were observed in 37.09%, 25.80%, and 16.12% of the isolates, respectively. Resistance to rifampicin occurred most frequently.</p>	
<p>Ampicillin Gentamicin Ciprofloxacin Tetracycline Chloramphenicol Nalidixic acid Trimethoprim/sulfamethoxazole Streptomycin</p>	<p><i>E. coli</i>, (n=141)</p>	<p>Susceptibility test: agar duffuson (CLSI)</p> <p>ESBL confirmatory test (CLSI)</p>	<p><b>Venus clam</b> <i>Chamelea gallina</i> (n=77)</p>	<p>F</p>	<p>Italy, Adriatic coast</p>	<p>47 strains (33.3%) were resistant to at least one drug and 16 (11%) were MDR, i.e. resistant to 3 or more antibiotics. Resistance to tetracycline (25.5%) was the most frequent, followed by resistance to ampicillin (17%) and streptomycin</p>	<p>(Vignaroli et al., 2016)</p>

						(14%). Most (21/24) ampicillinresistant isolates produced ESBL.	
streptomycin-spectinomycin trimethoprim	<i>E. coli</i> (n=37)	Culture method: fecal samples (n=271)  Typing method: PCR (16S rRNA), sequencing  Susceptibility test: Virulence genes	Sea Lion (wild and captive)  (n=37)	C	Australia	Sequencing of gene cassette arrays identified genes conferring resistance to streptomycin-spectinomycin (aadA1) and trimethoprim (dfrA17, dfrB4). Class II integrases were not detected in the <i>E. coli</i> isolates. The frequent detection in captive sea lions of <i>E. coli</i> with resistance genes commonly identified in human clinical cases suggests that conditions experienced in captivity may contribute to establishment. Identification of antibiotic resistance in the microbiota of Australian sea lions provides crucial information for disease management. Our data will inform conservation management strategies and provide a mechanism to monitor microorganism dissemination to sensitive pinniped populations.	(Delpont et al., 2015)
Ampicillin Erythromycin Streptomycin Chloramphenicol Ciprofloxacin Nitrofurantoin	50 faecal isolates <i>E. faecalis</i> (n=20), <i>E. hirae</i> (n=20),	Susceptibility test: agar diffusion (CLSI)  PCR for selected	<b>Fur seal</b> (n=11): <i>Arctocephalus australis</i> (n=9) <i>Arctocephalus tropicali</i> (n=2)	C	Brazil	Thirty (60 %) of strains were resistant at least one antimicrobial. Resistance to erythromycin most commonly observed (26 %), followed by nitrofurantoin (14 %),	(Santestevan et al., 2015)

Norfloxacin Vancomycin Gentamycin Tetracycline	<i>E. casseliflavus</i> (n=6), <i>Enterococcus</i> spp. (n=4)	resistance gene				tetracycline (8 %), norfloxacin (6 %) and ciprofloxacin (6 %). No resistance to ampicillin, chloramphenicol, streptomycin, gentamycin, and vancomycin. Tetracycline- and erythromycin-resistant strains tested by PCR for <i>tet(M)</i> and <i>tet(L)</i> , and <i>erm(B)</i> respectively. Two of four tetracycline-resistant isolates harbored the <i>tet(M)</i> gene and none of them had <i>tet(L)</i> . Of 13 erythromycin-resistant strains, two (15.38 %) <i>E. casseliflavus</i> contained <i>erm(B)</i> gene.	
Ampicillin Florfenicol Tetracycline Nitrofurantoin Enrofloxacin Trimethoprim-Sulfamethoxazole Gentamicine Penicillin Erythromycin Oxolinic acid	<i>Streptococcus agalactiae</i> (n=2)	Suceptibility testing: disk diffusion (CLSI methods)	<b>Fish</b> (n=2) Gulf Killifish ( <i>Fundulus grandis</i> )	O	USA Louisiana	Resistant to oxolinic acid, enrofloxacin and gentamicine	(Soto et al., 2015)
Teicoplanin Cephalexin Penicillin Oxacillin	<i>V. parahaemolyticus</i> (n=6) <i>V. alginolyticus</i> (n=22)	Susceptibility test: agar duffuson according to	<b>56 samples:</b> <b>Fish</b> (n=33), Red scorpionfish ( <i>Scorpaena scrofa</i> )	OCH	Italy	All strains isolated resistant to one or more antibiotics tested.	(Smaldone et al., 2014)



			<b>Gasteropods</b> (10) Red-mouthed rock shell ( <i>Thais haemastoma</i> )			<i>Aeromonas</i> spp. against four antibiotics (Teicoplanin, Penicillin, Oxacillin, Vancomycin)	
Ampicillin Aztreonam Ceftazidime Ceftazidime/clavulanic acid Cefotaxime Cefotaxime/clavulanic acid Imipenem Meropenem Piperacillin Piperacillin/tazobactam Ticarcillin Gentamicin Tobramycin Tetracycline Ciprofloxacin Chloramphenicol	<i>Aeromonas</i> spp. (n=147)	Susceptibility test: agar disc diffusion (CLSI) and sequencing for specific resistance genes	<b>Mediterranean mussels</b> ( <i>Mytilus galloprovincialis</i> ) (n=28)	F	Croatia	20% of the <i>Aeromonas</i> isolates had multiple resistance, most frequently detected against penicillins, piperacillin/sulbactam and tetracycline. ESBL-encoding genes were detected in 21 isolates, with <i>bla</i> CTX-M-15 gene identified in 19 and <i>bla</i> SHV-12 in 12 isolates. Among them, 10 isolates simultaneously harboured <i>bla</i> CTX-M-15 and <i>bla</i> SHV-12, while 3 isolates additionally carried an AmpC $\beta$ -lactamase <i>bla</i> FOX-2 gene. <i>bla</i> PER-1 gene was identified in a single isolate also harbouring the <i>bla</i> CTX-M-15 gene. While <i>bla</i> SHV-12 was chromosomally encoded, <i>bla</i> CTX-M-15 was located on conjugative IncFIB-type plasmids. <i>Int11</i> and <i>int12</i> genes were detected in 57.1% and 33.3% of ESBL-producing isolates.	(Maravic et al., 2013)
Enterococci: Vancomycin Teicoplanin	154 isolates, <i>E. coli</i> (n=10) <i>E. faecium</i> (n=120)	Susceptibility test: agar disc diffusion (CLSI) and	<b>Sea urchins</b> (n=104)	O	Azores islands, Portugal	Enterococci: High percentages of resistance found for erythromycin, ampicillin, tetracycline and	(Marinho et al., 2013)

<p>Ampicillin Streptomycin Gentamicin Kanamycin Chloramphenicol Tetracycline Erythromycin Quinupristin– dalfopristin Ciprofloxacin</p> <p><i>E. coli</i>: Ampicillin Amoxicillin + clavulanic acid Cefoxitin Cefotaxime Ceftazidime Aztreonam Imipenem Gentamicin Amikacin Tobramycin Streptomycin Nalidixic acid Ciprofloxacin Sulfamethoxazole –trimethoprim Tetracycline Chloramphenicol</p>	<p><i>E. hirae</i> (n=14) <i>E. faecalis</i> (n=8) <i>E. gallinarum</i> (n=2)</p>	<p>sequencing for specific resistance genes</p>	<p>Black sea urchin (<i>Arbacia lixula</i>) (n=64) Sea urchin (<i>Paracentrotus lividus</i>) (n=22) Purple sea urchin (<i>Sphaerechinus granularis</i>) (n=18)</p> <p><b>Sea cuchumbers (146)</b> <i>Holothuria mammata</i> (n=35) <i>Holothuria sanctori</i> (n=111)</p>			<p>ciprofloxacin. The <i>erm(A)</i> or <i>erm(B)</i>, <i>tet(M)</i> and/or <i>tet(L)</i>, <i>vat(D)</i>, <i>aac(6')</i>- <i>aph(20'')</i> and <i>aph(3')</i>-IIIa genes were found in isolates resistant to erythromycin, tetracycline, quinupristin/dalfopristin, high-level gentamicin and high-level kanamycin, respectively.</p> <p>Resistance in <i>E. coli</i> isolates was detected for streptomycin, amikacin, tetracycline and tobramycin. The <i>aadA</i> gene was found in streptomycin-resistant isolates and <i>tet(A)</i> + <i>tet(B)</i> genes in tetracycline- resistant isolates.</p>	
<p>Amikacin Amoxicillin + Clavulanic acid Ampicilline Cefazolin Ceftiofur Ceftizoxime Enrofloxacin Erythromycin</p>	<p>Isolates (n=126) from wounds, abscesses and major organ systems of stranded or live animals, Including</p>	<p>MIC determination in broth microdilution according to CLSI</p>	<p><b>Sea otters</b> (n=104) <i>Enhydra lutris</i> 87 dead and 17 live-stranded or captured</p>	C	USA	<p>Gram-positive bacterial isolates were susceptible to most antimicrobials with a gram-positive spectrum.</p> <p><i>S. infantarius</i> was resistant to amikacin, enrofloxacin and ciprofloxacin.</p>	(Brownstein et al., 2011)

Gentamycin Oxacillin Rifampin Tartacycline Ticarcillin- Clavulanic acid Trimethoprim + Sulfamethoxazole Cefotaxime Ceftazidime Ciprofloxacin Clindamycine Doxycycline Florfenicol Imipenem Piperacillin Tylosine	<i>E. coli</i> <i>Staphylococcus</i> spp. <i>Streptococcus</i> spp. <i>Bordetella</i> sp. <i>Salmonella</i> spp. <i>Vibrio cholera</i> <i>Arcanobacterium</i> spp. <i>Erysipelothrix</i> spp.					<i>Arcanobacterium</i> spp. had no detectable resistance.  <i>Erysipelothrix rhusiopathie</i> showed resistance to the highest number of antimicrobials.	
Penicillin G, Piperacillin, Ticarcillin, Cefotaxime, Ceftazidime, Ceftiofur, Amikacin, Gentamicin, Ciprofloxacin, Enrofloxacin, doxycycline, Chloramphenicol, and Sulfamethoxazole	N=130 isolates comprising 77 G- rods 38 G+ cocci 14 G+ rods 1 G- coccus	Cloacal swabs  Susceptibility test: agar diffuson (Kirby-Bauer)	<b>Sharks</b> (n=58): Nurse sharks (n=15), <i>Ginglymostoma</i> <i>cirratum</i> Bull sharks (n=28), <i>Carcharhinus</i> <i>leucas</i> , Blacktip sharks (n=4), <i>Carcharhinus</i> <i>limbatus</i> , Lemon shark (n=1), <i>Negaprion</i> <i>brevirostris</i> , Spinner sharks (n=7), <i>Carcharhinus</i> <i>brevipinna</i> ,	C (top predators)	Six sites in coastal Belize and USA	Resistance to at least one drug found in each study sites and in all fish species.  Multidrug resistance seen in most of the study sites.	(Blackburn et al., 2010)

			Dogfish (n=3), <i>Mustelus canis</i> ,  <b>Teleosts</b> (n=7): Redfish, <i>Sciaenops</i> <i>ocellata</i>				
Ampicillin Cephalotin Chloramphenicol Enrofloxacin Erythromycin Florfenicol Gentamicin Nalidixic acid Nitrofurantoin Oxolinic acid Oxytetracycline Trimethoprim- sulfamethoxazole	<i>Pseudomonas</i> spp. (n=57) <i>Aeromonas</i> spp. (n=72)	Disc diffusion on Mueller- Hinton agar (CLSI)	<b>Turbot</b> (n=36) <i>Psetta maxima</i>	C	Spain	All strains were sensitive to enrofloxacin. The <i>Aeromonas</i> spp. were also sensitive to nitrofurantoin, oxolinic acid and oxytetracycline. For all other agents the percentage of resistant strains varied from 1.4 to 42.1. The most prevalent resistance were seen for ampicilline, chloramphenicol, erythromycin, florfenicol, nalidixic acid and trimethoprim-sulfamethoxazole.	(Martinez et al., 2010)
Erythromycin Spiramycin Oxytetracycline Furazolidone Kanamycin Nalidixic acid Chloramphenicol Ampicillin Sulfamethoxazole Amoxicillin Colistin Doxycycline Florfenicol Flumequine	<i>Aeromonas hydrophila</i> (n=50) <i>Vibrio parahaemolyticus</i> (n=18) <i>V. alginolyticus</i> (n=4) <i>V. cholera</i> (n=6) <i>Chromobacterium violaceum</i> (n=4)	Disc diffusion according to Kirby-Bauer	<b>Mud crab</b> (n=50) <i>Scylla serrata</i>	O	Malaysia	No resistance seen for oxolinic acid.  Highest percentage of resistance seen against lincomycin (94.5), ampicilline (90.1), amoxicilline (86.8), and oleandomycin (78.0).	(Najiah et al., 2010)



Fosfomycin Lincomycin Nitrofurantoin Novobiocin Oleandomycin Oxolinic acid Tetracycline	<i>Acinetobacter baumaii</i> (n=2) <i>P. aeruginosa</i> (n=1) <i>Hafnia alvei</i> (n=2) <i>Morganella morganii</i> (n=1) <i>E. coli</i> (n=1) <i>P. shigelloides</i> (n=1) <i>Shewanella putrefaciens</i> (n=1)						
Ampicillin, Ceftriaxone, Chloramphenicol, Nalidixic acid, Kanamycin, Streptomycin, Tetracycline, and Trimethoprim	<i>E. coli</i> <i>V. parahaemolyticus</i> <i>V. vulnificus</i> (n=not specified)	Culture method: Sample of shrimp composite  Susceptibility test: Growth on agar without antibiotics and with two-fold dilution of antibiotics (CLSI), Expansion of results in % growing on supplemented agar and growth on these media by the three	Wild-caught Shrimps (n=7), Species name not given	O	USA, South Carolina	Generally less resistance in bacteria from wild caught vs farmed shrimps.  Percentages of bacteria with reduced susceptibility to ceftriaxone, chloramphenicol, and tetracycline were 2.8%, 1.4%, and 1.1%, respectively.  Phenotypic resistance to: Ampicillin in <i>E. coli</i> and <i>V. parahaemolyticus</i>  <i>V. vulnificus</i> showed no resistance for examined antibiotics.	(Boinapally and Jiang, 2007)

		identified isolates					
Ampicillin Chloramphenicol Colistin Gentamicin Kanamycin Nalidixic acid Nitrofurantoin Streptomycin Tetracycline Ticarcilline Trimethoprim Sulfamethoxazole	<i>Plesiomonas shigelloides</i> (n=2)  <i>Salmonella</i> sp. (n=2)	Disc diffusion on Mueller-Hinton agar	Brown trout ( <i>Salmo trutta</i> ) (n=30), Pike ( <i>Esox lucius</i> ) (n=12), all from fresh water, environmental samples	C	Spain	<i>P. shigelloides</i> strains shared resistance to ampicillin, streptomycin, ticarcillin and sulphamethoxazole. The <i>Salmonella</i> strains were resistant to ampicillin, streptomycin, tetracycline, ticarcilline and sulfamethoxazole	(Gonzalez et al., 1999)
Ampicillin Carbenicillin Gentamicin Kanamycin Nalidixic acid Streptomycin Tetracycline	<i>Plesiomonas shigelloides</i> (n=?)	Disc diffusion on Mueller-Hinton agar and MIC determination in Mueller-Hinton broth (NCCLS)  Curing of plasmids by ethidium bromide.	<b>Blue crab</b> (n=16+12) <i>Callinectes sapidus</i>	O	USA	Isolates were susceptible to gentamicin, nalidixic acid and tetracycline, and resistant to ampicillin, carbenicillin, kanamycin and streptomycin.  All isolates carried plasmids conferring streptomycin resistance.	(Marshall et al., 1996)
Chlorotetracycline Halofunginone Oxytetracycline Salinomycin Streptomycin	Faecal streptococci	Inoculation on Tryptic Soy Agar with increasing concentration of AB.	<b>Water</b> (n=143) from pristine sources, Faecal bacteria likely from wild animals	-	USA Virginia	Resistance to halofunginone, salinomycin and streptomycin observed.	(Wiggins, 1996)

### 11.1.4 - Table 4. Antimicrobial resistance in insects

Antimicrobial agents	Bacterial species	Methodology for sampling and determination of resistance	Animal species	Omnivorous (O) Carnivorous (C) Herbivorous (H)	Country	Comments	Reference
Ampicillin Piperacillin Cefuroxime Cephalexin Cefepime Doripenem Imipenem Ertapenem Meropenem Ciprofloxacin Levofloxacin Norfloxacin Ofloxacin Netilmicin Tobramycin Amicacin Chloramphenicol Metronidazole Mupirocin Tigecycline	<i>E. coli</i> (n=not stated)	Culture method: Cultivation and purification and further identification of bacteria by MALDI TOF MS  Susceptibility test: Agar diffusion and microdilution (EUCAST) and E-test	Mosquitoes: <i>Culex pipenes</i> (n=42)	O/H?  Both males and females feed on various sugar sources, such as nectar, honeydew and juices from fruits.  Only females feed on blood, and will do so preferentially, over sugar, when they have mated.	Slovakia	Beta-lactamase producing <i>E. coli</i> isolates; in particular ampicillin resistance.	(Hleba et al., 2017)
Carbapenem  Colistin (gene)	<i>E. coli</i> (NDM and MCR-1) n=not stated	Culture method: Total DNA from pulverized samples and bacterial isolates were screened by PCR  Typing methods: MLST, PCR-sequencing, BLAST-analysis	Flies: Order Diptera (n=150)	O  Detritivores – consumption of decaying organic matter, most prevalent feeding behaviour	China	Direct evidence of carbapenem-resistant <i>E. coli</i> .  Colistin-resistant <i>E. coli</i> also indicated by expression of the <i>mcr-1</i> gene. Flies showed the biggest difference between DST (62/120) and <i>bla</i> <sub>NDM</sub> -positive strains	(Wang et al., 2017b)

		Susceptibility test: Agar dilution method (CLSI and EUCAST)				(31/120).	
Tetracycline Oxytetracycline Carbenicillin Ampicillin Ceftazidime Gentamycin Rifampicin	<i>E. coli</i> (n=220)	Culture method: Cultivation of bee gut microbiota identified by qPCR  Typing method: 16srRNA, diagnostic PCR and sequencing  Susceptibility test: Etest method (bioMérieux)	Honeybees: <i>Apis</i> (n=150)  Female bumble bees: <i>Bombus</i> <i>bimaculatus</i> , <i>Bombus</i> <i>impatiens</i> and <i>Bombus</i> <i>perplexis</i> (n=5)	H  H	U.S.A.  Bees from Switzerland , the Czech Republic and New Zealand were included for comparison	Relatively high incidence of tetracycline and oxytetracycline resistance observed in bacterial isolates from Connecticut bees compared to bees from other locations. PCR screens showed that tetracycline resistance was associated with mobile elements such as transposons and plasmids.	(Tian et al., 2012)

## 11.2 - Antimicrobial resistance in wild terrestrial mammals

### 11.2.1 Table 5. Antimicrobial resistance in wild boars (wild boars are considered Omnivorous)

Antimicrobial agents	Bacterial species N	Methodology for sampling and determination of reistance	Animal species N	Country	Results	Reference
Methicillin Penicillin Oxacillin Cefotixin Clindamycin	<i>S. aureus</i> (n=30)	Samples from mouth and nose of 45 wild boars were collected during hunt activity.	<i>Sus scrofa</i> n=45	Portugal	One MRSA CC398 (spa- type t899) isolate was detected (oxacillin MIC = 32 mg/L and <i>mecA</i> -positive), which	(Sousa et al., 2017)

<p>Gentamicin Fusidic acid Ciprofloxacin Tetracycline Linezolid</p>		<p>Susceptibility test: disk diffusion</p> <p>PCR, MLST, <i>spa</i>-typing</p>			<p>presented resistance to penicillin, tetracycline, and ciprofloxacin and contained the genes of immune evasion cluster (IEC) system (type B).</p> <p><b>Resistance against other antimicrobial agents:</b>  Penicillin :3, Oxacillin: 4  Cefotoxin: 1, Clindamycin: 2  Gentamicin: 1, Fusidic acid: 1, Ciprofloxacin: 2  Tetracycline: 1, Linezolid: 1  Susceptible to all tested antimicrobials: 18</p>	
<p>Vancomycin Teicoplanin Ampicillin Streptomycin Gentamicin Kanamycin Chloramphenicol Tetracycline Erythromycin Ciprofloxacin Trimethoprim-sulfamethoxazole Linezolid</p> <p>MICs of vancomycin and teicoplanin were determined by agar dilution technique.</p>	<p><i>Enterococcus</i> spp. (n, not stated)</p>	<p>Culture method: 348 cloacal/ rectal samples</p> <p>For identification of bacteria biochemical conventional methods (Gram staining, catalase, and bile esculin test) and PCRs with specific primers for different enterococcal species (<i>E. faecalis</i>, <i>E. faecium</i>, <i>E. hirae</i>, <i>E. durans</i>, <i>E. casseliflavus</i>, and <i>E. gallinarum</i>) were performed.</p> <p>Susceptibility test: agar disk diffusion (CLSI)</p> <p>PCR for detection of acquired vancomycin</p>	<p>Red-legged partridges (127), white storks (81), red kites (59), and <b>wild boars (81)</b></p>	<p>Spain</p>	<p>One VRE-a isolate was identified in one wild boar. This isolate was identified as <i>E. faecium</i>, harbored vanA gene included into Tn1546 (truncated with IS1542/IS1216), and belonged to the new ST993. This isolate contained the erm(A), erm(B), tet(M), dfrG, and dfrK genes. Ninety-six VRE-i isolates were identified (89 <i>E. gallinarum</i> and seven <i>E. casseliflavus</i>), with the following prevalence: red kites (71.2 %), white storks (46.9 %), red-legged partridges</p>	<p>(Lozano et al., 2016)</p>

		resistance genes (vanA, vanB, vanD, vanE, and vanG), and intrinsic vancomycin resistance genes (vanC-1 and vanC-2/3, linked to the species <i>E. gallinarum</i> and <i>E. casseliflavus/flavescens</i> , respectively.  Typing method: MLST			(7.9 %), and <b>wild boars (4.9 %)</b> .	
Ciprofloxacin Erythromycin Streptomycin Tetracycline	<i>Campylobacter</i> species (n=55)	Culture Method: 363 fresh faecal samples from hunted wild animals were collected, incubated on selective agar and identified classical biochemical methods.  Typing Method: Multiplex PCR (16S rRNA), conventional PCR  Susceptibility test: Agar plate dilution method (EUCAST)	Red deer: <i>Cervus elaphus</i> (n=179)  Fallow deer <i>Dama dama</i> (n=45)  Mouflon <i>Ovis musimon</i> (n=13)  Wild boar: <i>Sus scrofa</i> (n=126)	Spain           O	Resistance to erythromycin (4.8%), ciprofloxacin (37.5%), tetracycline (52.9%) and streptomycin (55%) were detected. <i>C. lanienae</i> presented a significantly higher number of susceptible isolates to ciprofloxacin and tetracycline than <i>C. coli</i> .	(Carbonero et al., 2014)
Ampicillin Amoxicillin+ clavulanic acid Cefoxitin Cefotaxime Ceftazidime Aztreonam	<i>E. coli</i> (n=not specified)	Culture Method: 67 faecal samples from different locations were collected and cultured overnight on	Wild ungulates  Red deer: <i>Cervus elaphus</i> (n=42)	Portugal	According to NRI cut-offs, 10% of the population showed a non-wild-type phenotype against at least one antibiotic, also including	(Dias et al., 2015)

<p>Imipenem Amikacin Streptomycin Nalidixic acid Ciprofloxacin Co-trimoxazole Tetracycline Chloramphenicol</p>		<p>MacConkey agar plates and subjected to PCR</p> <p>Typing Method: BOX-PCR fingerprinting (Dice similarity coefficients/UPGMA clustering method)</p> <p>Susceptibility test: Disc method (EUCAST)</p>	<p>Roe deer (n=4)</p> <p>Wild boar (n=21)</p>		<p>tetracycline (9%), cotrimoxazole (6%), streptomycin (4%), ampicillin (2%) and amoxicillin/clavulanic acid (1%). No Statistically different levels of resistance were identified between <i>E. coli</i> recovered from the three wild ungulates. Screening of <i>Salmonella</i> spp., revealed that its prevalence was very low (1.5%)</p>	
<p>Amoxicillin-clavulanate Cefoxitin Amikacin Apramycin Imipenem Aztreonam Sulfamethoxazole Gentamicin Ampicillin Ciprofloxacin Cefotaxime Ceftazidime Tetracycline Streptomycin Trimethoprim Chloramphenicol Florfenicol Kanamycin Nalidixic acid</p>	<p><i>E. coli</i>, 6 isolates (4 isolates from wild boars, and 2 from Iberian Ibexes)</p>	<p>Culture Method: Faecal samples and immunomagnetic separation to obtain <i>E. coli</i> O157</p> <p>Typing Method: multiplex PCR, PFGE</p> <p>Susceptibility test: Agar diffusion method, Broth microdilution method, Multiplex PCR targeting nine virulence factors</p>	<p>Hunter-harvested wild boars n=117, Iberian ibexes n=160</p>	Spain	<p>Four wild boars and two Iberian ibexes carried <i>E. coli</i> O157:H7, which was not found in livestock faces. All <i>E. coli</i> O157:H7 isolates were susceptible to all antimicrobial agents tested.</p>	(Navarro-Gonzalez et al., 2015)
<p>Benzylpenicillin Cefoxitin Chloramphenicol</p>	<p><i>S. aureus</i> (n=135)</p>	<p>Culture method: Samples from skin and/or nares (with nasal swabs</p>	<p><i>Sus scrofa</i> (n=713)</p>	Spain	<p>No MRSA was isolated. Resistance against other</p>	(Porrero et al., 2014a)

Ciprofloxacin Clindamycin Erythromycin Fusidic acid Gentamicin Kanamycin Linezolid Mupirocin Quinupristin-dalfopristin Rifampin Streptomycin Sulfamethoxazole Tetracycline Tiamulin Trimethoprim Vancomycin		being the better option for sampling). Direct plating on Baird Parker agar with rabbit plasma fibrinogen.  Antimicrobial susceptibility testing: antimicrobial susceptibility by broth microdilution (EUCAST)  Typing method: PCR: <i>mecA</i> and <i>mecC</i> , <i>spa</i> typing, MLST			antimicrobial agents: Benzylpenicillin:35 Chloramphenicol:1 Sulfamethoxazole:1 Streptomycin:15 Tetracyclin: 4 Trimethoprim:1	
Fosfomycin Oxacillin	<i>S. aureus</i> (n=8)	Culture method: 117 nasal swabs. Sampling on blood agar  Susceptibility testing: broth microdilution and E-test  Typing method: PCR, PFGE, MLST, <i>spa</i> -typing, microarray analysis	(n=117)	Germany	Antimicrobial susceptibility testing and microarray-based genotyping confirmed the absence of important virulence/resistance genes. Except for <i>fosB</i> (a putative fosfomycin/bleomycin resistance gene), which was present in six isolates, no antibiotic resistance genes were detected.	(Meemken et al., 2013)
Susceptibility testing to 27 different antimicrobial agents.	Five Gram-negative strains of <i>Escherichia coli</i> , <i>Serratia odorifera</i> , <i>Hafnia alvei</i> and <i>Pseudomonas</i> sp. were isolated.	Culture method: Sampling on MacConkey agar.  Typing method: Integrons were detected in bacterial genomic DNA by the multiplex PCR	1 shot wild boar	Poland	Four of these strains had class 2 integrase ( <i>intI2</i> ), and one harbored class 1 integrase ( <i>intI1</i> ). The results showed that a number of multiresistant, integron-containing bacterial strains of different genera may inhabit a	(Mokracka et al., 2012)



		method. Integron-positive strains were then identified with API 20E or API 20NE by sequencing 16S rDNA. rep-PCR  Susceptibility testing: Disc diffusion test (EUCAST).			single individual of a wild animal, allowing the possibility of transfer of antimicrobial resistance genes.	
Ciprofloxacin Sulfamethoxazole Ampicillin Cefotaxime Tetracycline Streptomycin Trimethoprim Kanamycin Nalidixic acid	<i>E. coli</i> Eight wild boars (12.7%) were carriers of <i>E. coli</i> resistant to the antimicrobial agents tested.	Culture method: Fecal samples  Typing method: PCR  Susceptibility testing: disk diffusion and microdilution, according to the EUCAST	individual (n=143) feces samples from hunter harvested wild boars	Spain	The frequency of antimicrobial resistance was low (0% to 7.9%). However, resistance to an extended-spectrum cephalosporin and fluoroquinolones was detected.	(Navarro-Gonzalez et al., 2013b)
23 different clinically relevant antimicrobial agents	<i>S. enterica</i> (n=2) <i>C. coli</i> (n=2) <i>Campylobacter</i> spp. (n=8) <i>E. faecium</i> (n=20) <i>E. faecalis</i> (n=12) <i>E. coli</i> (n=40)	Culture method: Fecal samples  Typing method: PCR and Serotyping  Susceptibility testing: Disk diffusion and microdilution according to the EUCAST	Urban wild boars (n=41)	Spain (Barcelona)	Resistance was most frequent in <i>E. faecium</i> (95% of the isolates were resistant to at least one antimicrobial agent), followed by <i>E. faecalis</i> (50%) and <i>E. coli</i> (10%). For the first time resistance to linezolid in bacteria carried by wildlife is reported.	(Navarro-Gonzalez et al., 2013a)
ampicillin cefazolin ceftiofur dihydro-streptomycin	<i>Campylobacter</i> <i>Salmonella</i> STEC O157 <i>L. monocytogenes</i> (n=not specified)	Culture method: Fecal samples  Typing method: PCR: 16S rDNA for typing for <i>Campylobacter</i>	<b>Hunted wild boars (n=121)</b> , 128 wild deer	Japan	Some <i>Campylobacter</i> spp. and <i>Salmonella</i> spp. isolates were resistant to antimicrobials, whereas all	(Sasaki et al., 2013)

gentamicin kanamycin apramycin oxytetracycline bicozamycin chloramphenicol erythromycin colistin nalidixic acid enrofloxacin trimethoprim fosfomycin		Slide agglutination for Salmonella PCR for Stx genes  Susceptibility testing: microdilution according to CLSI			the STEC O157 and O26 isolates were pan susceptible to all antimicrobials tested. High resistance rates to dihydrostreptomycin (DSM) and/or oxytetracycline (OTC) in <i>Campylobacter</i> and <i>Salmonella</i> isolates were observed.	
Ampicillin Amoxicillin Clavulanic acid Cefazoline Cefotaxime Ceftiofur Streptomycin Gentamycin Neomycin Kanamycin Sulphamethoxazole Trimethoprim– sulphamethoxazole Nalidixic acid Enrofloxacin Chloramphenicol Tetracycline Colistin	<i>Salmonella</i> spp. (n=54)	Culture method: Faeces and serum samples  Typing method: Serological study, ELISA  Suseceptibility test: disk diffusion (CLSI)	wild boar ( <i>S. scrofa</i> ) (n=499 hunted wild boars), marten ( <i>Martes</i> <i>martes</i> ), badger ( <i>Meles</i> <i>meles</i> ), fox ( <i>Vulpes</i> <i>vulpes</i> ) and wolf ( <i>Canis</i> <i>lupus</i> )	Italy	A total of 16 different antimicrobial resistance profiles were observed among the 54 wild boar isolates. The most frequent resistance profile was the streptomycin, sulphamethoxazole (6– 12.0%) profile, followed by the sulphamethoxazole , colistin (5–10%), and s ulphamethoxazole, trimethoprim– sulphamethoxazole (4–8%) profiles. Among the three isolates presenting an MDR profile, one <i>S. enterica</i> subsp. <i>enterica</i> 4,5,12:1:- possessed an ASSuT profile with additional resistance to trimethoprim– sulphamethoxazole, and one <i>S. Typhimurium</i> isolate possessed a cefotaxime,	(Zottola et al., 2013)

					streptomycin, and sulphamethoxazole profile.	
<p>Agar diffusion: Amoxicillin-clavulanate Cefoxitin Amikacin Apramicin Imipenem</p> <p>Broth microdilution method (MIC): Sulfamethoxazole Gentamicin Ampicillin Ciprofloxacin Cefotaxime Ceftazidime Tetracycline Streptomycin Trimethoprim Chloramphenicol Florfenicol Kanamycin Nalidixic acid</p>	<i>Salmonella</i> spp. (n=57)	<p>Culture method: Fecal samples</p> <p>Typing method: Serological typing of isolates based on the Kauffmann-White scheme, Phage typing of bacterial isolates</p> <p>Susceptibility test: Agar diffusion, microdilution</p>	214 hunted <i>S. scrofa</i>	Spain	The finding of a <i>S. Mbandaka</i> strain resistant to sulfamethoxazole, streptomycin and chloramphenicol and a <i>S. enteritidis</i> strain resistant to ciprofloxacin and nalidixic acid in wild boars is cause for public health concern.	(Navarro-Gonzalez et al., 2012)
<p>Ampicillin Cefotaxime Chloramphenicol Ciprofloxacin Florfenicol Gentamicin Kanamycin Nalidixic acid Streptomycin Sulfamethoxazole Tetracycline Trimethoprim</p>	Isolates (n=186) of enteropathogenic <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i>	<p>Culture method: Originate from fecal samples</p> <p>Typing method: Bioserotyping of isolates</p> <p>Susceptibility test: microdilution (CLSI)</p>	<i>Y. enterocolitica</i> from different sources, namely 77 human feces (45 Sweden, 25 Germany,	The isolates were from Switzerland, but the study was performed in Germany	Alle <i>Y. enterocolitica</i> isolates from wild boars were susceptible against the tested antimicrobial agents (streptomycin, tetracycline, and sulfamethoxazole), but not against ampicillin. All <i>Y. pseudotuberculosis</i> isolates were susceptible against streptomycin, tetracycline, and	(Bonke et al., 2011)

			7 Croatia) and 109 nonhuman sources [61 pigs (27 Germany, 27 Switzerland, 7 Finland), 30 pork (17 Germany, 13 Sweden), <b>14 wild boars (Switzerland)</b> , 2 monkeys (Croatia), and 2 chinchilla (Germany)		sulfamethoxazole and ampicillin.	
Ampicillin Amikacin Amoxicillin-clavulanic acid Cefotaxime Ceftazidime Aztreonam Cefoxitin Gentamicin Tobramycin Streptomycin Sulfonamides Tetracycline Trimethoprim-	<i>S. enterica</i> isolates (n=58) recovered (20 <i>S. Typhimurium</i> , 17 <i>S. rissen</i> , 14 <i>S. enteritidis</i> and 7 <i>S. Havana</i> )	Culture method: Fecal samples  Typing method: Serotyping  Susceptibility test: agar diffusion, microdilution  PCR: The presence of genes encoding SHV, TEM, OXA, and PSE-1 type $\beta$ -lactamases was studied by specific PCRs	Bísaro pigs (n=35) and wild boars (n=77))	Portugal	Most <i>S. Typhimurium</i> isolates (15/20 of Bísaro pigs and wild boars) showed ampicillin, chloramphenicol, streptomycin, tetracycline, sulfonamide, and amoxicillin-clavulanic acid resistances. Of the 17 <i>S. rissen</i> isolates of both origins, 13 were resistant to ampicillin, tetracycline and trimethoprim-sulfamethoxazole. Among	(Caleja et al., 2011)

sulfamethoxazole Nalidixic acid Ciprofloxacin Chloramphenicol Imipenem					the <i>S. Enteritidis</i> isolates of Bísaro pigs, eight were nalidixic acid-resistant and three were sulfonamide-resistant. Most of these resistant isolates carry integrons containing some of the resistant genes. Thus, it is crucial to track the evolution of multiresistant <i>S. enterica</i> isolates in different types of animals.	
Ampicillin, Amoxicillin-clavulanic acid Erythromycin Streptomycin Sulphametoxazol Trimethoprim Trimethoprim-sulfamethoxazole Aztreonam Cefotaxim Ciprofloxacin Chloramphenicol Colistin Erythromycin Furazolidon Gentamicin Nalidixic acid Tetracycline	<i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i> strains (n=18 isolates from wild boars)	Culturing from tonsils samples  Susceptibility test: agar diffusion (CLSI)  Typing method: PCR	Wild boars (n=153)	Samples were from Switzerland	Most <i>Y. enterocolitica</i> isolates were resistant against Ampicillin, Amoxicillin-clavulanic acid, Erythromycin, Streptomycin, but susceptible against other tested antimicrobial agents. Resistance in <i>Y. pseudotuberculosis</i> was only detected against erythromycin.	(Fredriksson-Ahoma et al., 2011)
Ampicillin Chloramphenicol Streptomycin Kanamycin Gentamicin Erythromycin	<i>Lactobacillus</i> spp (121): four species: (i) <i>L. mucosae</i> (n = 57), (ii) <i>L. reuteri</i> (n = 47), (iii) <i>L. fermentum</i> (n = 12),	Culture method: samples from small intestine and ceca of wild boars	Hunted wild boars (n=3)	Austria	All isolates were intrinsic vancomycin resistant. Initial phenotypic screening revealed that all were Susceptible to erythromycin (2 mg/ml),	(Klose et al., 2014)

quinupristin/ Dalfopristin Clindamycin	and (iv) <i>L. murinus</i> (n = 5).	Susceptibility test: broth microdilution method (cut-off values defined by the EFSA Panel on Additives and Products or Substances used in animal feed (FEEDAP, 2012).  Typing method: PCR-sequencing			but 30 were resistant to tetracycline (32 mg/ml). PCR screening detected tet(W) in 12 and tet(M) in one of heterofermentative strains, as well as the aph(30)-III kanamycin gene in <i>L. murinus</i> .	
<b>For Garm-positive</b> bacteria: amoxicillin ampicillin cephalothin cephoxitin clindamycin erythromycin gentamicin oxacillin penicillin G cotrimoxazole tetracycline vancomycin <b>For GN:</b> amoxicillin+clavula nic acid ampicillin cephalothin cephoxitin ciprofloxacin chloramphenicol enrofloxacin gentamicin clotrimoxazole tetracycline tobramycin	Enterobacteriaceae Entetrococci (n=36) Stapylococci (n=186)	Culture method: Swabs from oral cavity, nasal cavity, ear canals, anus, prepuce and vagina.  Susceptibility test: agar diffusion (NCCLS)  Typing method: Api ID32 Staph, Api ID32E and rapid ID32 Strep (bioMérieux, France)	Feral pigs Feral pigs (n=34) were live-captured in traps and all animals were humanely contended and released after sampling	Brazil	<b>Staphylococci:</b> all strains sensitive towards four drugs and highest resistance toward ampicillin (17%). <b>Enterococci:</b> presented the highest sensitivity against vancomycin (98%), ampicillin (94%) and tetracycline (90%), and highest resistance pattern toward oxacillin (99%), clindamycin (83%), and cotrimoxazole (54%). <b>GN-bacteria:</b> the highest resistance was observed with <i>Serratia marcescens</i> against CFL (98%), AMC (66%) and AMP (60%) and all drugs was most effective against E. coli SUT, TET (100%), AMP, TOB (98%), GEN, CLO (95%), CFO, CIP (93%).	(Lessa et al., 2011)

<p>amoxicillin–clavulanic acid ampicillin cephalothin ceftazidime chloramphenicol ciprofloxacin gentamicin nalidixic acid streptomycin trimethoprim–sulfamethoxazole sulfonamides compounds tetracycline</p>	<p><i>E. coli</i> (n=290)</p>	<p>Culture method: rectal swab</p> <p>Susceptibility test: agar diffusion and broth dilution</p> <p>PCR and sequencing: for detection of resistance gene</p> <p>Plasmid characterization</p>	<p>A total of (n=293) rectal swabs were collected from <i>S. scrofa</i>,</p>	<p>Czech Republic and Slovakia</p>	<p>Antibiotic resistance was recorded in 17 isolates (17 / 290, 6%). Five strains (2%) produced ESBL and were multiresistant (with resistance to 3–8 antibiotics), and in two strains, class 2 integrons were found.</p>	<p>(Literak et al., 2010b)</p>
<p>ampicillin amoxicillin–clavulanic acid cefoxitin ceftazidime cefotaxime aztreonam imipenem gentamicin amikacin tobramycin streptomycin nalidixic acid ciprofloxacin sulfamethoxazole + trimethoprim tetracycline chloramphenicol</p>	<p><i>E. coli</i></p>	<p>Culture method: 77 fecal samples</p> <p>Susceptibility test: agar disc diffusion</p> <p>PCR and sequencing of AMR-genes</p>	<p>Hunted wild boars</p>	<p>Spain</p>	<p>ESBL-producing <i>E. coli</i> isolates have been isolated from eight of seventy seven faecal samples (10.4%) of wild boars in Portugal. The ESBL types identified by PCR and sequencing were blaCTX-M-1 (6 isolates) and blaCTX-M-1 + blaTEM1-b (2 isolates). Further resistance genes detected included tet(A) or tet(B) (in three tetracycline-resistant isolates), aadA (in three streptomycin-resistant isolates), cmlA (in one chloramphenicol-resistant isolate), sul1 and/or sul2 and/or sul3 (in all sulfonamide-resistant isolates).</p>	<p>(Poeta et al., 2009)</p>

ampicillin amoxicillin-clavulanic acid cefquinome ceftiofur cephalothin cefazolin chloramphenicol enrofloxacin gentamicin neomycin spectinomycin streptomycin tetracycline trimethoprim-sulfamethoxazole spectinomycin streptomycin penicillin spiramycin (despite the fact that <i>E. coli</i> is naturally resistant to both latter agents)	<i>E. coli</i> (n=620)	Culture method: samples from the jejunums, ileums, and colons  Typing method: PFGE	wild boars (n=21) hunted in five geographic locations	Germany	All <i>E. coli</i> clones from wild boars were susceptible to all antimicrobial agents tested. the comparison of the MICs for susceptible <i>E. coli</i> clones from both wild boars and domestic pigs revealed that wild boars carried <i>E. coli</i> clones, which had significant lower MICs than susceptible <i>E. coli</i> clones from domestic pigs.	(Schierack et al., 2009)
vancomycin teicoplanin ampicillin streptomycin gentamicin kanamycin chloramphenicol tetracycline erythromycin quinupristin-dalfopristin ciprofloxacin	<i>E. faecium</i> (n=134) One isolate per sample was studied further ( <i>E. faecium</i> PG5V and PG48V). The mechanisms of resistance for other antibiotics were analyzed in these two vancomycin resistant isolates, as well as the presence of genes related with virulence factors.	Culture method: n=67 fecal samples  Susceptibility testing: disk diffusion, agar dilution (CLSI methods)  PCR and DNA-sequencing	Wild boars (n= not mentioned)	Spain	<b>Two vancomycin resistant</b> isolates. The tet(M), tet(L), and erm(B) genes, associated with tetracycline or erythromycin resistance, were identified in both isolates, and the presence of aph(3')-IIIa gene (associated with kanamycin resistance) was detected in one of them. <i>E. faecium</i> PG48V exhibited an ampicillin minimum	(Poeta et al., 2007b)



					inhibitory concentration (MIC) of 64 µg/ml. The two vanA-containing isolates harbored the Tn916/Tn1545-like mobile element, and the Tn5397-like transposon was also found in isolate PG48V.	
vancomycin teicoplanin ampicillin streptomycin gentamicin kanamycin chloramphenicol tetracycline erythromycin quinupristin- dalfopristin ciprofloxacin	134 enterococci (n=67 <i>E. faecium</i> , N=54 <i>E. hirae</i> , n=2 <i>E. faecalis</i> , n=2 <i>E. durans</i> and n=9 <i>Enterococcus</i> spp.) were recovered from wild boars (n=67) (two isolates/sample).	Culture method: fecal samples  PCR: using primers and conditions for the different enterococcal species  Susceptibility test: Disk diffusion (CLSI) and the agar dilution CLSI method for ampicillin  PCR for detection of genes for Macrolide [erm(A), erm(B), erm(C)], streptogramin [vat(D) and vat(E)], tetracycline [tet(M), tet(K), tet(L), tet(S), tet(O)], aminoglycoside [aph(30)-IIIa, aac(60)-aph(200), ant(6)-Ia], vancomycin [vanA, vanB, vanC-1, vanC-2/3], and chloramphenicol [catA]	Hunted wild boars (n=67)	Portugal	High percentages of resistance were detected for erythromycin, tetracycline, and ciprofloxacin (48.5%, 44.8%, and 17.9%, respectively), and lower values were observed for high-level-kanamycin, -streptomycin, chloramphenicol, and ampicillin resistance (9%, 6.7%, 4.5%, and 3.7%, respectively). No isolates showed vancomycin or high-level-gentamicin resistance.	(Poeta et al., 2007a)

ampicillin ceftiofur chlortetracycline clindamycin danofloxacin enrofloxacin erythromycin florfenicol gentamicin neomycin oxytetracycline penicillin spectinomycin sulphacholoropyridazine sulphadimethoxime sulphathiazole tiamulin tilmicosin trimethoprim/ sulphamethoxazole	<i>Clostridium hathewayi</i> (pathogenic to both humans and animals), <i>E. hirae</i> , <i>Bacteroides uniformis</i> , <i>Streptococcus bovis</i> , <i>Proteus mirabilis</i> , <i>S. epidermidis</i> , <i>Alcaligenes denitrificans</i> , <i>E. coli</i> (n=not stated)	Culture sample: cecal contents harvested at slaughter from an adolescent feral boar.  Chemostat culture: The continuous-flow feral pig culture was developed using published protocols for establishment of the previously developed redefined porcine continuous-flow (RPCF) culture. PCR ans sequencing: 16S rRNA gene for phylogenetic Confirmation  Susceptibility test: microdilution (CLSI), E-test	Adolescent feral boar (n=1)	USA	None of the bacterial species showed tylosin resistance except the only isolated <i>C. hathewayi</i> strain, which was resistant to erythromycin, chloramphenicol, ciprofloxacin, azithromycin, ceftriaxone, ceftizoxime, clindamycin and imipenem.  Of the other bacteria tested, only the <i>Bacteroides</i> spp. showed resistance to gentamicin, ciprofloxacin, ceftriaxone and imipenem.	(Ramlachan et al., 2007)
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### 11.2.2 - Table 6. Antimicrobial resistance in large terrestrial mammals (excluded wild boars):

Antimicrobial agents	Bacterial species	Methodology for sampling and determination of resistance	Animal species N	Omnivorous (O) Carnivorous (C) Herbivorous (H)	Country	Results	Reference
Amoxicillin Cefotaxime Chloramphenicol Clindamycin Erythromycin Levofloxacin	<i>S. agalactiae</i> (n=25)	Culture method: Skin swabs  Typing method: MALDI-TOF MS, PCR, RAPD PCR, PFGE	African and Asian elephants (n=23)	H	Germany	Despite generally broad antimicrobial susceptibility of <i>S. agalactiae</i> , many showed tetracycline resistance <i>in vitro</i> . <i>S. agalactiae</i> plays an important role in bacterial	(Eisenberg et al., 2017)

Moxifloxacin Penicillin G Tetracycline		Susceptibility test: broth microdilution method (CLSI).				infections not only in cattle and humans, but also in elephants.	
Ampicillin Ceftiofur Ceftriaxone Amoxicillin + Clavulanic acid Cefoxitin Tetracycline Chloramphenicol Sulfisoxazole Trimethoprim + sulfamethoxazole Gentamicin Nalidixic acid Ciprofloxacin	<i>E. coli</i> (n=146)	Culture method: fecal sample  Typing method: PCR  Susceptibility test: Broth microdilution (CLSI)	Feral horses of Sable Island (n=508)	H	Canada	<i>E. coli</i> was recovered from 146 (28.7%) individuals, and the majority of isolates (97%) were susceptible to all drugs tested. Resistance to tetracycline was most common, including organisms isolated from 4 (2.7%) of the colonized horses. A single isolate resistant to ampicillin, ceftriaxone, and ceftiofur was identified, which possessed the CTX- M-1 gene.	(Timonin et al., 2017)
Ampicillin Amoxicillin + clavulanate Ceftazidime Cefotaxime Cefoxitin Imipenem Nalidixic acid Ciprofloxacin Gentamicin Amikacin Tobramycin Chloramphenicol Trimethoprim + sulfamethoxazole Tetracycline	<i>E. coli</i> (n=89)	Culture Method: Faecal samples incubated with BHICTX, inoculated onto MacConkey-CTX agar. <i>E. coli</i> isolates identified by Gram-staining, triple sugar iron, indol and by PCR for uidA (beta- glucuronidase enzyme)	Red deer <i>Cervus elaphus</i> (n=122)	H	Spain	89 <i>E. coli</i> isolates recovered from 217 faecal samples from red deer and small mammals. Low antimicrobial resistance levels among faecal <i>E. coli</i> from animals observed. However, a multi-resistant ESBL-producing <i>E. coli</i> with an unusual class 1 integron carrying clinically relevant antibiotic resistance genes were detected.	(Alonso et al., 2016)

		<p>gene detection</p> <p>Typing Method: PCR and DNA sequencing</p> <p>Susceptibility test: Disc diffusion method (CLSI). Additional screening by the double disk synergy test.</p>					
<p>Ampicillin Ampicillin+ sulbactam Aztreonam Cefazolin Ceftazidima Ceftriaxone Cefepime Piperacillin Trimethoprim+ sulphamethoxazole</p>	<p><i>E. coli</i> (n=22)</p>	<p>Culture Method: Cultured by conventional techniques in MacConkey agar without antibiotics</p> <p>Typing Method: PCR</p> <p>Susceptibility test: MicroScan WalkAway 96 plus using standardized minimum concentration breakpoints for susceptibility (CLSI)</p>	<p>Red deer <i>Cervus elaphus</i> (n=22)</p>	H	Mexico	<p>Characterization of <i>E. coli</i> strains in red deer demonstrated the presence of potentially pathogenic strains in this host. The presence of hybrid-resistant STEC/EPEC, STEC/ETEC and STEC/EPEC/ETEC strains were also observed.</p>	<p>(Carrillo-Del Valle et al., 2016)</p>

Gentamicin cephalexin erythromycin ciprofloxacin amoxicillin trimethoprim-sulfa- methoxazole	<i>Staphylococcus</i> spp. (n=100)	Culture method: Swabs and biopsies of diverse lesions (abscess, cornea, ear, fluids, fracture, lung, lymph node, oral mucosa, nail, rectum, skin, and urine) were inoculated into sheep blood agar, incubated and isolates identified by standard tests.  Typing method: 16S rRNA partial sequence analysis  Susceptibility test: Disk diffusion (CLSI)	Wild animals –  Cockatiel: <i>Nymphicus hollandicus</i>  Crab-eating fox: <i>Cerdocyon thous</i>  Crab-eating raccoon: <i>Procyon cancrivorus</i>  Hoary fox: <i>Lycalopex vetulus</i>  Maned wolf: <i>Crysocyon brachyurus</i>  Rabbit: Leporidae  (n=not stated)	H  C  C  C  C  H	Brazil	The most common specie was <i>S. pseudintermedius</i> (61%) and resistance to erythromycin (57%), trimethoprim-sulfamethoxazole (50%) and amoxicillin (46%) was detected most frequently. In total, 40% of <i>Staphylococcus</i> spp. exhibited MDR phenotypes.	(de Godoy et al., 2016)
Amoxicillin Kanamycin Streptomycin Tetracycline Trimethoprim Sulfonamides Chloramphenicol	<i>E. coli</i> (n=52, buffalo only)	Culture Method: Fresh fecal samples were collected, frozen and transported to INSERM lab	African Buffalo: <i>Syncerus caffer caffer</i> (n=1000)  Cattle (n=several hundreds)	H  H	Zimbabwe  France (INSERM lab)	A significant gradient of antibiotic resistance was identified from isolated buffalo to buffalo in contact with cattle and cattle populations expressed as the Murray score; <i>Enterobacteriaceae</i>	(Mercat et al., 2016)

Amoxicillin and clavulanic acid Ticarcillin Cefoxitin Cefepime Cefotaxime Ceftazidime Streptomycin Gentamicin Kanamycin Nalidixic acid and Ofloxacin		France for culturing and isolation by MALDI-TOF  Typing Method: MALDI-TOF, rep-PCR, random amplification of polymorphic DNA (RAPD), multiplex PCR  Susceptibility test: Agar dilution (Muray score), multiplex PCR				(0.146, 0.258, and 0.340, respectively). The presence of tetracycline-, trimethoprim-, and amoxicillin-resistant subdominant <i>E. coli</i> strains (0, 5.7, and 38%, respectively) was also identified. The dissemination of tetracycline, trimethoprim, and amoxicillin resistance genes ( <i>tet</i> , <i>dfrA</i> , and <i>bla</i> <sub>TEM-1</sub> ) in 26 isolated subdominant <i>E. coli</i> strains also evidenced.	
Oxacillin Florfenicol Chloramphenicol (PCR-detection of genes against many other antimicrobial agents)	<i>S. aureus</i> (n=155)	Culture Method: Fecal samples, nasal, skin, wound or abscess swabs were collected. <i>S. aureus</i> isolates were recovered by culture, 124 of which were available for genotyping.  Typing Method: StaphyType DNA	16 bird and 28 mammal species  Particoloured bat: <i>Vespertilio murinus</i> (n=1)  Bank vole, <i>Myodes glareolus</i> (n=N/A)	O/C/H	Germany, Austria and Sweden	Resistance rates in wildlife strains were rather low and <i>mecA</i> -MRSA isolates were rare (n = 6). <i>mecC</i> -MRSA (n = 8) were identified from a fox, a fallow deer, hares and hedgehogs. The common cattle-associated lineages CC479 and CC705 were not detected in wildlife. Notably, a third common cattle lineage, CC97, was found to be common	(Monecke et al., 2016)

		<p>microarray, Genotyping Kits 2.0 (Alere Technologies), PCR, MLST, <i>Spa</i> typing (RIDOM)</p> <p>Susceptibility test: Automated agar dilution tests using the VITEK-2 device (CLSI). Virulence associated markers were determined by above-mentioned typing methods in some cases.</p>	<p>Brown rat: <i>Rattus norvegicus</i> (n=N/A)</p> <p>European marmot, <i>Marmota marmota</i> (n=14)</p> <p>Red squirrel, <i>Sciurus vulgaris</i> (n=1)</p> <p>Racoon, <i>Procyon lotor</i> (n=3)</p> <p>European badger: <i>Meles meles</i> (n=28)</p> <p>Beech marten: <i>Martes foina</i> (n=6)</p> <p>Mink: <i>M. lutreola/N. vison</i> (n=1)</p> <p>Least weasel, <i>Mustela nivalis</i> (n=1)</p> <p>Red fox: <i>Vulpes vulpes</i> (n=445)</p>			<p>among cervids. No <i>S. argenteus</i> or <i>S. schweitzeri</i>-like isolates were found.</p>	
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			<p>Lynx: <i>Lynx lynx</i> (n=331)</p> <p>Wild cat, <i>Felis silvestris</i> (n=1)</p> <p>Wild boar: <i>Sus scrofa</i> (n=160)</p> <p>Moose: <i>Alces alces</i> (n=505)</p> <p>Roe deer: <i>Capreolus</i> <i>Capreolus</i> (n=437)</p> <p>Sika deer, <i>Cervus nippon</i> (n=4)</p> <p>Red deer: <i>Cervus elaphus</i> (n=8)</p> <p>Fallow deer: <i>Dama dama</i> (n=10)</p> <p>Reindeer: <i>Rangifer</i> <i>tarandus</i> (92)</p>				
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			<p>Chamois: <i>Rupicapra rupicapra</i> (n=3)</p> <p>Mouflon: <i>Ovis orientalis</i> (n=31)</p> <p>European brown hare: <i>Lepus europaeus</i> (n=178)</p> <p>Wild rabbit, <i>Oryctolagus cuniculus</i> (n=5)</p> <p>Harbour porpoise: <i>Phocoena phocoena</i> (n=1)</p>				
<p>Penicillin Amoxicillin + clavulanic acid Cephalothin Cefotaxime Tetracycline Ciprofloxacin Enrofloxacin Rifampicin Clindamycin Azithromycin Clarithromycin Erythromycin</p>	<p><i>T. pyogenes</i> (n=97)</p>	<p>Culture Method: Samples were taken from various infections in domestic animals and European bison and cultured on agar.</p>	<p>Cattle (n=31) Pigs (n=26) Goats (n=9) Sheep (n=5) European bison (n=25)</p>	<p>H  O  H  H</p>	<p>Poland</p>	<p>The study reveals that all strains were susceptible to tested betalactams, and a statistically significant correlation between the resistance to enrofloxacin, tetracycline, macrolides, clindamycin, and a strain origin was found. The isolates from European bison were more susceptible than those from livestock, however the resistance to</p>	<p>(Rzewuska et al., 2016)</p>

		<p>Typing Method: PCR</p> <p>Susceptibility test: Strip diffusion method, Etest (CLSI) and PCR was used for detection of genes encoding seven putative virulence factors.</p>	Antelope (n=1)	H  H		tetracycline and fluoroquinolones was detected. The <i>plo</i> and <i>fimA</i> genes were detected in all strains.	
<p>Ampicillin Piperacillin Cefuroxim Cefotaxime Cefpodoxim Ceftazidime Cefepime Piperacin + Tazobactam Imipenem Meropenem Ertapenem Trimethoprim + Sulfamethoxazol Tigecyclin Gentamicin Amikacin Ciprofloxacin Fosfomycin Nitrofurantoin</p>	<i>E. coli</i> (n=83)	<p>Culture method: Rectal swab and fecal samples</p> <p>Typing method: MLST, MALDI-TOF</p> <p>Susceptibility test: Disk diffusion (EUCAST)</p> <p>Multidrug resistance test of the production of ESBL using a chromogenic agar plate</p>	<p>10 social groups of Lemur Verreaux's sifakas, 8 of which were adjacent. Samples from two groups living about 2 km away from the principal study population were also included. Groups ranged in size from 3 to 7 individuals, comprising 36–38 animals in total. (n=48)</p>	H	Madagascar  The study was performed by the German Primate Center.	<p><i>E. coli</i> strain-sharing is most prevalent within groups of wild lemurs, while intergroup relationships affect population-wide spread. The ST131 isolates which recovered in this study were non-ESBL-producing. The absence of antibiotic resistance indicates that <i>E. coli</i> spill over into the population might be low, despite relatively high human impact.</p>	(Springer et al., 2016)

Cefoperazone vancomycin amphotericin B	<i>Campylobacter</i> (n=97)	<p>Culture method: Fecal or cloacal swab samples were collected.</p> <p>Typing method: Genomic analysis. These genomes were part of the 100K Pathogen Genome Project using previously published methods. DNA extraction, library preparation, and next-generation sequencing.</p> <p>Susceptibility test: Antibiotic resistance genes were analyzed in every genome using the Resistance Gene Identifier software and the Comprehensive Antibiotic Resistance database (CARD)</p>	Non human primates, (n=not stated)  chickens, sheep, cows, and goats	H	USA	<p>Whole-genome analysis: Two major_beta-lactamase genes were observed frequently in these genomes (<i>oxa-184</i>, 55%, and <i>oxa-61</i>, 29%), where <i>oxa-184</i> was associated only with crows and <i>oxa-61</i> was associated with generalists. Mutations in <i>gyrA</i>, indicative of fluoroquinolone resistance, were observed in 14% of the isolates. Tetracycline resistance (<i>tetO</i>) was present in 22% of the isolates, yet it occurred in 91% of the abortion isolates. Virulence genes were distributed throughout the genomes; however, <i>cdtC</i> alleles recapitulated the crow-only and generalist clades. A specific <i>cdtC</i> allele was associated with abortion in livestock and was concomitant with <i>tetO</i>.</p>	(Weis et al., 2016)
Amoxicillin + clavulanic acid Ampicillin Ciprofloxacin Penicillin G	<i>E. coli</i> (n=115)	Culture Method: Faecal samples were	Herring gulls: <i>Larus argentatus</i> (n=30)	C	Ireland	The most common resistant phenotypes were tetracycline, ampicillin- and streptomycin-resistant isolates. Additionally,	(Carroll et al., 2015)

<p>Streptomycin Tetracycline Penicillin G Meropenem</p>		<p>cultivated on MacConkey agar plates</p> <p>Typing Method: PCR, S1-PFGE</p> <p>Susceptibility test: Disc diffusion (EUCAST), modified Hodge test (MHT)</p>	<p>Black-headed gulls: <i>Larus ridibundus</i> (n=30)</p> <p>Lesser black-back gulls: <i>Larus fuscus</i></p> <p>Hybrid deer species: <i>Cervus elaphus</i> x <i>Cervus Nippon</i> (n=30)</p> <p>Starlings: <i>Sturnus vulgaris</i> (n=26)</p>	<p>C</p> <p>C</p> <p>H</p> <p>O</p>		<p>plasmids were identified in all samples that exhibited multidrug-resistant phenotypes. These finding supports earlier reports that wildlife can serve as reservoirs, vectors and sentinels of AMR in the environment.</p>	
<p>Ampicillin Amoxicillin + clavulanic acid Cefoxitin Cefotaxime Ceftazidime Aztreonam Imipenem Amikacin Streptomycin Nalidixic acid Ciprofloxacin Co-trimoxazole Tetracycline Chloramphenicol</p>	<p><i>E. coli</i> (n=not specified)</p>	<p>Culture Method: 67 fecal samples from different locations were collected and cultured overnight on MacConkey agar plates and subjected to PCR</p> <p>Typing Method:</p>	<p>Wild ungulates</p> <p>Red deer: <i>Cervus elaphus</i> (n=42)</p> <p>Roe deer (n=4)</p> <p>Wild boar (n=21)</p>	<p>H</p> <p>H</p> <p>O</p>	<p>Portugal</p>	<p>According to NRI cut-offs, 10% of the population showed a non-wild-type phenotype against at least one antibiotic, also including tetracycline (9%), cotrimoxazole (6%), streptomycin (4%), ampicillin (2%) and amoxicillin/clavulanic acid (1%). No statistically different levels of resistance were identified between <i>E. coli</i> recovered from the three wild ungulates. Screening of <i>Salmonella</i> spp.,</p>	<p>(Dias et al., 2015)</p>

		BOX-PCR fingerprinting (Dice similarity coefficients/ UPGMA clustering method)  Susceptibility test: Disc method (EUCAST)				revealed that its prevalence was very low (1.5%)	
Methicillin	<b><i>E. coli</i></b> including EHEC STEC 1 fergusonii <b><i>Yersinia</i></b> <i>enterocolitica</i> <i>ruckerii</i> <i>intermedia</i> <i>frederiksenii</i> <b><i>Enterobacter</i></b> <i>cloacae</i> <i>aerogenes</i> <i>amnigenes</i> <i>nimipressuralis</i> <b><i>Providencia</i></b> <i>rustigani</i> <b><i>Ewingella</i></b> <i>americana</i> <b><i>Rahnella</i></b> <i>aquatilis</i> <b><i>Serratia</i></b> <i>fonticola</i> <i>quinivorans</i> <b><i>Aeromonas</i></b> <i>hydrophila</i> <i>sobria</i>	Culture Method: Cultures obtained from faecal samples were identified in accordance with Polish norms and reference procedures to detect microbes by Polish Committee for Standardization). Bacterial species were determined	Red deer: <i>Cervus elaphus</i> (n=120)	H	Poland, Slovakia, Hungary	13 of the 458 (2.84%) microorganisms isolated were identified as EHEC (Enterohaemorrhagic <i>E. coli</i> ) strains, and of these one strain, produced the Shiga toxin. No strain was identified as having ESBL (Extended-Spectrum Beta-Lactamase) resistance. Other bacteria identified include <i>Yersinia enterocolitica</i> (4, 0.67%) and <i>S. aureus</i> (4, 0.67%), but without methicillin resistance, and <i>L. monocytogenes</i> (8, 1.75%).	(Gnat et al., 2015)

	<i>ichthiosmia</i> <b>Kluyvera</b> <i>intermedia</i> <b>Enterococcus</b> <i>faecalis</i> <i>cecorum</i> <i>mundtii</i> <i>casseliflavus</i> <i>durans</i> <i>faecium</i> <b>Staphylococcus</b> <i>epidermidis</i> <i>aureus</i> <b>Listeria</b> <i>monocytogenes</i> (n=458)	using commercial tests.  Typing Method: PCR, Oxacillin Screen Agar plates (CLSI)  Susceptibility test: MRSA - Oxacillin Screen Agar plates (CLSI)					
Amoxicillin-clavulanate Cefoxitin Amikacin Apramycin Imipenem Aztreonam Sulfamethoxazole Gentamicin Ampicillin Ciprofloxacin Cefotaxime Ceftazidime Tetracycline Streptomycin Trimethoprim Chloramphenicol Florfenicol Kanamycin Nalidixic acid	<i>E. coli</i> (n=2 Iberian ibexes, n=4 boars )	Culture Method: Faecal samples were collected and processed according to the ISO 16.654:2001 protocol which applies immunomagnetic separation to obtain <i>E. coli</i> O157	Hunter-harvested wild boars: <i>Sus scrofa</i> (n=117)  Iberian ibexes: <i>Capra pyrenaica</i> (n=160)  5 herds of cattle: <i>Bos taurus</i> (n=380)  Horses: <i>Equus ferus caballus</i> (n=32)	O  H  H  H	Spain	Four wild boars and two Iberian ibexes carried <i>E. coli</i> O157:H7, which was not found in livestock feces. All <i>E. coli</i> O157:H7 isolates were susceptible to all antimicrobial agents tested.	(Navarro-Gonzalez et al., 2015)

		<p>Typing Method: multiplex PCR, PFGE (Applied Math, St-Martens-Latem, Belgium)</p> <p>Susceptibility test: Agar diffusion method, Broth microdilution method, Multiplex PCR targeting nine virulence factors</p>					
<p>amoxicillin ampicillin cephalexin cefoxitin ceftiofur ciprofloxacin amphenicol chloramphenicol combined sulfonamide sulfametaxazol + trimethoprim streptomycin gentamicin tetracycline</p>	<p><i>E. coli</i>  (n=26)</p> <p><i>Salmonella</i> spp.  (n=2)</p>	<p>Culture method: Rectal swabs were collected and seeded on MacConkey agar. Isolates were identified by an API 20E identification system.</p> <p>Typing method: PCR, API 20E identification</p>	<p>Coati: <i>Nasua nasua</i> (n=10)</p> <p>Opossum: <i>Didelphis Marsupialis</i> (n=3)</p> <p>Grey short-tailed opossum: <i>Monodelphis domestica</i> (n=1)</p> <p>Bats:</p>	<p>0</p> <p>0</p> <p>0</p> <p>0</p>	Brazil	<p>The study reports evidence of site-specific AMR as well as virulence patterns in a state park as opposed to other regions. Strains from the Amazonas state exhibited resistance almost exclusively to aminopenicillins, while strains from the state park exhibited resistance to cephalosporins, sulfonamide, aminoglycoside, tetracycline and fluoroquinolone, in</p>	(Iovine et al., 2015)

		<p>system, serotyped in the Reference Center for <i>Salmonella</i> Serotyping (Brazil)</p> <p>Susceptibility test: Disk diffusion method (CLSI)</p>	<p><i>Molossus molossus/ Glossophaga soricina/ Desmodontinae</i> sp./ <i>Tonatia saurophila/ Lophostoma</i> sp. (n=18)</p> <p>Rodents: <i>Mesomys hispidus/ Oecomys</i> sp. (n=3)</p> <p>Marsupial: <i>Metachirus</i> sp. (n=1)</p>	<p>H (O)</p> <p>O?</p>		<p>addition to strains exhibiting multidrug resistance.</p>	
<p>Ciprofloxacin Erythromycin Streptomycin Tetracycline</p>	<p><i>Campylobacter</i> species (n=55)</p>	<p>Culture Method: 363 fresh faecal samples from hunted wild animals were collected, incubated on selective agar and identified with classical biochemical methods.</p> <p>Typing Method:</p>	<p>Red deer: <i>Cervus elaphus</i> (n=179)</p> <p>Fallow deer <i>Dama dama</i> (n=45)</p> <p>Mouflon <i>Ovis musimon</i> (n=13)</p> <p>Wild boar: <i>Sus scrofa</i> (n=126)</p>	<p>H</p> <p>H</p> <p>H</p> <p>O</p>	<p>Spain</p>	<p>Resistance to erythromycin (4.8%), ciprofloxacin (37.5%), tetracycline (52.9%) and streptomycin (55%) were detected. <i>C. larieniae</i> presented a significantly higher number of susceptible isolates to ciprofloxacin and tetracycline than <i>C. coli</i>.</p>	<p>(Carbonero et al., 2014)</p>



		Multiplex PCR (16S rRNA), conventional PCR.					
		Susceptibility test: Agar plate dilution method (EUCAST)					
Cefotaxime Tetracycline Streptomycin Sulfamethoxazole Trimethoprim Chloramphenicol Nalidixic acid Ciprofloxacin	<i>E. coli</i> (n=not stated)	Culture Method: Cell stocks recovered from fecal samples were grown on Brain Heart Infusion agar.  Typing Method: 2D-GEL combined with MS (MALDI-TOF/TOF) and bioinformatics.  Susceptibility test: Identification of virulence	Iberian wolf: <i>Canis lupus signatus</i> (n=not stated)	C	Portugal	<i>E. coli</i> strain WA57, isolate is a cefotaxime-resistant strain that produces extended-spectrum beta-lactamases. The study reports significant differences in the abundance of 40 protein spots ( $p < 0.01$ ) from the extracellular, periplasmic, cytoplasmic, and membrane sub-proteomes and the whole-cell proteome of WA57 exposed and non-exposed to cefotaxime. Comparative proteomics analysis revealed complex changes in expression and metabolism that occur when WA57 is stressed with cefotaxime. Abundance of	(Goncalves et al., 2014)

		factors/ resistance genes by bioinformatics.				chaperone, porin and export proteins is particularly affected showing that the stress response and transport functions might directly influence the antibiotic resistance of this strain.	
Penicillin Ampicillin Amoxicillin/ clavulanate Daptomycin Levofloxacin Erythromycin Linezolid Minocycline Nitrofurantoin	<i>Enterococcus</i> (n=57)	Culture Method: Rectal swabs were seeded in M- <i>Enterococcus</i> agar.  Typing Method: semi- automated WIDER system (CLSI), MS by MALDI- TOF, PCR, nucleotide sequencing, PFGE.  Susceptibility test: WIDER system (CLSI), PCR,	Alpacas (n=40)  Ilamas (n=10)	H  H	Chile (sample collection ) Spain (some Lab Analysis?)	The study shows that the most prevalent species was <i>E. hirae</i> (82%), followed by other <i>non-E. faecalis</i> and <i>non-. faecium</i> species. Selected isolates exhibited susceptibility to almost all studied antibiotics, and virulence factors were not detected by PCR. Of note, Some discrepancies were detected among the identification methods used, and the most reliable were the <i>rpoB</i> , <i>pheS</i> , and <i>aac(6)-I</i> nucleotide sequencing.	(Guerrero- Olmos et al., 2014)

		nucleotide sequencing.					
<p>Penicillin Ampicillin Amoxicillin + Clavulanic acid Cefoxitin ceftriaxone enrofloxacin ciprofloxacin tetracycline doxycycline trimethoprim–sulfamethoxazole erythromycin streptomycin kanamycin gentamicin tobra-mycin</p>	<p><i>S. aureus</i> (n=not specified)</p>	<p>Culture Method: Samples collected were (i) swabs from nasal cavities, (ii) different organs and (iii) an abscess in an enlarged submandibular lymph nodes. These were cultured in Mueller–Hinton broth.</p> <p>Typing Method: PCR, <i>spa</i> typing, Multilocus Sequence Typing (MLST) and <i>SCCmec</i> typing.</p> <p>Susceptibility test: Disc</p>	<p>Chamois: <i>Rupicapra r. rupicapra</i> (n=2)</p> <p>Roe deer: <i>Capreolus capreolus</i> (n=1)</p>	<p>H</p> <p>H</p>	<p>Italy</p>	<p>A marked <i>S. aureus</i> genetic heterogeneity was detected. Notably, t1328, ST22 isolates, obtained from the liver of a chamois kid, was a methicillin-resistant <i>S. aureus</i> (MRSA) harbouring a <i>SCCmec</i> cassette type IV. The set of virulence marker and toxin genes investigated showed profiles characteristic of the <i>S. aureus</i> lineages detected, including those of the human adapted ST (CC) 22 and ST (CC) 45 isolates.</p>	<p>(Luzzago et al., 2014)</p>

		diffusion (CLSI), PCR.					
cefotaxime tetracycline ampicillin sulfamethizole sulphamethoxazole/ trimethoprim 19:1 amoxicillin-clavulanic acid 2:1 gentamicin enrofloxacin erythromycin ampicillin rifampicin	<i>E. coli</i>  (n=120)  <i>E. faecium</i>  (n=120)	Culture Method: Faecal samples were collected.  Typing Method: qPCR, MALDI-TOF MS, multiplex PCR.  Susceptibility test: Disc diffusion (CLSI), Enumeration of resistant/total coliforms (EUCAST).	Buffalo: <i>Syncerus caffer</i> (n=35)  Wildebeest: <i>Connochaetes</i> (n= 40)  Zebra: <i>Equus</i> (n=40)  Cattle: <i>Bos taurus</i> (n=20)	H  H  H  H	Tanzania	Vancomycin-resistant Enterococci were detected in wild life samples, and <i>E. coli</i> resistant to cefotaxime and enrofloxacin were observed among isolates from all wild life, but not from cattle. Culture independent estimates of the number of <i>sulII</i> gene copies obtained by qPCR did not differ between wildlife from the two sample sites, while <i>tetW</i> differed.	(Katakweba et al., 2015)
Benzylpenicillin Cefoxitin Chloramphenicol Ciprofloxacin Clindamycin Erythromycin Fusidic acid Gentamicin Kanamycin Linezolid	<i>S. aureus</i> (n=230)	Culture Method: Samples collected from the skin and/or nares were cultured on Baird	Eurasian griffon vulture: <i>Gyps fulvus</i> (n=40)  Iberian ibex: <i>Capra pyrenaica</i> (n=157)  Red deer:	C  H  H	Spain	The study reports that the proportion of methicillin-susceptible <i>S. aureus</i> carriers were 5.0, 22.9, 19.8, and 17.7% in Eurasian griffon vulture, Iberian ibex, red deer, and wild boar, respectively (P=0.057). A higher proportion of	(Porrero et al., 2014a)

Mupirocin Quinupristin-dalfopristin Rifampin Streptomycin Sulfamethoxazole Tetracycline Tiamulin Trimethoprim Vancomycin		Parker agar and isolates confirmed by PCR.  Typing Method: spa typing, multilocus sequence typing (MLST).  Susceptibility test: Broth microdilution (EUCAST).	<i>Cervus elaphus</i> (n=273)  Wild boar: <i>Sus scrofa</i> (n=713)	O		isolates (P=0.000) were recovered from nasal samples (78.5%) than skin samples (21.5%), but the 9.3% of red deer and 18.3% of wild boar would have been undetected if only nasal samples had been tested. 63 different spa types were identified, including 25 new spa types.	
Penicillin Florfenicol Oxytetracycline Erythromycin Vancomycin Metronidazole Tylosin	<i>Clostridium perfringens</i> (n=26)  <i>Clostridium difficile</i> (n=2)	Culture Method: Feecal samples were collected from animals, cultured on agar and bacteria isolated.  Typing Method: Multiplex-PCR, PCR Ribotyping.	Crab-eating fox: <i>Cerdocyon thous</i> (n=11),  Cougar: <i>Puma concolor</i> (n=9)  Oncilla: <i>Leopardus tigrinus</i> (n=4)  Ocelot <i>Leopardus pardalis</i> (n=3)  Maned wolf:	C  C  C  C	Brazil	<i>Clostridium perfringens</i> was isolated from 26 samples (76.5%) Notably, only one strain (6.2%), isolated from an ocelot, was resistant to oxytetracycline. All other strains tested (n =16) were susceptible to florfenicol, metronidazole, penicillin, vancomycin, erythromycin and tylosin. <i>Clostridium difficile</i> was isolated from only two (5.9%) samples.	(Silva et al., 2014)

		Susceptibility test: Serial agar dilution method (CLSI).	<p><i>Chrysocyon brachyurus</i> (n=4)</p> <p>Yagouarundi <i>Jaguarundi</i> <i>Puma</i> (n=1)</p> <p>Margay / Tree ocelot: <i>Leopardus wiedii</i> (n=1)</p> <p>Hoary fox: <i>Lycalopex vetulus</i> (n=1)</p>	C  C  C			
Oxacillin Ciprofloxacin Rifampicin Tetracycline Penicillin	<i>E. coli</i> (n=92)	<p>Culture Method: Fresh fecal samples were collected and cultured on McConkey agar.</p> <p>Typing Method: PCR, nitrocefin test.</p> <p>Susceptibility test: Disc</p>	<p>Hybrid deer: <i>Cervus elaphus</i> x <i>Cervus Nippon</i> (n=)</p> <p>Herring gulls <i>Larus argentatus</i> (n=)</p>	H  C	Ireland	The study identified bacterial isolates resistant to $\beta$ -lactam compounds in both animal species. The prevalence of resistant isolates was higher in herring gulls (87%) compared to deer (31%). Resistance to this class of antibiotic was found only in non-pathogenic <i>E. coli</i> in herring gulls and in both pathogenic and non-pathogenic <i>E. coli</i> strains in deer.	(Smith et al., 2014b)

		diffusion (CLSI).					
ampicillin cephalothin amoxicillin-clavulanate ceftazidime trimethoprim/sulfamethoxazole gentamicin nalidixic acid ciprofloxacin streptomycin tetracycline chloramphenicol aztreonam cefepime meropenem ertapenem	<i>E. coli</i> (n=75)	Culture method: fecal sample  Typing method: PCR, Multiplex PCR.  Susceptibility test: double disc synergy test (DDST), disc diffusion method (CLSI).	Non-human <b>primates</b> , mice, people and domestic animals (dogs, cats)  (n=43)	O	Côte d'Ivoire	In conclusion, ESBL and PMQR genes frequently found in humans and domestic animals in the villages were rather exceptional in wildlife living in the protected area. Although people enter the park, the strict biosecurity levels they are obliged to follow probably impede transmission of bacteria between them and wildlife.	(Albrechtova et al., 2014)
ampicillin cephalotin amoxicillin-clavulanic acid ceftazidime trimethoprim-sulfamethoxazole gentamicin nalidixic acid ciprofloxacin streptomycin tetracycline chloramphenicol	<i>E. coli</i>  <i>K. pneumoniae</i>  <i>Citrobacter</i> spp.  (n=not stated)	Culture method: Fecal samples.  Typing method: PCR.  Susceptibility test: Double-disc synergy test (DDST) for production of ESBL (CLSI, 2008).	Gorillas (n=72) Agile mangabeys (n=9) Chimpanzees (n=7) African buffalos (n=4) Forest elephants (n=4) Red River hogs (n=2) Peter's duikers (n=2)	Most probably include all three :O/C/H	Central African Republic	We found a considerable prevalence of multiresistant Enterobacteriaceae isolates with ESBL and PMQR genes in humans (10% and 31%, respectively). Among wildlife the most significant findings were CTX-M-15-producing <i>Klebsiella pneumoniae</i> in a habituated gorilla and a multiresistant <i>E. coli</i> isolate with gene qepA in an unhabituated gorilla.	(Janatova et al., 2014)

			<p>Lowland bongos (n=1)  Sitatunga (n=1)  Blue duiker (n=1)  PHP employees (trackers, assistants) (n=16)  Villagers (Bayanga, Yandounmbe (n=14)  Mossapoula)  Researchers coming from Europe (n=2)</p>			<p>Other isolates from wildlife were mostly represented by qnrB-harboring <i>Citrobacter</i> spp. The relatedness of resistant <i>E. coli</i> was investigated in a PFGE-based dendrogram; isolates from gorillas showed less than 80% similarity to each other and less than 80% similarity to human isolates. No ESBL-producing isolates were found in animals treated by ceftiofur. Although we did not detect any bacterial clone common to wildlife and humans, we detected an intersection in the spectrum of resistance genes found in humans and gorillas, represented by bla<sub>CTX-M-15</sub> and qepA.</p>	
<p>ampicillin  augmentin  trimethoprim  sulfamethoxazole  chloramphenicol  nalidixic acid  ciprofloxacin  tetracycline  kanamycin  streptomycin  imipenem  ceftriaxone  ceftazidime</p>	<p><i>Salmonella enterica</i>  (n=196)</p>	<p>Culture method:  Peritoneal and cloacal swabs, feces and liver.   Typing method:  Pulsed-field gel electrophoresis (PFGE) analysis.   Susceptibility test:  Etest method (bioMérieux, CLSI).</p>	<p>Jaguar Crocodile  Lion  Poultry  (n=not stated)</p>	<p>C  O  H</p>	<p>South Africa</p>	<p>Data showed similarities between <i>S. Enteritidis</i> strains isolated from humans and captive wild animals, suggesting a probable common source for strains from humans and animals.</p>	<p>(Smith et al., 2014a)</p>



<p>Ampicillin chloramphenicol ciprofloxacin doxycycline gentamicin neomycin streptomycin tetracycline trimethoprim- sulfamethoxazole Ceftiofur</p>	<p><i>E. coli</i> (n=440)</p>	<p>Culture method: Fecal samples were collected, incubated and isolates identified by PCR.</p> <p>Typing method: PCR</p> <p>Susceptibility test: Disk diffusion method (CLSI)</p>	<p>African elephant: <i>Loxodonta africana</i> (n=48)</p> <p>Banded mongoose <i>(Mungos mungo)</i> (n=2)</p> <p>Bushbuck: <i>Tragelaphus scriptus</i> (n=1)</p> <p>Cape buffalo: <i>Syncerus caffer</i> (n=8)</p> <p>Chacma baboon <i>(Papio ursinus)</i> (n=18)</p> <p>Crocodile: <i>Crocodylus niloticus</i> (n=2)</p> <p>Domestic cattle: <i>Bos primigenius</i> (n=7)</p> <p>Giraffe: <i>Giraffa camelopardalis</i> (n=2)</p> <p>Greater kudu: <i>Tragelaphus strepsiceros</i> (n=3)</p>	<p>All 14 different animal species will most probably include H, C and O</p>	<p>Botswana</p>	<p>The study shows that of the 150 fecal samples analysed, 41.3% contained <i>E. coli</i> isolates that were resistant to one or two of antibiotics tested, and 13.3% of isolates demonstrated multidrug resistance. Resistance was widespread, but not ubiquitous and isolates from wildlife demonstrated similar patterns of resistance to human <i>E. coli</i> from environmental and clinical sources in the study area. MDR was significantly higher in carnivores, water-associated species, and species inhabiting urban areas.</p>	<p>(Jobbins and Alexander, 2015)</p>
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			<p>Guineafowl: <i>Numida meleagris</i> (n=1)</p> <p>Impala: <i>Aepyceros melampus</i> (n=18)</p> <p>Leopard: <i>Panthera pardus pardus</i> (n=1)</p> <p>Otter: <i>Aonyx capensis</i> (n=4)</p> <p>Sable: <i>Hippotragus niger</i> (n=2)</p> <p>Spotted hyena: <i>Crocuta crocuta</i> (n=1)</p> <p>Vervet monkey: <i>Chlorocelms pygerythrus</i> (n=1)</p> <p>Warthog: <i>Phacochoerus africanus</i> (n=21)</p> <p>Waterbuck: <i>Kobus ellipsiprymnus</i> (n=4)</p>				
Oxacillin Cefoxitin	<i>Staphylococcus aureus</i>	Culture method:	Hares: (n=152)	Most probably O/H/C	Austria	The study reports the five MRSA isolates contained the mecC gene, were PVL	(Loncaric et al., 2013)

penicillin cefquinome tetracycline ciprofloxacin gentamicin chloramphenicol erythromycin clindamycin teicoplanin trimethoprim/sulf- amethoxazole linezolid rifampicin		Small intestine or other organs at postmortem, nasal and perineal swabs from other animals were obtained for bacteriological analysis.  Typing method: PCR, spa typing, MLST, 16S rDNA sequence analysis  Susceptibility test: Agar disc diffusion (CLSI)	40 different wild animals:  European brown hare  European otter  European hedgehog  Eurasian lynx			negative, carried SCCmec type XI and belonged to ST130 (where ST stands for sequence type), with spa types t843, t10513 or t3256, or to ST2620, with spa type t4335. The MRnSA isolate, most closely related to <i>S.</i> <i>stepanovicii</i> , carried <i>mecA</i> and <i>blaZ</i> genes related to SCC <i>mec</i> XI. MRSA isolates exhibited resistance to the beta-lactams only.	
Amikacin Streptomycin Neomycin Gentamicin Kanamycin Ampicillin Amoxicillin + clavulanic acid Cefotaxim Cefalotin Chloramphenicol Colistin Nalidixic acid Enrofloxacin Ciprofloxacin	<i>Salmonella</i> (n=130)	Culture Method: Sampling of mesenteric lymph nodes, faeces and viscera on dead/live animals was performed and	Canids: <b>Canidae</b> (63)  Mustelids <i>mustela</i> (25)  Birds: <b>Aves</b> (24)  Rodent ungulates (5)	C  C  O  O	Italy	Of the 130 strains of Salmonella isolated, <i>S. Typhimurium</i> was the most common serotype. Almost all the analyzed strains (97.7%) showed resistance/intermedia te resistance to at least one class of antibiotics and the highest resistance values	(Botti et al., 2013)

Trimethoprim + sulfamethoxazole Oxytetracycline Tetracyclin		incubated classical microbiological culture method.  Typing Method: Phage typing (Centro di Referenza Nazionale per le Salmonellosi, Italy, according to standard methods)  Susceptibility test: Disk-diffusion test (Kirby-Bauer Method, CLSI)				were observed for the tetracycline class.	
vancomycin teicoplanin ampicillin chloramphenicol tetracycline erythromycin quinupristin-dalfopristin ciprofloxacin	Enterococci (n= 227)  <i>E. coli</i> (n= 195)	Culture Method:  237 faecal samples were obtained	Iberian wolf: <i>Canis lupus signatus</i> (n=not stated)	C	Portugal	The study shows high rates of resistance for tetracycline and erythromycin among the enterococci isolates, and most of resistant isolates harboured the <i>tet(M)</i> and/or <i>tet(L)</i> and	(Goncalves et al., 2013a)

strepto-mycin gentamicin kanamycin ciprofloxacin amoxicillin plus clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam imipenem gentami-cin amikacin tobramycin strep-tomycin nalidixic acid		from free- ranging animals  Typing Method: PCR  Susceptibility test: Disc diffusion (CLSI)				<i>erm</i> (B) genes, respectively. The occurrence of resistant enterococci and <i>E. coli</i> isolates in the faecal flora of Iberian wolf, including the presence of resistant genes in integrons, and virulence determinants was also observed.	
vancomycin teicoplanin ampicillin chloramphenicol tetracycline erythromycin quinupristin- dalfopristin ciprofloxacin streptomycin gentamicin kanamycin amoxicillin plus clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam imipenem amikacin tobramycin streptomycin	<i>Enterococci</i> spp. (n=27)  <i>E. coli</i> (n=18)	Culture Method:  Fresh fecal samples obtained from specimens of wild animals and cultured on agar plates. Classical biochemical methods was performed for species identification and	Iberian Lynx: <i>Lynx pardinus</i> (n=30)	C	Spain	The study reported high percentages of resistance to tetracycline and erythromycin (33% and 30%, respectively) were detected among enterococcal isolates. Additionally, virulence genes were detected in one <i>E. faecalis</i> isolate (cpd, cyLB, and cyL) and one <i>E.</i> <i>hirae</i> isolate (cyL). High percentages of resistance were detected in <i>E. coli</i> isolates to tetracycline (33%), streptomycin (28%), nalidixic acid (28%), and	(Goncalves et al., 2013b)

nalidixic acid ciprofloxacin sulfamethoxazole -trimethoprim		confirmed by PCR  Typing Method: PCR  Susceptibility test: Disk diffusion method (CLSI),  PCR (Virulence genes)				sulfamethoxazole- trimethoprim (SXT, 22%).	
amikacin gentamicin kanamycin streptomycin spectinomycin ceftiofur ceftriaxone ciprofloxacin enrofloxacin norfloxacin sulfadiazine sulfamethazine amoxicillin ampicillin chloramphenicol tetracycline	<i>E. coli</i> (n=220)	Culture Method: Feca l samples were collected from 50 Sika deer farms and cultured on Mac- Conkeyagar.  Typing Method: PCR  Susceptibility test: Broth micro- dilution	Sika deer: <i>Cervus Nippon</i> (n=not stated)	H	China	This study shows that nearly all the isolates were resistant to at least four of the tested antimicrobials. More than 90% of the <i>E. coli</i> isolates carried at least one of the tested virulence genes. About 85% of the <i>E. coli</i> isolates carried one or more antimicrobial- resistant genes responsible for resistant phenotypes of sulfonamides, streptomycin/spectio nomycin or tetracycline.	(Li et al., 2013)

		(CLSI), PCR (Virulence genes)					
colistin amoxicillin- clavulanate cefoxitin amikacin apramicin imipenem aztreonam gentamicin ceftazidime chloramphenicol florfenicol	<i>E. coli</i> (n=194)	Culture Method:  Fecal samples were collected and cultured on MacConkey agar.  Typing Method: PCR  Susceptibility test: Disc diffusion (EUCAST/ Rosco Diagnostica), Broth microdilution (EFSA/EUCA- ST)	Wild boar: <i>Sus scrofa</i> (n=143) Iberian ibex <i>Capra pyrenaica</i> (n=184)  Cattle (n=380)  Horse (n=32)	O  H  H  H	Spain	The study assessed antimicrobial resistance in indicator <i>E. coli</i> isolates. The frequency of antimicrobial resistance was low (0% to 7.9%). However, resistance to an extended- spectrum cephalosporin and fluoroquinolones was detected.	(Navarro- Gonzalez et al., 2013b)
ampicillin streptomycin ciprofloxacin erythromycin linezolid	<i>Enterococcus</i> spp. (n=115)	Culture Method: 103 rectal or	Fox: (n=54)  Beech marten: (n=9)	Most probably include all categories: O, H, C	Poland	The study reports that all strains, regardless of source, were susceptible to 13-lactams,	(Nowakiewicz et al., 2014)

<p>quinupristin/dalfopristin gentamicin teicoplanin penicillin nitrofurantoin tetracycline vancomycin chloramphenicol enrofloxacin rifampicin bacitracin kanamycin</p>		<p>cloacal swabs from animals of various species and 12 milk samples from cattle were collected and cultured on agar and identified by commercial microtest EN-COCCUStest.</p> <p>Typing Method: 16S-23S rRNA intergenic spacer region (ITS-PCR)</p> <p>Susceptibility test: Disk diffusion (CSLI), Agar screen test (Statistica version 8.0)</p>	<p>European hamster (n=2)</p> <p>Rat (n=1)</p> <p>Beaver (n= 1)</p> <p>Bat (n=1)</p> <p>Hedgehog (n=2)</p> <p>Russian tortoise (n=17)</p> <p>Dog (n=2)</p> <p>Cat (n=2)</p> <p>Cattle (n=12)</p> <p>Poultry (n=12)</p>			<p>gentamicin, linezolid, and teicoplanin; the highest resistance was to kanamycin, quinupristin, and rifampicin. Despite the relatively low level of resistance in the strains isolated from wild and exotic animals, the large number of intermediately susceptible strains in these groups is an indication of the evolutionary character of the development of resistance, suggesting that these animals may be potential reservoirs of Enterococcus strains resistant to a wide panel of currently used antibiotics.</p>	
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		(Statsoft Polska, Krakow, Poland)					
ampicillin, amoxicillin + clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam imipenem gentamicin amikacin tobramycin streptomycin nalidixic acid ciprofloxacin sulfamethoxazole etrimethoprim - (SXT) tetracycline chloramphenicol	<i>E. coli</i> (n=22)  <i>Enterococcus</i> spp. (n=50)	Culture Method: For <i>E. coli</i> isolation, faecal samples were seeded in Levine agar plates. For enterococcal isolation, faecal samples were sampled in SlanetzeBartl eyagar plates.  Typing Method: PCR  Susceptibility test:  Agar disk diffusion,	Red foxes: <i>Vulpes vulpes</i> (n=42)	C	Portugal	The report showed a high percentage of <i>E. coli</i> isolates exhibited resistance to streptomycin, tetracycline, trimethoprim-sulfamethoxazole or ampicillin (54.27%), and they harboured the aadA, tet(A) and/or tet(B), sul1 and blaTEM resistance genes, respectively. <i>E. faecium</i> was the most prevalent species (50%) among enterococcal isolates. A high percentage of enterococcal isolates showed tetracycline resistance (88%) harbouring different combinations of tet(M) and tet(L) genes. The erm(B) or the aph(30)-IIIa gene were identified in most of our erythromycin- or kanamycin-resistant	(Radhouani et al., 2013)

		PCR (virulence genes)				enterococci, respectively.	
ampicillin cefazolin ceftiofur dihydro- streptomycin gentamicin kanamycin apramycin oxytetracycline bicozamycin chloramphenicol erythromycin colistin nalidixic acid enrofloxacin trimethoprim fosfomicin	<i>Campylobacter</i> spp.  <i>Salmonella</i> spp.  Shiga toxin- producing <i>E. coli</i> (STEC) O157 and O26  <i>L. monocytoge- nes</i>  (n=not specified)	Culture Method: Rectal contents were collected from animals in meat- processing facilities cultured and identified by classical methods.  Typing Method: PCR  Susceptibility test:  Agar dilution method (CSLI)	Wild Boars: <i>Sus scrofa</i> (n=121)  Wild Deer <i>Cervus Nippon</i> (n=128)	O  H	Japan	The most common <i>Campylobacter</i> species in this study was <i>C. lanienae</i> and <i>C. hyointestinalis</i> . The nine <i>Salmonella</i> serovars isolated were <i>S.</i> <i>enterica</i> subsp. <i>enterica</i> serovar Agona (three isolates), <i>S.</i> Narashino (two), <i>S. Enteritidis</i> (one), <i>S. Havana</i> (one), <i>S.</i> <i>Infantis</i> (one), and <i>S.</i> <i>Thompson</i> (one). Five (16%) and 6 (29%) isolates of <i>C. lanienae</i> and <i>C.</i> <i>hyointestinalis</i> , respectively, were resistant to enrofloxacin. STEC O157 and O26 and <i>L. monocytogenes</i> were isolated from 2.3% (95% CI: 0- 5.0), 0.8% (95% CI: 0-2.3), and 6.1% (95% CI: 1.7-10.5) of the rectal content samples of wild deer,	(Sasaki et al., 2013)

						respectively, but not from wild boars	
amoxicillin clindamycin cephalotin penicillin oxacillin tetracycline ampicillin erythromycin sulphazotrim gentamicin cephoxitin vancomycin cloranphenicol amoxicilin + clavulanic acid gentamicin cephoxitin tobramicin cotrimoxazole enrofloxacin ciprofloxacin	<i>E coli</i> <i>Proteus vulgaris</i> <i>Proteus mirabilis</i> <i>Pseudomona-daceas</i> <i>Serratia marcescens</i> CoNS <i>Micrococcus</i> spp. <i>S. intermedius</i> <i>Candida albicans</i> <i>Candida parapisilossis</i> <i>Aspergillus sp.</i> <i>Penicillium sp.</i> <i>Malassezia pachydermatis</i> <i>Trichoph metagrophytes</i> <i>Trichosporon asahii</i> <i>Cryptococcus laurentii</i> (n=not specified)	Culture Method: Standard Stuart medium transport microbiologic al swabs were used to collect samples from oral/nasal/ear r cavities, foreskin (prepuce) and perineal areas, then cultured on an appropriate agar.  Typing Method: Bacteria were identified using Api ID32 Staph, Api ID32	Brazilian Maned-wolf: <i>Chrysocyon brachyurus</i> (n=8)	C	Brazil	The study showed that <i>E. coli</i> samples from prepuce, anus and ear showed multiresistance toward erythromycin and tetracycline in particular, followed by <i>Proteus mirabilis</i> and <i>P. vulgaris</i> strains isolated from anus and ear. Among Gram-positive bacteria, strains of coagulase-negative staphylococci showed multi-resistance mainly toward erythromycin and amoxicillin.	(Vieira-da-Motta et al., 2013)

		<p>Strep and Api ID32E, and interpreted by miniAp automated system (bioMérieux, France).</p> <p>Susceptibility test: disk diffusion on agar method (CSLI)</p>					
<p>penicillin tetracycline erythromycin clindamycin cotrimoxazole oxacillin aminoglycosides quinolones linezolid glycopeptides fosfomicin rifampicin</p>	<p><i>S. aureus</i> (n=100)</p>	<p>Culture method: Mucosal swabs feaces, oral and genital swabs</p> <p>Susceptibility test: Agar plates supplemented with aztreonam disk (standard not stated)</p> <p>Typing method:</p>	<p>Madagascar (Lemur n=25) Uganda (chimpanzees n=58) Côte d'Ivoire (chimpanzees n=21)</p>	<p>H O</p>	<p>Madagascar (Lemur) Uganda (chimpanzees) Côte d'Ivoire (chimpanzees)</p>	<p>The penicillin resistance in isolates from wild chimpanzees (Taï National Parc, n=14) and lemurs (Kirindy, n=21) was 21.4% (n=3) and 9.5% (n=2), respectively. In contrast, captive populations from Ngamba and Entebbe (n=49) carried more frequently isolates resistant to penicillin (55.1%, n=27), tetracycline (30.6%, n=15), erythromycin (1%, n=1),</p>	<p>(Schaumburg et al., 2013)</p>

		Sequence based genotyping ( <i>spa</i> typing, multilocus sequence typing), PCR				clindamycin (1%, n=1) and cotrimoxazole (2.0%, n=1). No resistance was detected against oxacillin, aminoglycosides, quinolones, linezolid, glycopeptides, fosfomycin and rifampicin.	
Genes Norfloxacin Enrofloxacin Levofloxacin Ciprofloxacin Cefazolin Cefotaxime Ceftazidime Gentamicin Kanamycin Amikacin	<i>Trueperella pyogenes</i> (n=36)	Culture Method: Samples from face, nose, mouth, neck, leg and buttock as well as some internal organs were collected and cultured on BHI agar and identified by 16S rRNA gene sequence.  Typing Method:	Forest musk deer (n=36)	H	China	The study reported that isolates with less virulence determinants (VDs) obtained from sick forest musk deer mainly belonged to Type 1, and the isolates with robust VD repertoire obtained from dead forest musk deer were included in Type 3. Additionally, resistant isolates exhibited significant lower virulence than susceptible ones.	(Zhao et al., 2013)

		BOX-PCR , 16S rRNA gene sequence					
		Susceptibility test:					
		Microbroth dilution technique (CSLI)					
ampicillin ciprofloxacin chloramphenicol doxycycline neomycin rifampin streptomycin sulfamethoxazole	<i>E. coli</i> (n=236)	Culture method: Fresh fecal samples	Gorillas (n=119)	O	Gabon	In areas of low human density, human-wildlife <i>E. coli</i> transmission seems to be low. The presence of antibiotic-resistant isolates in gorillas may be better explained by other mechanisms for resistance acquisition, such as horizontal gene exchange among bacteria or naturally acquired resistance.	(Benavides et al., 2012)
ampicillin amoxicillin plus clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam	<i>E. coli</i> (n=not specified)	Culture Method: 128 faecal samples were collected from animals and	Iberian lynx: <i>Lynx pardinus</i> (n=30 (wild) and 98 (captive))	C	Portugal	The study shows the occurrence of unrelated multiresistant <i>E. coli</i> in faecal microbiota of captive specimens. Cefotaxime-	(Goncalves et al., 2012a)

<p>imipenem gentamicin amikacin tobramycin streptomycin nalidixic acid ciprofloxacin sulfamethoxazole -trimethoprim tetracycline chloramphenicol</p>		<p>seeded in Levine agar. Bacterial isolates were detected by classical biochemical methods and API 20E system (BioMe´rieux France).</p> <p>Typing Method: PCR</p> <p>Susceptibility test: Disc diffusion/double-disc diffusion test (CLSI), PCR (resistant genes)</p>				<p>resistant <i>E. coli</i> isolates were observed only in faecal samples of captive animals. ESBL-producing isolates, resistant genes in integrons and virulence determinants were also identified.</p>	
<p>ampicillin amoxicillin plus clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam imipenem gentamicin amikacin tobramycin streptomycin nalidixic acid</p>	<p><i>E. coli</i> (n=13)</p>	<p>Culture Method: 237 fresh fecal samples were obtained from free-ranging animals and seeded in Levine agar. Bacterial isolates were identified by classical</p>	<p>Iberian wolf: <i>Canis lupus signatus</i> (N=not stated)</p>	<p>C</p>	<p>Portugal</p>	<p><i>E. coli</i> isolates with TEM-52, SHV-12, CTX-M-1, and CTX-M-14-type ESBLs were detected in 5.5% of the samples investigated in the study; thereby revealing the presence of ESBL-producing <i>E. coli</i> isolates, in a wild ecosystem.</p>	<p>(Goncalves et al., 2012b)</p>

<p>ciprofloxacin sulfamethoxazole- trimethoprim tetracycline and chloramphenicol</p>		<p>biochemical methods and API 20E system (BioMe´rieux France).</p> <p>Typing Method: PCR</p> <p>Susceptibility test: Disc diffusion/double- disc diffusion test (CLSI), PCR (AMR genes)</p>					
<p>tetracycline erythromycin ampicillin</p>	<p>Enterococci (n=284)</p>	<p>Culture Method: Fecal samples were collected, cultured on bile aesculin azide agar. Strains were identified by MALDI Biotyper (Bruker Daltonics).</p> <p>Typing Method: Agarose gel electrophoresis (visualized by UV light)</p>	<p>Chamois: <i>Rupicapra rupicapra</i> (n=9)</p>	<p>H</p>	<p>Slovakia</p>	<p>Low frequency of resistance was observed in studied enterococcal populations (about 5 % for tetracycline and erythromycin and 0 % for ampicillin). This indicates that enterococcal population in feces of the majority of studied animals did not encounter mobile genetic elements encoding antibiotic resistance probably due to spatial separation</p>	<p>(Vandzurova et al., 2012)</p>



		Susceptibility test: Dilution method on Mueller–Hinton agar (CSLI)				and/or due to low exposure to the antibiotics.	
ampicillin penicillin kanamycin gentamycin streptomycin erythromycin tetracycline chloramphenicol colistin fosfomycin vancomycin cefazolin cephalothin cefmetazole cefotiam cefoperazon latamoxef sodium cefotaxime nalidixic acid norfloxacin ofloxacin ciprofloxacin sulfamethoxazole + trimethoprim sulfamethizole	<i>E. coli</i>  (n=285)	Culture methods: 265 fresh fecal samples  Typing method: The predominant isolates were characterized by serotyping, virulence gene profiling, plasmid profiling, pulsed- field gel electrophoresis (PFGE), and microbial sensitivity tests.  Susceptibility test: Disc diffusion and agar dilution (CLSI)	Japanese macaques  N=13 troops	O	Japan	The prevalence of antimicrobial- resistant <i>E. coli</i> was 6.5% (n=62) in wild macaques, and these isolates were resistant to cephalothin. We conclude that wild Japanese macaques in Shimokita Peninsula were unlikely to act as a reservoir of pathogenic <i>E. coli</i> for humans and that antimicrobial- resistant <i>E. coli</i> in wild macaques may be derived from humans.	(Ogawa et al., 2011)
Genes (ampicillin resistance)	<i>Firmicutes</i> <i>Clostridiales</i> (n=160)	Culture Method: Rectal swabs or faecal samples were collected from animals and	Polar bears: <i>Ursus maritimus</i> (n=10)	C	Norway (Arctic Svalbard)	The study shows that in a 16S rRNA gene clone library constructed, all sequences obtained from 161 clones showed affiliation with the phylum <i>Firmicutes</i> , with 160 sequences	(Glad et al., 2010)

		<p>cultured on chocolate agar.</p> <p>Typing Method: PCR, 16S rRNA sequencing</p> <p>Susceptibility test: PCR (ampicillin resistant genes)</p>				<p>identified as <i>Clostridiales</i> and one sequence identified as unclassified <i>Firmicutes</i>. The proportion of amp<sup>r</sup> bacteria ranged from 0% to 44%. All of 144 randomly selected amp<sup>r</sup> isolates tested positive for enzymatic b-lactamase activity.</p>	
Ampicillin, tetracycline	<i>E. coli</i> (n=not stated)	<p>Culture method: Vaginal swab samples Since cultivation was not feasible, polymerase chain reaction and DNA sequencing were used to detect and characterize these resistance genes Typing method: PCR</p> <p>Susceptibility test: Resistance genes, sequences available in the ClustalW multiple sequence alignment programs.</p>	<p>Baboons (n=34)</p> <p>Mangabeys (n=9)</p>	<p>O</p> <p>O</p>	Kenya	<p>Bacteria were tested for the presence of two types of resistance genes: tetracycline resistance (tet) genes and erythromycin resistance (erm) genes. These genes are frequently found in human isolates of the two types of bacteria that were a substantial part of the normal microbiota of primates (Firmicutes and Bacteroidetes). The tet(M) and tet(W) genes were found most commonly, and the tet(Q) gene was found in over a third of the samples from baboons. The ermB and ermF genes were found only in a minority of the samples.</p>	(Jeters et al., 2009)

						The ermG gene was not found in any of the specimens tested.	
tetracycline streptomycin ampicillin trimethoprim- sulfamethoxazole nalidixic acid ciprofloxacin amoxicillin- clavulanic acid gentamicin tobramycin chloramphenicol cefotaxime aztreonam ceftazidime amikacin cefoxitin imipenem	<i>E. coli</i> (n=112)	Culture Method: 72 fecal samples were collected and seeded on Levine agar and bacterial isolates identified by classical biochemical methods.  Typing Method: PCR  Susceptibility test: Agar disk diffusion method, double disk diffusion test (CLSI)	Wild animals (14 birds of prey, 10 owls, 7 foxes, 6 wild rabbits, 5 genets, 4 forest wildcats, 3 storks, 3 deer, 3 otters, 2 wolves, 2 mouflons, 1 badger, 1 partridge, 1 hedgehog, 1 pigeon, 1 ferret, 1 quail, 1 wild boar, 1 salamander, 1 snake, 1 winter wren, 1 jay, 1 magpie, and 1 Mediterranean turtle)	Most probably include all categories: O, H, C	Portugal	The study revealed the following percentages of resistance were obtained: tetracycline, streptomycin, ampicillin, and trimethoprim-sulfamethoxazole (SXT) (range 19–35%); nalidixic acid (14%); ciprofloxacin (9%); amoxicillin-clavulanic acid, gentamicin, tobramycin, chloramphenicol (range 4.5–7%); cefotaxime, and aztreonam (1.8%); ceftazidime (0.9%); and amikacin, cefoxitin, and imipenem (0%). A <i>bla</i> <sub>TEM</sub> gene was found in 22 of the 25 ampicillin-resistant isolates, and the gene encoding CTX-M-14 beta-lactamase was identified in the two cefotaxime-resistant isolates	(Costa et al., 2008)
Dalbavancin Vancomycin Teicoplan	Enterococci spp.	Culture method: Faecal samples of wild animals...	Human, poultry, pets and	Most probably	Portugal	All vancomycin-susceptible <i>Enterococcus</i> spp. were inhibited by ≤0.25 mg/l	(Poeta et al., 2008)

		Typing method: PCR  Susceptibility test: Microbroth dilution method (CLSI)	Wild animals (not specified) from different natural parks (n=66)	O/H/C		dalbavancin. Although vancomycin-resistant- enterococci (VRE) showed higher dalbavancin MIC values (16 mg/l), the isolates that exhibited the VanC resistance phenotype were inhibited at dalbavancin concentrations $\leq 0.125$ mg/l. Only vanA isolates were not inhibited by low concentrations of dalbavancin since vanA strains showed higher dalbavancin MIC values (16 mg/l)	
Ampicillin Cephalothin Chloramphenicol Ciprofloxacin Doxycycline Gentamycin Nalidixic acid Neomycin Streptomycin Trimethoprim- sulfaxazole Tetracycline	<i>E. coli</i>  (n =343)	Culture method: Fecal swab samples  Typing method: repetitive extragenic palindromic polymerase chain reaction (rep-PCR).  Susceptibility test: Disc-diffusion method (CLSI)	Gorillas (41)  Livestock (cattle, goats, sheep) N = 48	O  H	Uganda	Thirty-five percent of isolates from humans, 27% of isolates from livestock, and 17% of isolates from gorillas were clinically resistant to at least one antibiotic used by local people, and the proportion of individual gorillas harboring resistant isolates declined across populations in proportion to decreasing degrees of habitat overlap with humans.	(Rwego et al., 2008)
chloramphenicol ampicillin	<i>E. coli</i> Two collections of	Culture method: freshly deposited	Baboons	O	Tanzania	Antibiotic resistance in <i>E. coli</i> strains from wild baboon hosts is less	(Routman et al., 1985)

streptomycin kanamycin tetracycline	<i>E. coli</i> from human hosts and one from free-ranging African yellow baboons were examined for the ability to utilize various sugars (biotype) and for resistance to antibiotics. (n=496)	feces of baboons under observation.  Susceptibility test: Disk diffusion (no standard given)  Typing method: Not stated.				frequent than in strains from contemporary human hosts, but it is similar to levels found in <i>E. coli</i> collected in the preantibiotic era. About 8.5% of the strains were resistant to at least one of the antibiotics tested.	
Genes	Enterococci (n=)  <i>E. coli</i> (n, not stated)	Culture method: Water samples and fecal swabs were collected.  Typing method: PCR  Susceptibility test: Antibiotic resistance analysis; ARA (SAS software version 8; SAS Institute), PCR	Rabbits (n=971)  Dogs (872)  Birds (n=922)  Cats (n=48)  Unknown animal (n=224)		USA	The study suggests that bird and wild animal feces, soil amendments, and/or fecal coliform growth in the storm drain are the major contributors to the fecal bacterial pollution in downstream areas.	(Jiang et al., 2007)
amoxicillin sulfamethoxazole chloramphenicol kanamycin streptomycin	<i>E. coli</i> (n=341)	Culture method: Fresh fecal samples were collected, cultured and bacterial	150 wild birds and mammals  128 extensively	H, C, O (not specified species)	France	The study reports a gradient of resistance ranging from absence to high prevalence (resistance score of	(Skurnik et al., 2006)

tetracycline nalidixic acid		strains identified by API 20E strips (API, La Balme-les-Grottes, France).  Typing method: PCR  Susceptibility test: Disc diffusion (the French Society of  Microbiology antibiogram committee, CASFM, www.sfm.asso.fr).	reared farm animals  42 pet dogs			18.7%) and a gradual increase in the prevalence of class 1 integrons (from 0% to 16%), both correlated with the increase in exposure to humans. In wild animals with little contact with humans, resistance, when present, was not mediated by integrons.	
Vancomycin Teicoplanin Ampicillin Streptomycin Gentamicin, Kanamycin Chloramphenicol Tetracycline Erythromycin Quinupristin-dalfopristin Ciprofloxacin	<i>Enterococci faecium</i> (n=45)  <i>Enterococci faecalis</i> (n=73)  <i>Enterococci hirae</i> (n=14)  <i>Enterococci casseliflavus</i> (n=4)  <i>Enterococci gallinarum</i> (n=2)	Culture Method: Faecal samples were obtained from animals and cultured. Colonies with typical enterococcal morphology were identified by cultural characteristics and biochemical tests (API ID20 Strep system, BioMe ´rieux).	<b>77 wild animals</b> (14 birds of prey, 10 owls, seven foxes, six wild rabbits, five European genets, four forest wildcats, four salamanders, three storks, three magpies, three deer, three vipers, three otters, two wolves, two mouflon, two badgers, one	O, H, C	Portugal	The study reported that 44 of the 140 isolates (31.4%) showed susceptibility to all the antibiotics tested (5.5% of <i>E. faecalis</i> ; 62.2% of <i>E. faecium</i> ; and 78.6% of <i>E. hirae</i> ). Neither ampicillin-resistance nor acquired vancomycin-resistance was detected. Notably, 1.4% of the isolates showed high-level-resistance for gentamicin or streptomycin. Tetracycline and erythromycin resistances were shown	(Poeta et al., 2005)

	<i>Enterococci</i> spp. (n=2)	Typing Method: PCR  Susceptibility test: Disk diffusion method (NCCLS), PCR (antibiotic resistance genes + virulence factors)	partridge, one hedgehog, one pigeon, one ferret, one quails and one wild boar)			in 28.6% and 20.1% of the isolates, respectively. A wide variety of virulence genes were detected in most of <i>E. faecalis</i> isolates but were rarely found in <i>E. faecium</i> and not detected in the other species.	
Amoxicillin/ Clavulanic acid Ampicillin Ceftiofur Chloramphenicol Enrofloxacin Florfenicol Gentamicin Nalidixic acid Neomycin Oxytetracycline Streptomycin Sulphamethoxazol-e Trimethoprim Avilamycin Bacitracin Erythromycin Flavomycin Narasin Vancomycin Virginiamycin	Verocytotoxic <i>E. coli</i> (n=179)  <i>Campylobacter</i> spp. (n=1)  <i>Salmonella</i> spp. (n=0)  <i>E. faecalis</i> (n=30)  <i>E. faecium</i> (n=8)	Culture Method: Faecal samples were collected from animals, cultured. Colonies were identified by phase-contrast microscopy, and other assays (eg. ME02_046, National Veterinary Institute, complying with the Nordic Committee on Food Analysis, ISO 17025).  Typing Method: PCR	Red deer: (n=135)  Moose (n=127)  Roe deer (n=206)  Reindeer (n=153)	H  H  H  H	Norway	Antibiotic resistance was observed in 13 (7.3%) of the 179 <i>E. coli</i> isolates tested. The proportion of <i>E. coli</i> -resistant isolates was higher in wild reindeer (24%) than in the other cervids (2.2%). <i>E. faecalis</i> or <i>E. faecium</i> were isolated from 19 of the samples, none of these being reindeer. All the strains isolated were resistant against one (84%) or more (16%) antibiotics. A total of 14 <i>E. faecalis</i> -strains were resistant to virginiamycin only.	(Lillehaug et al., 2005)

		Susceptibility test: Broth dilution-based VetMIC-plates method (National Veterinary Institute, Sweden), Etest strips (Biodisk, Sweden) PCR (genes)					
Ampicillin Amoxicillin/clavulanic acid Cephalothin Cefuroxime; Cefotaxime Aztreonam Imipenem Gentamicin Streptomycin Nalidixic acid Ciprofloxacin Chloramphenicol Tetracycline Trimethoprim Sulphamethoxazole	<i>E. coli</i> (n=98)  Enterobacter agglomerans group (n=48)  <i>Yersinia</i> spp. (n=29)  <i>Serratia</i> spp. (n=11)	Culture Method: Fecal samples were obtained from animals cultured and analysed accordingly.  Typing Method: PCR  Susceptibility test: agar dilution method (NCCLS)	Felled moose: <i>Alces alces</i> (n=16)  White-tailed deer: <i>Odocoileus virginianus</i> (n=7)  Voles: <i>Clethrionomys glareolus</i> (n=23)	H  H  H	Finland	Of the 15 antibiotics, the only resistance observed was to cefuroxime and streptomycin (one <i>E. coli</i> sample). Most of the cefuroxime resistance was, as judged from MIC profiles, most likely caused by a class A (Bush group 2e) cefuroximase similar to the chromosomal <i>Proteus vulgaris</i> enzyme, and most probably indigenous as opposed to the most common transferable class-A $\beta$ -lactamases: the cerufoxime-resistant strains were tested by using PCR for the	(Osterblad et al., 2001)



						presence of TEM and SHV8, but only one strain contained a TEM-type enzyme and none carried SHV.	
ampicillin cephalothin chloramphenicol entamicin nalidixic acid neomycin streptomycin sulphamethoxazole/trimethoprim tetracycline	<i>Salmonella</i> spp.  (n=1012)	Culture method: Fecal samples (approximately 1 g) or cloacal swabs  Typing method: Confirmation of <i>Salmonella</i> spp. isolates and serological typing using standard methods.  Susceptibility test: The disc diffusion method (Bauer et al., 1966) was used to determine the antibiograms of all <i>Salmonella</i> spp. isolates.	Porcupine Tiger Local brocket Deer Rainbow boa Macajuel Regal python Cook's tree boa Rabbit Cook's tree boa Anaconda Rainbow boa Macajuel Laura "Lora" Mapepire balsain  (n=1186)	O H C	Trinidad	Sixty-five (99%) of 66 <i>Salmonella</i> spp. isolates exhibited resistance to one or more of the nine antimicrobial agents tested. Resistance was high to cephalothin (92%), moderate to streptomycin (35%) and tetracycline (29%), but significantly low to gentamicin (2%), chloramphenicol (0%), and sulphamethoxazole/trimethoprim (0%).	(Gopee et al., 2000)
amikacin ampicillin augmentin cefazolin cefipime cefotaxime clavulanic acid ceftazidime chloramphenicol ciprofloxacin	24 species of Enterobacteriaceae	Culture Method: Frozen bacterial isolates were cultured in a retrospective analysis and identified by	77 species (14 families) of nondomesticated Australian mammals	Most likely O, C, H	Australia	The study shows a low but widespread prevalence of antimicrobial resistance in wild isolates. Only amikacin, ciprofloxacin, meropenem and gentamicin inhibited growth in all 946 samples. Extensive	(Sherley et al., 2000)

erythromycin fusidic acid gentamicin meropenem methicillin nitrofurantoin novobiocin oxacillin penicillin piperacillin rifampicin teicoplanin tetracycline ticarcillin tobramycin trimethoprim vancomycin Kanamycin nalidixic acid neomycin spectinomycin streptomycin		biochemical profiles.  Typing Method: Not stated.  Susceptibility test: Agar disk method (The Canberra Hospital/ National Committee for Clinical Laboratory Standards, NCCLS)				variation in the combination of antibiotics to which isolates were resistant to, as well as multiple antibiotic resistance were observed. Geographical location and host group significantly influenced the antibiotic resistance profile of an isolate, whereas bacterial species influenced both the resistance profile of an isolate and the number of antibiotics it was resistant to.	
fosfomycin tetracycline minocycline streptomycin ampicillin penicillin G chloramphenicol kanamycin nalidixic acid norfloxacin cefdinir trimethoprim	Shiga toxin (Stx)-producing <i>E. coli</i> (STEC) (n=not stated)	Culture Method: 43 fecal samples were collected from animals. DHL agar and Sorbitol MaConkey agar were used as selective media for STEC.  Typing Method: RAPD DNA	Deer: Cervidae (n=not stated)	H	Japan	The study demonstrates that STEC isolates from deer might belong to the same category of STEC strains known to be pathogenic for humans. However, direct evidence for transmission of STEC from deer to humans was not evident.	(Asakura et al., 1998)

		<p>fingerprinting, PCR</p> <p>Susceptibility test: Disc diffusion, Sensi-Disc® (Becton Dickinson), PCR</p>					
<p>ampicillin streptomycin tetracycline chloramphenicol kanamycin sulfadimethoxin</p>	<p><i>E. coli</i> (n=874)</p>	<p>Culture Method: Fecal samples of animals were cultured directly onto drug-supplemented media.</p> <p>Typing Method: Conjugation test</p> <p>Susceptibility test:  Agar dilution method</p>	<p>Japanese serow: <i>Capricornis crispus</i> (n=283)</p>	H	Japan	<p>The study shows that only 12 (4.9%) serows were shown to have drug-resistant <i>E. coli</i>. No transferable R plasmid was detected among 87 resistant strains isolated from wild serows. In contrast, all 33 captive serows except one showed resistant <i>E. coli</i>. Of 161 drug-resistant strains from captive serows, 50 (31.1%) were found to carry R plasmids. Notably, wild serows seemed to readily change to harbor resistant <i>E. coli</i> almost as soon they were reared in human areas without direct exposure to drugs.</p>	(Kinjo et al., 1992)

<p>Ampicillin Cephalotin Streptomycin Sulphathiazole Tetracycline Nalidixic acid Chloramphenicol Gentamicin Kanamycin</p>	<p><i>E. coli</i> (n=74)</p>	<p>Culture Method: 81 fecal samples were collected and plated directly on media with antimicrobial drugs for isolation bacteria.</p> <p>Typing Method: DNA isolation, electrophoresis</p> <p>Susceptibility test: Disc method (Bauer, 1966)</p>	<p>Red deer: <i>Cervus elaphus</i></p> <p>Roe deer: <i>Capreolus capreolus</i></p> <p>Chamois: <i>Rupicapra rupicapra</i></p> <p>Alpine marmot: <i>Marmota marmota</i></p> <p>(n=not stated)</p>	<p>H</p> <p>H</p> <p>H</p> <p>O</p>	<p>Italy</p>	<p>The study reports that 9 of 31 specimens from red deer (29%) contained resistant strains. Different animals were likely colonized by the same resistant strain of <i>E. coli</i>. Conjugative R plasmids were found in four strains isolated from the marmot, roe deer and chamois.</p>	<p>(Caprioli et al., 1991)</p>
<p>tetracycline kanamycin ampicillin cephalothin streptomycin chloramphenicol nalidixic acid gentamicin</p>	<p><i>Salmonella</i> and <i>Shigella</i> spp.  (n=92)</p>	<p>Culture method: Fecal samples</p> <p>Typing method: DNA gel electrophoresis</p> <p>Susceptibility test: Agar method (No standard EUCAST/CLSI). Transfer of plasmid-mediated resistance was tested by the mating of 50 selected</p>	<p>Baboons (n=103)</p>	<p>O</p>	<p>Kenya</p>	<p>Resistance was significantly higher among enteric bacteria from the third group of baboons living in close proximity to a tourist lodge and having daily contact with unprocessed human refuse. Conjugation studies and analysis of the cell DNA by gel electrophoresis showed that in many cases resistance was plasmid-borne and transferable. These data suggest that wild nonhuman</p>	<p>(Rolland et al., 1985)</p>

		resistant strains with a nalidixic acid-resistant laboratory strain, <i>E. coli</i> C600.				primates in frequent contact with human debris have a higher proportion of antibiotic-resistant enteric bacteria than do conspecifics without this contact. The findings further suggest that such groups of wild animals may constitute a heretofore overlooked source of antibiotic resistance in the natural environment.	
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### 11.2.3 - Table 7. Antimicrobial resistance in small terrestrial mammals

Antimicrobial agents	Bacterial species	Methodology for sampling and determination of resistance	Animal species	Omnivorous (O) Carnivorous (C) Herbivorous (H)	Country	Results	Reference
trimethoprim ampicillin ciprofloxacin cefotaxime	<i>E. coli</i> (n, not stated)	Culture method: Faeces from animals trapped at costal and inland sites.  Typing method: Phylotyping by quadruplex PCR  Suceptibility tests: Agar dilution method (EUCAST)	Mice, voles and shrews (n=74)	O	UK	Isolates from costal site showed resistance level of 79% versus inland resistance level of 35% to at least one antibiotic.  46% of all animals carried ampicillin resistant <i>E. coli</i> .	(Furness et al., 2017)
clindamycin erythromycin moxifloxacin tetracycline metronidazole vancomycin	<i>C. difficile</i> (n=34)	Culture method: Twenty-seven farms were sampled. Pools of pig faecal samples (n = 210), samples of intestinal content from	Pig farms and pests like <b>Rats</b> ( <i>Rattus</i> sp.), <b>Mice</b> ( <i>Mus musculus</i> )  Pigeons ( <i>Columba livia</i> )	O C C  O	Canada	Resistance to clindamycin, erythromycin, moxifloxacin and tetracycline was variable (41.2, 52.9, 5.9 and 76.5% respectively). MDR was observed in 12 of 34	(Andres-Lasheras et al., 2017)

		<p>common farm pest species (n = 95) and environment-related samples (n = 93) were collected.</p> <p>Typing method: Ribotyping: PCR and PCR-RFLP toxinotyping</p> <p>Susceptibility test: E-test (CLSI)</p>	(n= see under Methodology for sampling and determination of resistance)			<p>(35.3%) of the isolates, showing all of them resistant to clindamycin, erythromycin and tetracycline.</p> <p>Rodents and pigeons may transmit toxigenic and antimicrobial-resistant <i>C. difficile</i> strains that are of the same ribotypes as those occurring in humans.</p>	
<p>amoxicillin + clavulanic acid ampicillin ceftazidime cefotaxime cefoxitin imipenem nalidixic acid ciprofloxacin gentamicin amikacin tobramycin chloramphenicol tetracycline trimetho + sulfamethoxazole</p>	<i>E. coli</i> (n=89)	<p>Culture method: Various wild small animals, rectum sampling for microbiological culture, selective agar.</p> <p>Typing method: PCR (<i>uidA</i>) and sequencing based detection of genes</p>	<p>Wood mice (n=12)</p> <p>black rats (n=40)</p> <p>European rabbits (n=42)</p> <p>white-toothed shrews (n=1)</p>	0	Southern Spain	<p><i>E. coli</i> recovered from 12 out of 95 samples from small animals. Only 1 strain from a wood mouse (<i>Apodemus sylvaticus</i>) showed resistance to antibiotics (in particular to quinolones).</p> <p><b>Multispecies</b></p>	(Alonso et al., 2016)

		Suceptibility tests: Disk diffusion (CLSI)					
Only provided as aminopenicillins, phenicols, aminoglycosides, sulfonamides, dihydrofolate reductase inhibitors, tetracyclines, cephalosporins, quinolones.	<i>S. enterica</i> serovar Typhimurium (n=117)  <i>Salmonella</i> (n=59)	Culture method: Fecal and mesenteric lymph node samples  Typing method: Sero- and phage typing, PCR  Suceptibility tests: Disk diffusion CLSI	Pig related environment wild bird faeces (n=22), rodents (n=5) and farm related environments (n=15)	0	North-eastern Spain	The majority of isolates (151 out of 176) resistant to at least one antibiotic class. The presentation of data does not distinguish between sources. The resistance pattern of the 42 samples not directly from pig not fully clear.	(Andres-Barranco et al., 2016)
Oxacillin Florfenicol Chloramphenic (PCR-detection of genes against many other antimicrobial agents)	<i>Staphylococcus aureus</i> (n=124)  <i>S. aureus</i> (n=155) isolates were recovered by culture, 124 of which were available for genotyping	Culture method: No cultivation based but DNA microarray based 28 specific genes covered . Faecal samples from 2855 animals, 16 bird and 28 mammal	A total of 2855 animals as well as a number of fecal samples: 16 bird species 28 mammal species. Fox Hares, Hedgehog	0	Germany, Austria and Sweden	Low resistance rates reported, mecA-MRSA were rare (n=6) MecC-MRSA were detected in a fox, hares and hedgehogs.  Of the 124 isolates from wildlife, 19,4% carried the <i>blaZ</i> gene, 6.5% carried the <i>mecC</i> gene or <i>blaZ</i> from the SCCmec XI. All other genes had prevalences below 5%.	(Monecke et al., 2016)



		<p>species, 124 isolates available for culture and genotyping.</p> <p>Typing method: DNA microarray hybridization, MLST</p> <p>Suceptibility tests: Performed on selected isolates (mecC for methicillin resistance- or florfenicol/chloramphenicol resistance genes cfr/fexA-positives); agar dilution (CLSI)</p>				<b>Study lack resolution to bacterial species level..</b>	
ticarcillin	Enterobacteria, <i>Klebsiella</i> spp. (n=36)	Culture method: Rectal swabs from 114 animals in Trouis-Sauts, isolated village in French Guinea. Microbial culture	Rodents (n=100) and marsupials (n=14) belonging to 16 species	O	French Guinea	<p><i>Klebsiella</i> spp. isolates naturally resistant to ticarcillin found in 31% of animals.</p> <p>Acquired resistance to ticarcillin found in 13.7% animals in the village and 2,4% of animals 600 m from the village.</p> <p>A TEM-1 penicillinase present in all strains.</p>	(Grall et al., 2015)

		Typing method: Quadruplex PCR, MLST  Suceptibility tests: PCR of several R-genes (R-standard not stated)				Most common integron gene cassettes found encoded resistance to trimethoprim, spectinomycin, streptomycin, chloramphenicol, ( <i>Mangler mat og met section</i> )	
amoxicillin + clavulanic acid ampicillin azithromycin cefoxitin ceftiofur ceftriaxone chloramphenicol ciprofloxacin gentamycin kanamycin nalidixic acid streptomycin sulfisoxazole, tetracycline, trimetho-sulfa	<i>E. coli</i> (n=397)  <i>Salmonella</i> spp. (n=3)	Culture method: Microbial culturing of colon content from rats from urban neighbourhood Vancouver. Selective culture  Typing method: Serotyping-API20E  Suceptibility tests: Broth microdilution (CLSI)	Norway ( <i>Rattus norvegicus</i> ) (n=593)  Black rats: <i>Rattus rattus</i> (n=40)	O	Canada	Of 724 rats trapped, 633 underwent analysis. 41 out of 397 isolates were resistant to one or less of the tested antibiotics, whereas 17 where multiresistant.  Salmonella was only detected in 3/633 rats. Resistance to streptomycin, trimethoprim, tetracycline and sulfisoxazole was seen among the 3 isolates.  <b>Claim low level of resistance compared to other rat/city oriented studies</b>	(Himsworth et al., 2015)
Vancomycin teicoplanin ampicillin streptomycin	<i>E. faecalis</i> , VRE (n=11)	Culture method: Faecal samples	Common voles (n=54), wood mice (n=29),	O	Spain	Study focused on vancomycin resistant Enterococcus (VRE) .	(Lozano et al., 2015)

<p>gentamycin kanamycin chloramphenicol tetracycline erythromycin ciprofloxacin trimetho-sulfa linezolid</p>		<p>thawed and cultured. 155 samples in media with or without vancomycin.</p> <p>Typing method: MLST, biochemical tests, and PCR and sequencing for species determination</p> <p>Suceptibility tests: Agar dilution, EUCAST, and disk diffusion CLSI</p> <p>The presence of 20 different R-genes were determined</p>	<p>Algerian mice (n=6), Black rats (n=46) Greater white toothed shrews (n=6), Garden mouse (n=1). Red squirrel (n=1)</p>			<p>VRE detected in 11 of 155 isolates of the following species: <i>E. faecalis</i>, <i>faecium</i>, <i>casseliflavus</i>, and <i>E. gallinarum</i>. The majority of isolates came from the black rats. Detected van genes: <i>vanB2</i>, <i>vanA</i>, <i>vanC2</i>, <i>vanC1</i>. Of 147 enterococci obtained on media free of vancomycin, 12 was erythromycin resistant and containing the <i>ermB</i> gene, Trimetho sulfa was detected in 11 isolates, 5 isolates were resistant to tetracycline and harboured the <i>tet(M)</i> gene.</p>	
<p>metronidazole clindamycin tetracycline ciprofloxacin enrofloxacin cephalothin</p>	<p>Obligate anaerobic bacteria (n=81) including <i>Bacteroides</i>, <i>Prevotella</i> and <i>Clostridium</i> species</p>	<p>Culture method: Culturing of obligate anaerobic bacteria from 81 clinical specimens including wild animals,</p>	<p>Snakes  coati-mundi (n=6)</p>	<p>C  O</p>	<p>Costa Rica</p>	<p>Most of the strains isolated from the wild animals were susceptible to the tested antibiotics, with some exceptions for metronidazole, ciprofloxacin and cephalothin.</p>	<p>(Mayorga et al., 2015)</p>

		Snakes and coati-mundi					
		Typing method: PCR, RapID 32A system, membrane fatty acid profile.					
		Suceptibility tests: E-test (CLSI), PCR detection of specific R-genes					
amoxicillin clavulanic acid ceftazidime ampicilin chloramphenicol tetracycline trimetho sulfa gentamicin ciprofloxacin	<i>E. coli</i> (n=234)	Culture method: Samples from cecal content from animals trapped on farms in forest and rice fields, selective cultivation.	<i>Rattus tanzenumi</i> (n=19)  <i>Bandicota indica</i> (n=16)  <i>Ratus norgeicus</i> (n=10)  <i>Rattus argentiventer</i> (n=9)  <i>Rattus exulans</i> (n=5)  Shrews:	O	Vietnam	Samples from 66 animals (234 isolates) examined. Prevalence of resistance to tetracycline, 25,7%. ampicillin 85,9%, trimetho sulfa 18,8%, chloramphenicol 22,5%, amoxicillin clavulanic acid 34,5%, and ciprofloxacin 7,3%. MDR was 8 times more likely on animals trapped on farms than those trapped in forest and rice fields.	(Nhung et al., 2015)

			<i>Suncus murinus</i> (n=7)				
ampicillin chloramphenicol streptomycin sulfisoxazole trimethoprim tetracycline gentamicin nalidixic acid ciprofloxacin cefotaxime	<i>Salmonella</i> spp. (n=125)	Culture method: Vicinity of pig farms with environmental samples of bird and rodent droppings. Enrichment and selective media.  Typing method: Sero- and phage typing, PFGE  Suceptibility tests: Disk diffusion (CLSI)	Wild birds (47 different species) and rodents rats (n=30) mice (n=58)  Environmental samples of bird and rodent droppings.	V	Spain	Most Salmonella (74%) isolated from wild bird samples did not show resistance.  Resistance detected in rodent samples (78%) and environmental samples (91%)-	(Andres-Barranco et al., 2014)
chloramphenicol nalidixic acid streptomycin tetracycline trimetho-sulfa colistin	<i>E. coli</i> (n=113)	Culture method: Intestinal contents collected from farm rabbits, wild rabbits and wild hares. Selective growth media.	Domestic and wild lagomorphs (rabbits ( <i>O. cuniculus</i> ) (N0102)  Hares ( <i>Lepus europaeus</i> ) (n=11)	H	Northern Italy	Study focused on plasmid mediated quinolone resistance in a collection of 113 MDR strains. Various resistance gene cassette arrays were found in class 1 integrons. 17 out of 113 isolates harboured the <i>oqxAB</i>	(Dotto et al., 2014)

		<p>Typing method: RapID E20 kit (Vitek). Resistance genes identified by PCR and sequencing. MLST</p> <p>Suceptibility tests: Agar disk diffusion and E-test, colistin (CLSI)</p>				<p>resistance gene. 16 out of 17 from farm rabbits. Colistin resistance found in 6 out of 113 strains.</p> <p>All tested strains resistant to tetracycline. More than 50% resistant to streptomycin, nalidixic acid and trimetho-sulfa. 40,7% of isolates resistance against at least 3 classes.</p>	
<p>penicillin oxacillin cefoxitin kanamycin gentamycin tobramycin tetracycline chloramphenicol trimetho-sulfa erythromycin clindamycin ciprofloxacin linezolid vancomycin mupirocin-fusidic acid</p>	<p><i>S. aureus</i> (n=13)</p>	<p>Culture method: Faecal samples from 101 wild small animals. Selective culture.</p> <p>Typing method: PCR detection of specific genes spa, agr and MLST typing.</p> <p>Suceptibility tests: agar disk diffusion, PCR detection</p>	<p>Common voles: <i>Microtus arvalis</i> (n=54)</p> <p>Wood mice: <i>Apodemus sylvaticus</i> (n=29)</p> <p>Algerian mice: <i>Mus spretus</i> (n=6)</p> <p>Brown rats: <i>Rattus norvegicus</i> (n=6)</p>	0	Spain	<p>13 of 101 faecal samples were positive for <i>S. aureus</i>. Unique samples studied further. 2 of 101 were MRSA from wood mouse. With the exception of the MRSA phenotype, the remaining isolates were susceptible to the antibiotics tested, with one exception of a penicillin resistant isolate carrying the <i>blaZ</i> gene.</p>	(Gomez et al., 2014)

		of R-genes (CLSI)	Greater white toothed shrews: <i>Crocidura russula</i> (n=5) Garden dormouse: <i>Eliomys quercinus</i> (n=1)				
ampicillin amoxicillin + clavulanic acid cefotaxime ceftazidime aztreonam imipenem gentamicin amikacin tobramycin streptomycin nalidixic acid ciprofloxacin trimetho sulfa tetracycline chloramphenicol	<i>E. coli</i> (n=77)	Culture method: 136 faecal samples from European wild rabbits in the Azores archipelago. Selective culturing.  Typing method: Biochemical tests, API20E system.  Suceptibility tests: Disk diffusion, CLSI PCR with specific primers for some resistance genes.	Wild rabbits (n=136)	H	Portugal	77 isolates further studied for resistance, 16,9% resistant to ampicillin, 1.3% to tetracycline, 42,9% to streptomycin, and low level resistance to the other tested antibiotics. Common resistance genes included <i>bla</i> TEM, <i>tetA</i> , <i>strA</i> , <i>strB</i> , <i>aacA</i> as well as some integron related genes.	(Marinho et al., 2014)

<p>ampicilin chloramphenicol chlortetracycline kanamycin streptomycin sulfadimethoxine</p>	<p><i>Enterobacteriaceae</i> Species included 26 isolates of <i>E. coli</i>, 38 isolates of <i>Klebsiella pneumoniae</i>, and 14 isolates of <i>Citrobacter freundii</i>.</p>	<p>Culture method: Collected 20 samples of faces in a cave from bats in Okinawa. Selective plating.</p> <p>Typing method: Not stated</p> <p>Suceptibility tests: Agar dilution Japanese society for chemotherapy</p>	<p>Least shoehorse bat (<i>Rhinolophus pumilus</i>)</p>	<p>C</p>	<p>Japan</p>	<p>20 feces samples randomly found in a cave- 78 isolates tested, one isolate resistant to chlortetracycline and streptomycin and 9 resistant to sulfadimethoxine, whereof 5 had a transmissible R plasmid.</p>	<p>(Obi et al., 2014)</p>
<p>ampicillin cefotaxime nalidixic acid tetracycline co-trimoxazole chloramphenicol ciprofloxacin gentamicin nitrofurantoinin amikacin imipenem ertapenem</p>	<p><i>E. coli</i> (ESBL) (n=65)</p>	<p>Culture method: Rectal swabs from rodents from 18 districts in Hong Kong. Culture and selective media.</p> <p>Typing method: Vitek GN1 system</p>	<p>Chestnut spiny rats (n=148)</p> <p>Indo-Chinese Forest rats (n=326)</p> <p>Brown rats (n=452)</p> <p>Black rats (n=39)</p>	<p>O</p> <p>O</p> <p>O</p> <p>O</p>	<p>Hong Kong</p>	<p>Faecal carriage of ESBL was found 68 of 965 samples.</p> <p>ESBL carriage less than 1% in chestnut spiny rats and indo-Chinese forest rats, 7.7% in black rats and 13,9% in brown rats. Carriage varied with district CTX-M type detected in 92% ESBL positive</p> <p>281 unique isolates found, including 77 ESBL producers.</p>	<p>(Ho et al., 2015)</p>



		Suceptibility tests: Disk diffusion (CLSI), PCR of resistance genes				Overall highest resistance level seen for brown rats, and lowest for chestnut spiny rats. Brown rats had multi-resistance in 33% of isolates. Isolates from brown rats had >30% isolates resistant to ampicillin, cefotaxime, nalidixic acid, and tetracycline.	
amikacin amoxicillin- + clavulanic acid ampicillin cefoxitin cetiofur ceftriaxone chloramphenicol ciprofloxacin gentamicin kanamycin nalidixic acid streptomycin sulfisoxazole tetracycline trimetho-sulfa	<i>E. coli</i> (n=not clearly stated)	Culture method: Colon, cecal and faecal samples from mice trapped in swine farms, for microbial culture, selective media.  Typing method: Not stated  Suceptibility tests: Broth microdilution CIPARS protocol (Canada)	Wild house mouse: <i>Mus musculus</i> (n=49)	O	Canada	The percentages positive samples to at least 1 antibiotic were 53% for colon sample to 73% for cecum sample. No resistance to amikacin, ciprofloxacin, or nalidixic acid. The frequency of tetracycline resistance varied from 44% in colon samples to 56% in faecal samples. The frequency of resistance to the other antibiotics ranged from 32% for streptomycin to 6% for chloramphenicol.	(Allen et al., 2013)

<p>amikacin streptomycin neomycin gentamycin kanamycin ampicillin amixicillin+clav. cefotaxime cefalothin chloramphenicol colistin nalidixic acid enrofloxacin ciprofloxacin trimetho-sulfa oxytetracycline tetracycline</p>	<p><i>Salmonella</i> spp. (n=130)</p>	<p>Culture method: Large collection of samples, including dead animals from the wild, hunted or from rehabilitation centres. Microbiological culture.</p> <p>Typing method: Serological typing.</p> <p>Suceptibility tests: Disk diffusion (CLSI)</p>	<p>2713 wild small animals in North-western Italy (canids (n=1222), mustelids (n=221), birds (n=1101), rodents (n=100), ungulates (n=69) including red fox, marten, common kestrel, badger, common pigeon, wild boar, carrion crow, red deer, scops owl, golden eagle, black grouse, tawny owl, short-toed snake eagle, peregrine falcon.</p>	<p>O/C/H</p> <p>Difficult to specify diets for all animal groups used.</p>	<p>Italy</p>	<p>4.3% of samples positive for salmonella. <i>S.</i> Thypimurium most common serotype.</p> <p>Disk diffusion test on 88 strains, 97,5 % showed resistance to at least one class of antibiotics, with highest resistance towards tetracycline class (&gt;80% of strains), streptomycin (47%) and ampicillin (14%) Resistance to most other antibiotics tested varied between 0-5%.</p>	<p>(Botti et al., 2013)</p>
<p>ampicillin chloramphenicol streptomycin sulphonamides trimethoprim tetracycline nalidixic acid ciprofloxacin</p>	<p><i>S. enterica</i> (n=6)</p>	<p>Culture method: Hedgehogs large intestine samples close to rectum, enrichment and culturing.</p>	<p>Hedgehogs (n=25)</p>	<p>O</p>	<p>Burkina Faso</p>	<p>Salmonella present in 96% of hedgehog samples. 6/25 isolates resistant to one or more of the tested antimicrobials. The detected resistance was to streptomycin and</p>	<p>(Kagambega et al., 2013)</p>

cefotaxime mecillinam		Typing method: API20E, serotyping, phage typing, PFGE.  Suceptibility tests: Standard disk diffusion method and E-test for ciprofloxacin (CLSI)				in one case sulphonamides  <b>Also other animals</b>	
32 different antibiotics	<i>E. coli</i> (n, not specified)	Culture method: Rectal swabs from 198 wild animals living in proximity to a village. Cultivation.  Typing method: PCR, sequencing, and MLST  Suceptibility tests: Disk diffusion method (standard not stated)	<i>Wild mammals species: Proechimys cuvieri, Nectonmys melanius, Oryzomys megacephalus, Oecomys bicolor, Rhinophylla pumilio, Carollia perspicillata, Molossus molossus</i>  (n>6 each)	H H H O O C	French Guinea	<i>E. coli</i> only recovered from 45% of wild animals (n=198), rare genotypes found and no antibiotic resistance detected.	(Lescat et al., 2013)

<p>oxacillin cefoxitin penicillin cefquinome tetracycline ciprofloxacin gentamicin chloramphenicol erythromycin clindamycin teicoplanin trimethoprim/sulfamethoxazole linezolid rifampicin</p>	<p>Staphylococci (n=6)</p>	<p>Culture method: Small intestine or other organs at postmortem, nasal and perineal swabs from other animals were obtained for bacteriological analysis.</p> <p>Typing method: PCR, spa typing, MLST, 16S rDNA sequence analysis</p> <p>Susceptibility test: Agar disc diffusion (CLSI)</p>	<p>Hares: (n=152)  40 different wild animals:  European brown hare  European otter  European hedgehog  Eurasian lynx</p>	<p>Most probably O/H/C</p>	<p>Austria</p>	<p>The study reports the five MRSA isolates contained the mecC gene, were PVL negative, carried SCCmec type XI and belonged to ST130 (where ST stands for sequence type), with spa types t843, t10513 or t3256, or to ST2620, with spa type t4335. The MRnSA isolate, most closely related to <i>S. stepanovicii</i>, carried <i>mecA</i> and <i>blaZ</i> genes related to SCCmec XI. MRSA isolates exhibited resistance to the beta-lactams only.</p>	<p>(Loncaric et al., 2013)</p>
<p>ampicillin streptomycin ciprofloxacin erythromycin linezolid quinupristin + dalbapristin gentamicin teicoplanin penicillin nitrofurantoin tetracycline</p>	<p><i>Enterococcus</i> spp. (n=115)</p>	<p>Culture Method: 103 rectal or cloacal swabs from animals of various species and 12 milk samples from</p>	<p>Fox: (n=54)  Beech marten: (n=9)  European hamster (n=2)  Rat (n=1)</p>	<p>Most probably O, H, C</p>	<p>Poland</p>	<p>The study reports that all strains, regardless of source, were susceptible to 13-lactams, gentamicin, linezolid, and teicoplanin; the highest resistance was to kanamycin, quinupristin, and rifampicin. Despite the relatively low</p>	<p>(Nowakiewicz et al., 2014)</p>

vancomycin chloramphenicol enrofloxacin rifampicin bacitracin kanamycin		cattle were collected and cultured on agar and identified by commercial microtest EN-COCCUStest.  Typing Method: 16S-23S rRNA intergenic spacer region (ITS-PCR)  Susceptibility test: Disk diffusion (CSLI), Agar screen test (Statistica version 8.0 (Statsoft Polska, Krakow, Poland))	Beaver (n= 1)  Bat (n=1)  Hedgehog (n=2)  Russian tortoise (n=17)  Dog (n=2)  Cat (n=2)  Cattle (n=12)  Poultry (n=12)			level of resistance in the strains isolated from wild and exotic animals, the large number of intermediately susceptible strains in these groups is an indication of the evolutionary character of the development of resistance, suggesting that these animals may be potential reservoirs of Enterococcus strains resistant to a wide panel of currently used antibiotics.	
ampicillin amoxicillin clavulanic acid, cefoxitin cetiofur ceftriaxone streptomycin kanamycin gentamicin	<i>E. coli</i> (n=523)	Culture method: Faecal isolates from trapped animals, culturing. (182 samples from 109 raccoons)	Raccoons: <i>Procyon lotor</i> (n=109)	0	Ontario, Canada	Reduced susceptibility to one or more antibiotic found in 19% of the 16 racoons from urban sites, 17% of 29 racoons at rural site, and 42 out of 130 samples from the Zoo site.	(Jardine et al., 2012)

<p>amikacin tetracycline chloramphenicol sulfisoxazole trimetho-sulfa nalidixic acid ciprofloxacin</p>		<p>Typing method: serotyping, PFGE.</p> <p>Suceptibility tests: Broth microdilution (NCCLS/CLSI)</p> <p>PCR of selected genes.</p>				<p>Resistance to extended spectrum cephalosporins were rare and observed in a few single individuals at the zoo (n=2) and the rural site (n=1).</p>	
<p>penicillin ampicillin oxacilin erythromycin tetracycline gentamicin novobiocin cefoxitin</p>	<p>Staphylococci (n=113)</p> <p>111 isolates were coagulase-negative. Main species among isolates <i>S. warneri</i>, <i>epidermidis</i>, and <i>S. pasteurii</i>.</p>	<p>Culture method: Samples isolated from thigh muscle meat of healthy farmed and wild rabbits.</p> <p>Typing method: MALDI biotyper to determine species.</p> <p>Suceptibility tests: Agar dilution (CLSI)</p>	<p>Wild rabbits: <i>Oryctolagus cuniculus</i> (n=30)</p>	H	Slovakia	<p>113 strains of staphylococci isolated. Only two isolates Coagulase positive, the remaining Coagulase negative. Only one isolate susceptible to all antibiotics tested. Major resistances: ampicilin (78.8%), erythromycin (58,4%), penicillin (51,3%), oxacilin (46%). Resistance to cefoxitin and gentamicin were rare. Fifteen percent of isolates were resistant to one antibiotic, simultaneous resistance to two, three, four and five antibiotics was confirmed in 22.1%, 23.9%, 21.2% and</p>	(Pipova et al., 2012)

						13.3% of isolates, respectively.	
azithromycin ciprofloxacin erythromycin gentamicin florfenicol nalidixic acid telithromycin clindamycin	<i>Campylobacter</i> (n=13)	Culture method: Livestock farms Faecal swabs. Selective culture  Typing method: Species specific PCR, MLST, PFGE, resistance gene location, AR profile.  Suceptibility tests: Microbroth dilution (CLSI), PCR of tet(O) and gyrA	Wild small animals (n=142)  Birds 19 bird species (n=188)	O	USA	<i>Campylobacter</i> found only in wld birds. Of the 188 birds sampled. 9 positive isolates for <i>Campylobacter</i> (prevalence 4.8%) from 3 birds: originated from House sparrow, rose-breasted grosbeak, white-throated sparrow. Two isolates from house sparrow resistant to tetracycline, ciprofloxacin and nalidixic acid. A plasmid location of the <i>tet(O)</i> gene was indicated in both cases. A single point mutation in the <i>gyrA</i> identified (fluoroquinolones resistance).	(Sippy et al., 2012)
amikacin amoxicillin-clavulanic acid ampicillin cefoxitin ceftriaxone chloramphenicol ciprofloxacin gentamicin kanamycin	<i>E. coli</i> (n=163) <i>S. enterica</i> (n=4)	Culture method: Faecal samples from wild small animals living in swine farms (9 farms), residential areas (10 areas) , landfills (8	Deer mouse: <i>Peromyscus</i> sp. and White footed mouse: <i>Peromyscus</i> (n=171 combined)  House mouse: <i>Mus musculus</i> (n=65)	O	Canada	Resistance in 25/52 trapped animals in swine farms, 6/69 in residential areas, 3/20 in landfills, and 1/22 in natural habitats. Animals trapped on farms had the highest level of resistance. Salmonella was found in 4/302 samples and pan-susceptible. Swine farm origin was significantly	(Allen et al., 2011)

nalidixic acid streptomycin sulfoxazole tetracycline trimethoprim-sulfa		landfills), and natural habitats 9 habitats), Selective culture.  Typing method: PCR  Suceptibility tests: Broth microdilution (CIPARS protocol, Canada), PCR for AMR genes	Meadow vole: <i>Microtus pennsylvanicus</i> (n=6)  Short-tailed shrew: <i>Bravina brevicauda</i> (n=32)  <i>Rattus norvegicus</i> (n=1)  Eastern chipmunk: <i>T. striatus</i> (n=27)			associated with the presence of resistant bacteria although they were found in all environments sampled.  Resistant fecal bacteria were found in small mammals living in all environments studied, indicating that environmental exposure to antimicrobials, antimicrobial residues, resistant bacteria, or resistance genes is widespread.	
ampicillin cefotaxime chloramphenicol ciprofloxacin florfenicol gentamicin kanamycin nalidixic acid streptomycin sulfamethoxazole tetracycline trimethoprim	Enteropathogenic <i>Yersinia</i> spp. (n=186)	Culture method: Strain collection obtained between 1995-2009  Typing method: Serotyping  Suceptibility tests: Broth microdilution (CLSI)	<b>Wild boars</b> (Switzerland), Monkeys (n=2) (Croatia) Chinchilla (n=2) (Germany) FOR <i>Y. enterocolitica</i>  and  Capybara (n=3), Mara (n=4) (both from Germany)	O, H	Switzerland, Croatia, Germany	<i>Studier med mange dyr og land kilder</i>  All <i>Y. enterocolitica</i> strains (n=186) susceptible to cefotaxime, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, kanamycin, nalidixic acid, and trimethoprim.  5/5 wild boars with <i>Y. enterocolitica</i> 2/O:9 strains showed resistance to ampicillin and 1/5 to streptomycin.	(Bonke et al., 2011)



		<p>PCR analyses of resistance genes</p> <p>Double disk diffusion (CLSI)</p>	<p><i>Y. pseudotuberculosis</i></p>			<p>For the 4&gt;/O:3 type 6/6 showed resistance to ampicillin.</p> <p>Only few isolates from Chinchilla and monkey but also with resistance to ampicillin and streptomycin. All ampicillin resistant isolates carried the <i>blaA</i> and <i>blaB</i> genes</p> <p>All tested isolates of <i>Y. pseudotuberculosis</i> (n=12) was sensitive to all tested antibiotics.</p>	
<p>ampicillin cefazolin streptomycin kanamycin gentamicin apramycin chloramphenicol oxytetracycline nalidixic acid enrofloxacin</p>	<p><i>E. coli</i> (n=81)</p>	<p>Culture method: Rectal swabs from rodents sampled in forest in national park, Hokkaido Cultivation, selective media.</p> <p>Typing method: PCR</p> <p>Suceptibility tests: Agar dilution (CLSI),</p>	<p>Wild mice Voles (n=109)</p> <p>Japanese field mice (n=52)</p> <p>Small Japanese field mice (n=19)</p>	O	Japan	<p>78 of the 81 isolates tested susceptible to all antibiotics tested. One isolate multiresistant (harbouring <i>blaTEM</i>, <i>strA</i> and <i>strB</i>, <i>aphA1</i>, <i>cat1</i>, and <i>tetB</i>. Two isolates resistant to tetracycline (<i>tetA</i>).</p> <p>Overall low prevalence of resistance among wild mice that inhabited the forest.</p>	(Ishihara et al., 2011)

		Detection of specific resistance genes by PCR.					
ampicillin chloramphenicol chlortetracycline kanamycin streptomycin nalidic acid sulfadimethoxine	<i>E. coli</i> (n=128)	Culture method: 128 isolates Microbial culture  Typing method: PCR  Suceptibility tests: Agar dilution (CLSI),  PCR for specific resistance genes.	Small Asian mongoose (n=26)  Japanese weasel (n=18)	C	Japan	19 out of 67 isolates from mongoose resistant to at least one antimicrobial, and 36 out of 61 isolates from Japanese weasel. The highest resistance level for mongoose isolates was to sulfadimethoxine 25,4%. For Japanese weasel chlortetracycline (39.9%), nalidic acid (34.45) and ampicillin (31.1%). <i>bla</i> TEM genes detected in 84% of amp resistant isolates. <i>tetA</i> and <i>tetB</i> were also frequently detected. ESBL not reported.	(Nakamura et al., 2011)
ampicillin apramycin chloramphenicol tetracycline trimethoprim	<i>E. coli</i> (n=345)	Culture method: Faecal samples (878 from bank voles +1140 from wood mice) Culturing and biochemical methods.	Bank voles (n=248)  Wood mice (n=308)	H	UK	A study including longitudinal variation. A total of 878 faecal samples from bank voles (248 animals) and 1140 wood mice samples (308 animals) were obtained. Animals were trapped/released and individually monitored over 2 years (microchip tagged).	(Williams et al., 2011)

		<p>Typing method: Serotyping, API 20E.</p> <p>Suceptibility tests: Disk diffusion (CLSI)</p>				<p>27% to 20% of animals sampled at least 5 times over the study period. Approx. 20% of <i>E. coli</i> isolates tested positive to at least one antimicrobial. 14.6% to at least 2 antimicrobials. High prevalence of ampicilin, chloramphenicol, tetracycline, and trimethoprim resistant <i>E. coli</i> observed.</p> <p>Higher prevalence of resistance in wood mice 241/1012 isolates resistant to at least one antibiotic, whereas 104 out of 702 in bank voles. The prevalence in both species increased over time. Seasonal peak in spring-summer-fall months.</p>	
17 antimicrobials including beta-lactams, aminoglycosides, tetracyclines, sulphonamides, chloramphenicol and fluoroquinolones	<i>E. coli</i> of CTX-M-ESBL type (n=211)	<p>Culture method: Brown rats collected in the city area of Berlin were sampled by rectal swabs.</p> <p>Typing method: MLST, PCR</p>	Brown rat: <i>Rattus norvegicus</i> (n=66)	O	Germany	<p>211 unique Isolates for agar disk diffusion tests. Prevalence of the CTX-M-ESBL type 33 % exhibited resistance to multiple antibiotics One isolate was of CTX-M-9 type.</p>	(Guenther et al., 2010a)

		Suceptibility tests: Agar disk diffusion Broth microdilution (CLSI)					
ampicillin streptomycin spectinomycin chloramphenicol gentamicin tetracycline	<i>E. coli</i> (n=188)	Culture method: Faecal isolates from animals from farmland and natural reserve. Microbial culturing  Typing method: PCR determination of bacterial species.  Suceptibility tests: Agar diffusion (CLSI) and PCR analyses of resistance genes	Yellow-necked mouse (n=408)  Wood mouse (n=95)  Striped field mouse (n=124)  Bank vole (n=572)  Field vole (n=40)  Common vole (n=38)  House mouse (n=1)  European pine vole (n=4)  Greater white-toothed shrew (n=1)	O	Germany	<i>E. coli</i> strains isolated from 188 of 1443 gut samples. Only 5.5% of isolate tested positive to the used antibiotics. Ampicillin and tetracycline resistance most common although overall low prevalence. PCR tests of some common resistance genes and <i>StrA</i> most common.	(Guenther et al., 2010b)

<p>amikacin amoxicillin clavulanic acid ampicillin chloramphenicol streptomycin trimetho sulfa tetracycline tobramycin</p>	<p>Salmonella (n=16)</p>	<p>Culture method: Faeces samples, standard culture, selective media.</p> <p>Typing method: serotyping</p> <p>Suceptibility tests: Resistance profile method not described. PCR of specific resistance genes.</p>	<p>Wild rabbits: <i>Oryctolagus cuniculus</i> (n=5)</p>	<p>H</p>	<p>Portugal</p>	<p>16 isolates from wild animals, one <i>S. enteritidis</i> recovered from rabbit sensitive to all tested antibiotics, <i>S. Thypimurium</i> isolates (n=3) most resistant to ampicillin, tetracycline, streptomycin and chloramphenicol. Genomic and proteomic focused analyses. Study not aimed at quantitative-surveillance as such.</p>	<p>(Pinto et al., 2010)</p>
<p>amoxicillin clavulanic acid chloramphenicol gentamicin streptomycin tetracycline ampicillin cephalexin cefotaxime penicillin G enrofloxacin trimetho sulfa</p>	<p><i>Salmonella</i> (n=5)</p>	<p>Culture method: Faecal samples (scats n=66) selective cultures</p> <p>Typing method: serotyping - AP20E and antiserum</p> <p>Suceptibility tests: Disk diffusion (CLSI)</p>	<p>Eurasian otters (<i>Lutra lutra</i>)</p>	<p>C</p>	<p>Portugal</p>	<p>67 samples tested, 5 positive for Salmonella (7,6%) of the species <i>S. enterica</i> and <i>S. gallinarum</i>. All <i>Salmonella</i> isolates resistant to multiple antibiotics. All isolates resistant to amoxicillin clavulanic acid, ampicillin, cephalexin, and penicillin G. All isolates susceptible to cefotaxime and trimetho sulfa.</p>	<p>(Oliveira et al., 2010)</p>

ampicillin amoxicillin clavulanic acid cefoxitin cefotaxime ceftazidime aztreonam imipenem gentamicin amikacin tobramycin streptomycin nalidixic acid ciprofloxacin trimethoprim sulfa tetracycline chloramphenicol  Enterococci: vancomycin teicoplanin ampicillin streptomycin gentamicin kanamycin chloramphenicol tetracycline erythromycin quinopristin- dalfopristin ciprofloxacin	<i>Enterococcus</i> species (n=64)  <i>E. coli</i> (n=44)	Culture method: Intestinal samples selective culture,  Typing method: Serotyping - API20E for <i>E.</i> <i>coli</i> and APIID20 strep for enterococci. Further PCR to the species level.  Suceptibility tests: Agar disk diffusion (CLSI), PCR of selected resistance genes	Wild rabbits: <i>Oryctolagus</i> <i>cuniculus</i> (n=77)	H	Portugal	44 <i>E. coli</i> and 64 enterococci isolated from 77 intestinal samples obtained. <i>E. faecalis</i> and <i>E. faecium</i> most dominant species.  <i>E. coli</i> . No ESBL isolates. 38 out of 44 susceptible to all tested antibiotics. Ampicillin, tetracyclin and trimethoprim sulfa most common in other 6 isolates. Th <i>bla</i> TEM, <i>tetA</i> and <i>tetB</i> detected among other resistance genes.  Enterococci. 29.7% of enterococci isolates were resistant to tetracycline, 20.3% to erythromycin 14.1% to ciprofloxacin, and 10.9% to kanamycin. No vancomycin resistance detected.	(Silva et al., 2010)
nalidixic acid amikacin ampicillin cefotaxime erythromycin norfloxacin penicillin sulfamethoxazole/	<i>E. coli</i> (7), <i>Proteus</i> <i>mirabilis</i> (9), <i>Citrobacter freundii</i> (6), <i>Edwardsiella</i> sp. (5), <i>Klebsiella</i> <i>pneumoniae</i> (5), <i>Pseudomonas</i> <i>aeruginosa</i> (6),	Culture method: Six captured animals. Samples taken from various parts of six animals, with	Virginia opossum ( <i>Didelphis</i> <i>virginiana</i> ) (n=6)	O	Mexico	Multidrug resistance was found in some of the strains or at least one of them, with the exception of <i>B. subtilis</i> . Highest levels of resistance to tetracycline, penicillin, and ampicillin.	(Barrios-Garcia et al., 2009)

trirnethoprim tetracycline	<i>Mannheimia haemolytica</i> (1), <i>S. aureus</i> (6), <i>S. epidermis</i> (9), <i>Streptococcus faecalis</i> (3), <i>Corynebacterium</i> sp. (2), <i>Bacillus subtilis</i> (n=2)	microbial cultured  Typing method: Not stated  Suceptibility tests: Disk diffusion (CLSI)					
vancomycin teicoplanin ampicillin streptomycin gentamycin kanamycin chloramphenicol tetracycline erythromycin quinopristin- dalfopristin ciprofloxacin	<i>E. faecium</i> , <i>E. gallinarum</i> and <i>E. casseliflavus</i> (n=not stated)	Culture method: Faecal samples (77) from 7 wild rabbits. 3 g initial sample per animal. Enrichment, culturing.  Typing method: an PCR  Suceptibility tests: Disk diffusion (CLSI), detection of vancomycin resistance genes by PCR.	Wild rabbits (n=77)	H	Portugal	<i>VanA</i> containing enterococcal strains in 3 out of 77 rabbit samples. (3.9% of analysed animals). Same strains also resistant to teicoplanin, tetracycline and erythromycin. 6 out of 11 vancomycin resistant strains also resistant to erythromycin, and 6/11 to ciprofloxacin.  The intestinal tract of wild rabbits could be a reservoir of vanA-containing enterococci.	(Figueiredo et al., 2009)
ampicillin amoxicillin	<i>E. coli</i> (n=37)	Culture method:	Mice, Voles, Shrews		Ontario, Canada	Animals living near farms were 5 times more likely to carry tetracycline	(Kozak et al., 2009)

clavulanic acid cetiofur ceftriaxone kanamycin gentamycin amikacin tetracycline chloramphenicol sulfisoxazole trimetho sulfa nalidixic acid ciprofloxacin		Intestinal content were added to liquid growth media, selective media and or enrichment  Typing method: Multiplex PCR  Susceptibility tests: Broth microdilution (Canadian Integrated programme for antimicrobial resistance surveillance), PCR detection of 15 selected genes	(n=86)			resistance than animals living in natural areas. Higher rates of resistance and more multiresistance in small animals living near farms. Wild small animals on farms varied from not detected to 24 % prevalence of tetracycline resistance (in 6 out of 22 animals). In natural areas, in most cases, resistance were not observed but 3 out of 37 isolates were ampicillin resistant and 2 out of 37 tetracycline resistant.	
amoxicillin + clavulanic acid ampicillin cephalothin ceftazidime chloramphenicol ciprofloxacin gentamicin nalidixic acid streptomycin trimetho-sulfa	<i>Enterobacteriaceae</i>	Culture method: Rectal swabs from wild rodents and bats. Selective culturing.  Typing method: Serotyping - API10S	Wild rodents (n=45) including Black rats (n=33) and 3 species of bats (n=24) chiropterans	O	Senegal	37 isolates from wild rodents, and 24 from bats further tested. Two resistant isolates from black rats; one to tetracycline with <i>tetA</i> gene and one multiresistant to sulphonamides, trimetho sulfa, and tetracycline with <i>su12</i> and <i>tetA</i> genes detected.	(Literak et al., 2009)



<p>sulphonamides tetracycline</p>		<p>Susceptibility tests: Disk diffusion CLSI, PCR of specific resistance genes and gene cassettes.</p>				<p>One isolate from bat, (<i>Micropteropus pusillus</i>) tetracycline resistant carrying the <i>tetA</i> gene.</p>	
<p>ampicillin amoxicillin apramycin doxycycline erythromycin gentamycin neomycin oxytetracycline spectinomycin tetracycline trimetho-sulfa sulphonamides</p>	<p>Enterobacteriaceae-e (<i>E. coli</i>, <i>Proteus</i>, <i>Klebsiella</i>) (n=133)</p>	<p>Culture method: Samples from trapped rats captured at the city port of Piraeus, Greece. Microbiological culture, selective media, staining.</p> <p>Typing method: Serotyping API20E</p> <p>Susceptibility tests: Disk diffusion (CLSI)</p>	<p>Brown rats: <i>Rattus norvegicus</i> (n=25)</p>	<p>O</p>	<p>Greece</p>	<p>Intestinal enterobacteria by mucosal scrubbings and mouth swabs with 133 isolates in total. 61,5% resistant to more than 8 of the 12 antibiotics tested. In most cases, more than 50% of the species/spp isolates were resistant to any given single antibiotic tested.</p>	<p>(Burriel et al., 2008)</p>
<p>amoxicillin chloramphenicol gentamycin</p>	<p><i>Morganella morganii</i>, <i>Stenotrophomonas maltophilia</i></p>	<p>Culture method: Samples from squirrels</p>	<p>Thirteen-lined ground squirrels:</p>	<p>O</p>	<p>WI, USA</p>	<p>The resistance patterns were characteristic for the species identified, Multiresistant strains of</p>	<p>(Cloud-Hansen et al., 2007)</p>

nalidixic acid streptomycin tetracycline	(n=85)	collected in the wild or born in animal facility (USA) Frozen cecal tissue samples, extraction and culturing  Typing method: PCR, Determination of isolates by 16S rRNA sequencing  Susceptibility tests: Broth dilution series (Standard clinical laboratory procedures, Hindler and Tamashiro, 2004)	<i>Spermophilus tridecemlineatus</i> (n=23)			<i>Morganella morgannii</i> and a multiresistant strain of <i>Stenotrophomonas maltophilia</i> reported. Study was not quantitative.	
Genes	Enterococci (n=3851)  <i>E. coli</i> (PCR-detection)	Culture method: Water samples and fecal swabs were collected.  Typing method: PCR	Rabbits (n=971)  Dogs (n=872)  Birds (n=922)  Cats	H	USA	The study suggests that bird and wild animal feces, soil amendments, and/or fecal coliform growth in the storm drain are the major contributors to the fecal bacterial pollution in downstream areas.	(Jiang et al., 2007)

		Susceptibility test: Antibiotic resistance analysis; ARA (SAS software version 8; SAS Institute), PCR	(n=48) Unknown animal (n=224)			Enterococci ARA results supported this conclusion and indicated that fecal bacteria from bird and wild animal feces as well as soil were the predominant source found in the watershed.	
vancomycin teicoplanin ampicillin streptomycin gentamicin kanamycin chloramphenicol tetracycline erythromycin quinupristin + dalfopristin ciprofloxacin	Enterococci (n= 140)	Culture method: Selective plating  Typing method: biochemical tests. API ID20 Strep, and species specific PCRs.  Susceptibility tests: Disk diffusion (NCCLS) and agar dilution (NCCLS) PCR of specific resistance genes.	Wild animals birds of prey (n=14), owls (n=10) foxes (n=7), rabbits (n=6), European genets (n=5), forest wild cats (n=4), storks (n=3), magpies (n=3), deer (n=3), vipers (n=3), otters (n=3), wolves (n=2), mufflon (n=2), badgers (n=2), partridge (n=1), hedgehog (n=1), pigeon (n=1), ferret (n=1), quail (n=1), wild boar (n=1).	O/H/ C	Portugal	In total, isolates from 77 wild animals examined. A total of 140 enterococci recovered. Most prevalent were <i>E. faecalis</i> (n=73) and <i>E. faecium</i> (n=45).  31.4% of all isolates susceptible to all antibiotics tested, Quinupristin-dalfopristin (50,7%) tetracycline (28.6%) and erythromycin (20,1%) were the most common resistance traits. Vancomycin resistance rarely detected. <i>Tet(M)</i> gene found in all tetracycline resistant isolates. The most resistant isolate recovered from an owl.	(Poeta et al., 2005)

benzylpenicillin cefoxitin chloramphenicol ciprofloxacin clindamycin erythromycin florfenicol fusidic acid gentamicin kanamycin linezolid mupirocin rifampin sulfamethoxazole streptomycin quinupristin- dalfopristin tetracycline thiamulin trimethoprim vancomycin	<i>S aureus</i> (n=361)	Culture method: Strain collection  Typing method: PCR of mecC gene, MLST  Susceptibility tests: Broth microdilution (EUCAST)	Various animals: wild animals (n=254)	O, H, C	Spain	Study focused on the occurrence of MRSA with the mecC gene. Retrospective investigation of a collection of 361 isolates including wild animals (n=254). 1% prevalence in 361 tested isolates None detected in wild small animals (not described), but in wild boar =1, fallow deer =2.	(Porrero et al., 2014b)
ampicillin chloramphenicol ciprofloxacin kanamycin streptomycin sulphamethoxazole tetracycline trimethoprim	Enterobacteriaceae (n=61)	Culture method: Rectal swabs cultured.  Typing method: serotyping - API20E strips  Susceptibility tests: MICs determined on ISO-Sensitest agar according to the method of the British	Magpies ( <i>Pica pica</i> ) (n=37) Rabbits ( <i>Oryctolagus cuniculus</i> ) (n=13)	O  H	UK	All 61 Enterobacteria tested from 13 rabbits were susceptible to the tested antibiotics with the exception of one isolate resistant to tetracycline. In contrast, 8 out of 20 magpies yielded antibiotic-resistant Enterobacteria with 25/97 isolates resistant to on or more antibiotic with tetracycline being most common.	(Livermore et al., 2001)

		society for antimicrobial chemotherapy  PCR of selected resistance genes.					
	<i>Enterobacteriaceae</i> (n=180)	Culture method: Faeces from two wild populations of rodents bank voles, and wood mice  Typing method: Not provided (brief comm., Nature)  Suceptibility tests: Broth dilution as well as Disk diffusion tests (BSAC guide to sensitivity testing)	Bank voles (n=38)  Wood mice (n=70)	H	UK	90% of coliforms were resistant to either amoxicillin/clavulanic acid, amoxicillin, and cefuroxime.	(Gilliver et al., 1999)
ampicillin chloramphenicol sulfamethoxazole trimethoprim gentamicin	Enterobacteria (n=not specified)	Culture method: Rectal swabs from trapped animals	Rats (n=32) Bats (n=27)	O	Indonesia (sampling)  Australia (analyses)	Variable resistance patterns between animal host species, bacterial species and island sampled. For instance 2 out of 20 <i>E. coli</i> isolates	(Graves et al., 1988)

cephalotin tetracycline		<p>Samples stored for 2-5 weeks at 30 degrees in Stuarts transport medium. Microbial culture in selective media.</p> <p>Typing method: Standard microbiological diagnostic procedures</p> <p>Susceptibility tests: Replica plating on agar plates</p>				<p>was tetracycline resistant (from 2 out of 12 rats) in Java. No <i>E. coli</i> isolates taken from another island was tetracycline resistant.</p>	
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