Neonatal sepsis and the adverse effects of antibiotic treatment – a systematic review

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Abstract

Objective: Antibiotic treatment in the neonatal period can be life-saving, but overuse is frequent. Recently studies have shown adverse effects from neonatal antibiotic treatment. This study aims to systematically review the litterature on the relationship between neonatal antibiotic treatment and changes in gut microbiota, the risk of necrotizing enterocolitis (NEC), and the risk of fungemia.

Methods: We performed a systematic search in the Pubmed, Embase, and Medline databases up to December 2014. We included studies that assessed the effect of neonatal antibiotic treatment on the following outcomes; (1) change in gut microbiota, (2) NEC, and (3) fungemia. Abstracts were considered for eligibility by two researchers.

Results: We included 14 studies in the microbiota category, twelve in the NEC category, and eleven in the fungemia category. The studies used very different designs and often had small study samples. Neonatal antibiotic treatment appeared to decrease gut microbiota diversity and the total bacteriall count, but findings were inconclusive on different bacteria. Neonatal antibiotic treatment, particularly prolonged treatment, appeared to increase the risk of NEC. Antibiotic treatment appeared protective of NEC in sepsis patients. Broad-spectrum antibiotic treatment appeared to increase the risk of fungemia.

Conclusions: Neonatal antibiotic treatment appears to have an effect on gut microbiota, the risk of NEC, and the risk of fungemia. However, the methodological quality was

poor in many studies, and more studies, preferably prospective with large study populations, are needed.

Abbreviations

EOS; early onset sepsis

LOS; late onset sepsis

LB; live born

GA; gestational age

BW; birth weight

VLBW; very low birth weight

GBS; *group B Streptococci*

E. coli; *Escherichia coli*

S. aureus; *Staphylococcus aureus*

CoNS; *coagulase-negative Staphylococci*

PROM; prolonged rupture of membranes

IAP; intrapartum antibiotic prophylaxis

CDC; Centers for Disease Control and Prevention

CVC; central venous catheters

PN; parenteral nutrition

NICU; neonatal intensive care unit

NEC; necrotizing enterocolitis

RCT; randomized controlled trial

OR; odds ratio

CI; confidence interval

CRP; C-reactive protein

PCT; procalcitonin

NNT; number needed to treat

MIC; minimum inhibitory concentration

PSC; peak serum concentration

TSC; trough serum concentration

V_D; volume of distribution

BNF; British National Formulary

NICE; British National Institute for Health Care and Excellence

AAP; American Academy of Pediatrics

1 Introduction

Neonatal sepsis is a rare, but potentially fatal blood stream infection occurring in the first month of life.[1] According to the World Health Organization, sepsis caused approximately 12% of the 2.9 million neonatal deaths in 2012.[2] In particular, premature infants are highly susceptible to infection in the neonatal period due to an immature immune system. The innate immune system was previously considered fully developed at birth, but recent studies have shown impaired skin and gut flora and lower activity of dendritic cells, cytokines, neutrophils, and the complement system in the neonatal period, especially in preterm infants.[3] Additionally, preterm neonates are often subject to prolonged hospitalization and invasive treatment, increasing the risk of infection.[4]

Neonatal sepsis is commonly divided into two types based on the onset of disease. Early onset sepsis (EOS) is typically defined as occurring in either the first 48-72 hours of life or the first week of life.[5-7] Late onset sepsis (LOS) is defined as sepsis occurring after this time. EOS and LOS have different modes of transmission, causative pathogens, and recommendations for treatment.

1.1 Epidemiology of neonatal sepsis

1.1.1 Early onset sepsis – incidence, risk factors, prevention, and outcome

The incidence of EOS has steadily decreased during the last 25 years in developed countries.[8] The current incidence lies between 0.5 – 1.2 per 1000 live born (LB) infants in the western world.[5,6,9] The majority of EOS patients have a gestational age (GA) ≥ 30

and birth-weight (BW) ≥ 1500 g.[5,8] However, the incidence of EOS is highest among preterm and very-low BW (VLBW) infants (BW < 1500 g).[6,10] The majority of EOS cases become symptomatic in the first day of life.

Gram-positive bacteria have been reported to cause between 60-80% of EOS-cases with Gram-negative bacteria causing the remainder of EOS.[5,6] The most common causative pathogens are *group B Streptococci* (GBS) followed by *Escherichia coli* (*E. coli*). In a US multi-centre study, Stoll *et al.* reported that GBS caused 43% and *E. coli* caused 29% of culture-confirmed EOS cases,[6] while Vergnano *et al.* found a GBS proportion of 58% and an *E. coli* proportion of 18% in an English multi-centre study.[5] While GBS EOS is more frequent among term infants, *E. coli* EOS is more frequent among preterm infants.[8,10] Other pathogens associated with EOS are *Staphylococcus aureus (S. aureus)*, *Coagulase-negative Staphylococci* (CoNS), *viridans-group Streptococci*, *group A Streptococci*, *Enterococci*, *Listeria*, *Bacteriodes*, and *Klebsiella*.

Pathogens are typically transmitted vertically during birth. Risk factors such as caesarean section, the use of epidural analgesia, premature birth (gestational age (GA) < 37 weeks), prolonged rupture of membranes (PROM; ≥ 12 hours), and chorioamnionitis are associated with EOS.[9,11] Chorioamnionitis, often presenting with an increased maternal temperature, can lead to PROM and premature birth, which could imply that there is an interaction between these risk factors.[12] Puopolo *et al.* found that maternal antibiotic treatment, especially with broad-spectrum antibiotics, increased the risk of EOS, but this effect disappeared when stratifying for treatment indication.[9]

A major cause of the declining EOS rate in countries such as the USA is screening and treatment of pregnant women with GBS colonisation.[13] There are two major approaches on how to conduct this screening. The British Royal College of Obstetrics recommend a risk based screening approach, and only recommend intrapartum antibiotic prophylaxis (IAP) to women who have GBS bacteriuria, a previous baby with GBS infection, intrapartum pyrexia (temperature > 38ºc) or known chorioamnionitis, PROM at a GA ≥ 37 weeks, or known GBS carriage in the current pregnancy.[14] The American Centers for Disease Control and Prevention (CDC) guidelines, on the other hand, recommend universal rectovaginal screening at a GA of 35 to 37 weeks and IAP for all GBS-colonized women.[15] Both guidelines recommend benzylpenicillin as the first choice IAP.

In Australia, the incidence of GBS-EOS dropped from 1.43 per 1000 LB infants in 1993 to 0.25 per 1000 LB after implementing universal screening.[13] Similar findings are reported in the US with a decrease from 1.7 per 1000 LB infants in 1990 to 0.6 in 1998.[16] However, similarly low rates of GBS-EOS are reported from countries without a universal GBS-screening policy.[17,18] Despite the reduction of the GBS-EOS rate in countries with an universal screening approach, some authors report an unchanged overall rate of EOS with an increase of EOS caused by Gram-negative bacteria among VLBW infants.[19-21] There is a concern that widespread IAP may lead to increased antibiotic resistance, and ampicillin as IAP has led to findings of ampicillin-resistant *E. coli* strains.[20,21] An effective GBS vaccine would be the best option for prevention of neonatal GBS infection.

EOS is associated with considerable morbidity and mortality.[22,23] Some studies have associated EOS with complications such as brochopulmonary dysplasia, intraventricular

haemorrhage, periventricular leukomalacia, and retinopathy of prematurity in VLBW infants.[20,23] EOS mortality rates have fallen in developed countries, and a study by Bizarro *et al.* reports a decrease in sepsis related mortality from 87% in 1928 to 3% in 2003.[8] Recent studies present EOS-attributable mortality rates between 11% and 18%, with higher rates among preterm infants.[6,8,21] EOS mortality rates vary between different causative pathogen, with higher mortality rates from Gram-negative EOS.[20] Mortality rates up to 40% have been reported in patients with *E. coli* EOS.[24] However, prematurity is a potential confounder of the relationship between gram-negative EOS and mortality.[6]

1.1.2 Late onset sepsis – incidence, risk factors, prevention, and outcome

LOS is typically nosocomially acquired and most frequently affects premature VLBW infants.[4] The incidence of LOS is increasing in the developed world, and many researchers believe this is due to the increased survival of preterm infants.[8] Vergnano *et al.* report an LOS incidence-rate of 3 per 1000 LB in an UK multi-centre study.[5] LOS-rates are largely dependent upon BW, and LOS affects 10% to 30% of VLBW infants.[4,25,26] The median age at onset of disease has been reported from 11-17 days.[4,27,28]

Gram-positive bacteria cause the majority of LOS cases with rates reported from 48% to 80% of LOS cases.[25,27,29] The ratio of Gram-positive to Gram-negative LOS is lower in developing countries than industrialized countries.[30] CoNS, and in particular *Staphylococcus epidermidis*, are the most common causative pathogens with reported rates from 48% - 65% of LOS cases.[4,25,27,31] Other reported LOS pathogens are *S. aureus*, *Enterococcus spp.*, *E.*

coli, *Klebsiella spp.*, GBS, *Enterobacter spp.*, *Serratia spp.*, *Pseudomonas spp.*, *Acinetobacter spp.*, and *Candida albicans*.

In addition to prematurity and low BW, there are several risk factors for LOS, mostly forms of invasive treatment.[4] An important risk factor is the use of indwelling catheters, such as percutaneous catheters, central venous catheters (CVC), and umbilical catheters.[4,25,27] This gives a passageway past the skin barrier for nosocomial bacteria such as CoNS. Additionally, indwelling catheters provide an ideal surface for development of bacterial biofilms, which are more resilient to antibiotic treatment and the host's immune system.[32] The risk of LOS increases with increasing duration of catheter use.[25,27]

Parenteral nutrition (PN) is often delivered through CVCs[33] and is independently associated with LOS in several studies.[25,27,30] An early onset of PN treatment, a swift return to BW, and a long duration of treatment are associated with an increased risk of LOS.[25] An increased duration of ventilator support has also been found to increase the risk of LOS.[25,27] Surgical treatment has also been associated with LOS.[27] Despite plausible explanations of a cause-effect relationship between invasive treatment and LOS, it is important to note that these treatment variables may be partial confounders as they are associated with factors that increase the risk of LOS such as prematurity, low BW, and severe disease.[4,26]

A few studies examine the association between LOS and maternal factors, and found no independent association between LOS and maternal age, marital status, prenatal care, high parity, PROM, mode of delivery, or antenatal antibiotic or steroid therapy.[25,30] Maternal chorioamnionitis has been associated with Gram-negative LOS.[29] EOS was not

associated with an increased risk of LOS in a registry-based study of > 34 000 VLBW infants.[28] IAP has been found to increase the risk of *E. coli* LOS, despite an unchanged overall rate of LOS.[21]

Minimizing the use of catheters and proper hand hygiene remain the primary strategies to prevent LOS. The CDC guidelines specify the importance of good hand hygiene to prevent intravascular-catheter related infection.[34] Kaplan *et al.* report a reduction in LOS rate from 18% to 14% in 24 neonatal intensive care units (NICU) after implementing an evidence-based approach to catheter use with attention to hygiene and prompt catheter removal.[35] A study by Janota *et al.* report that adding gloves to the CDC guidelines on hand hygiene successfully lowered the rate of LOS in their NICU.[36] While probiotics have proven effective in preventing necrotizing enterocolitis (NEC), a randomized controlled trial (RCT) on VLBW infants with probiotics as an intervention found no significant reduction in LOS risk.[37] A multi-centre RCT on VLBW infants found a decreased incidence of LOS in neonates who were given lactoferrin, a glycoprotein with antimicrobial activities found in mammalian milk.[38]

LOS is a significant cause of morbidity and mortality in preterm neonates.[25] LOS, and particularly Gram-negative LOS, is associated with intraventricular haemorrhage, bronchopulmonary dysplasia, patent ductus arteriosus, NEC, prolonged hospitalization, and prolonged respiratory support.[25,29] Mortality rates between 10-18% have been reported in VLBW infants with LOS.[25,39] Makhoul *et al.* examined the rate of mortality within three days of LOS in VLBW infants, and found a mortality rate of 6.5%.[40]

As with EOS, LOS mortality is highly dependent upon the causative pathogen.[40] Shah *et al.* compared mortality rates of patients with Gram-negative LOS, Gram-positive LOS, and no late-onset infection in a Canadian multi-centre study.[29] The mortality rate was independently associated with Gram-negative LOS compared with Gram-positive LOS (odds ratio (OR) 2.85; 95% confidence interval (CI) 2.24 – 5.16) or no infection (OR 3.40: 95% CI 2.24 – 5.16), but was not independently associated with Gram-positive LOS compared with no infection (OR 1.20; 95% CI 0.90 – 1.59). LOS caused by *Pseudomonas*, *Klebsiella*, and *Serratia* are associated with the highest mortality of all LOS pathogen, while CoNS is associated with the lowest mortality rates.[25,40]

1.2 Diagnosis of neonatal sepsis

1.2.1 Clinical signs and biomarkers of sepsis

Neonatal sepsis typically presents with clinical signs such as increasing respiratory distress, temperature instability, hypothermia (temperature < 36.0ºC), hyperthermia (temperature > 38.5ºC), lethargy, hypoperfusion (capillary refill time > 2 seconds), irritability, and feeding intolerance.[41,42] While such signs are common, most are nonspecific and have a low positive predictive value, partly due to the low prevalence of neonatal sepsis.[41,43] In addition, some neonates initially appear asymptomatic despite having an infection.[44] In a prospective study by Bekhof *et al.*, only respiratory insufficiency, lethargy, capillary refill time > 2 seconds, pallor/grey skin, and increased oxygen requirement were significantly associated with LOS.[43]

In NICUs biomarkers such as C-reactive-protein (CRP), complete blood-counts (CBC) and procalcitonin (PCT) are frequently used.[45] While these biomarkers have a high

sensitivity for neonatal infection, the specificity is imperfect, which combined with the low prevalence of neonatal sepsis, leads to a low positive predictive value. Additionally CRP has been shown to have a low sensitivity in the first 24 hours of life.[46] PCT rises more rapidly following infection and has been shown to reduce antibiotic consumption in neonates.[46,47] However, PCT can also be elevated due to other factors, such as maternal diabetes and birth, and has not proven superior to CRP in ascertaining true infection.[46,47] A Swiss study demonstrated that reduction in diagnostic tests such as CRP and CBC did not delay antibiotic treatment or affect mortality in a group of term born infants with increased risk of EOS.[48] Interleukin-1 and interleukin-6 have been found to be up-regulated up to two days before a clinical diagnosis of sepsis and had higher sensitivity than CRP on the day of diagnosis.[49] However, these tests remain expensive and are not in widespread use in NICUs yet.

1.2.2 Blood cultures

Neonatal sepsis is confirmed by demonstrable growth of bacteria in cultures from a normally sterile site. This is most commonly blood cultures, but many authors include cultures from cerebrospinal fluid.[5,6,10] It usually takes 24-36 hours before blood cultures become positive, and a very low proportion of tested neonates are diagnosed with culture-confirmed sepsis.[1] Blood cultures taken from neonates require samples of at least 0.5 ml, and failure to attain this volume is reported to be frequent.[50] Therefore, using blood culture results as the only definition of disease may underestimate the prevalence of neonatal sepsis.

On the other hand, false-positive results due to contamination with skin-bacteria such as CoNS are reported to be frequent.[51] As CoNS is a common cause of LOS in premature neonates, additional criteria are often used to separate contamination from true disease.[5,27] The Vermont Oxford Network Database require clinical signs of sepsis, a blood culture positive for CoNS, and antibiotic treatment ≥ 5 days to define a case as CoNS sepsis.[52] Beekmann *et al.* found that a model requiring two positive blood cultures for CoNS within five days, or one positive blood culture with clinical evidence of infection (low white cell count and hypothermia/hyperthermia or hypotension) achieved the best combined sensitivity (46%) and specificity (96%).[51] Some studies on EOS, particularly those that study term infants, classify all CoNS cases as contaminations for the sake of simplicity.[6]

While blood cultures are the gold standard in the diagnosis of neonatal sepsis, culture-negative sepsis is reported to cause the majority of EOS cases and a significant minority of LOS cases.[29,42,43] The diagnosis of culture-negative sepsis is based on clinical status and biomarkers such as CRP values.[48] In 2006, neonatologists within the Norwegian Pediatric Association suggested the following four criteria for the diagnosis of culture negative sepsis: i) clinical signs of infection, ii) maximum C-reactive protein level (CRP) > 30 mg/L, iii) minimum duration of 5 days antibiotic treatment and iv) exclusion of other explanations for the clinical picture. Other studies simply define culture-negative sepsis as sepsis in neonates with strong clinical suspicion and slightly elevated haematological markers. While these criteria attempt to standardize the diagnosis of culture-negative sepsis, there is a high risk of over-diagnosing neonates with clinical signs and exposing them to unnecessary antibiotic treatment.

1.2.3 Risk assessment algorithms

The imperfect sensitivity of blood cultures, the duration of time before results are available, and the low specificity of signs, symptoms, and biomarkers place clinicians in a difficult position. Missing potential disease can be fatal or cause significant morbidity. However, overuse of antibiotics in neonates may lead to adverse outcome. Therefore prediction models combining clinical and laboratory factors may determine which neonates are in need of further evaluation and reduce the number of neonates unnecessarily exposed to systemic antibiotic treatment.

Due to the vertical transmission of bacteria causing EOS, many algorithms that evaluate EOS-risk base their models on maternal factors. Puopolo *et al.* developed a risk stratification scheme for neonates with a GA ≥ 34 weeks using GA, maternal GBS status, rupture of membranes time, highest intrapartum temperature, and intrapartum antibiotic treatment to identify neonates at high risk of EOS.[9] Escobar *et al.* combined this model with objective neonatal data in the first 12 hours of life such as Apgar scores, need of respiratory support (mechanical ventilation, continuous positive airway pressure or oxygen supplementation), heart rate, respiratory rate, temperature, and respiratory distress.[53] Using these variables they divided their population into a high-risk group consisting of 4% of their population with a number needed to treat (NNT) of 118, a middle-risk group consisting of 11% of their population with a NNT of 823, and a low-risk group consisting of 85% of their population with a NNT of 9370. They proposed treating neonates in the high-risk group with antibiotics and observing neonates in the middle-risk group. This approach would theoretically reduce the percentage of antibiotic exposed neonates in their population from 6%-10% to 4%.

Verstraete *et al.* recently performed a systematic review of prediction models for LOS.[54] In their review, the study that performed best was a prediction model by Mahieu *et al.* Requiring at least two of the following factors; CRP ≥ 14 mg/L, neutrophil fraction > 50%, thrombocytopenia, fever > 38.2ºC, and exposure to parenteral nutrition ≥ 14 days, they achieved a sensitivity (95% CI) of 0.95 (0.86-0.99) and a specificity (95% CI) of 0.43 (0.30-0.56) for LOS.[55] However, this model did not perform as well in other NICUs than where it was developed. Bekhof *et al.* achieved a sensitivity of 97% and a specificity of 37% for LOS by requiring one of the following four factors to be present; increased respiratory support, capillary refill time ≥ 2 seconds, pallor/grey skin and/or a CVC.[43]

1.3 Antibiotic treatment

Because of the potentially fatal consequences of neonatal sepsis, antibiotics are administered empirically in many neonates. This means that antibiotics are administered before the clinician knows the causative pathogen or whether the neonate has an infection at all. This leads to a significant overuse of antibiotics in the neonatal population. To minimize the potentially adverse effects of antibiotic treatment, several guidelines are established to guide the prescription of systemic antibiotics in neonates.[56,57] The most commonly used antibiotics in neonates are aminoglycosides, penicillins, third-generation cephalosporins, and glycopeptides.[31,58]

1.3.1 Aminoglycosides

Aminoglycosides are a class of antibiotics that consist of tobramycin, gentamicin, netilmicin, and streptomycin among others.[59] They have been in use since 1944 and often provide Gram-negative coverage in empiric antibiotic regimens.[60] Aminoglycosides

achieve bactericidal effect through irreversibly binding to the 30S subunit of bacterial ribosomes, thereby inhibiting protein synthesis and altering the integrity of the bacterial cell membrane.[61] Aminoglycosides also have a post antibiotic effect, in which bacterial killing continues after the serum concentration has fallen below the minimum inhibitory concentration (MIC).[62] Despite aminoglycosides effectiveness and low rates of resistance, many clinicians are sceptical of aminoglycosides due to their potential nephrotoxicity and ototoxicity.[63]

A high ratio of peak serum concentrations (PSC) to the MIC is vital to achieve satisfactory bactericidal and post antibiotic effects.[62,64] In contrast, aminoglycoside toxicity occurs through saturation of proximal tubule cells in nephrotoxicity and cochlear cells in ototoxicity.[65,66] Saturation occurs with prolonged durations of aminoglycoside treatment and high average concentrations.[67] Many authors suggest maintaining trough serum concentrations (TSC) below 2.0 mg/L to prevent potential toxicity.[68-70]

Previously, administering small doses multiple times daily was the norm for aminoglycoside treatment in neonates.[69] However, this was irrational for a few reasons. Firstly, aminoglycosides are water-soluble drugs and neonates, in particular preterm neonates, have proportionally larger volumes of distribution (V_D) for aminoglycosides than children and adults.[71,72] Therefore, larger doses are needed to achieve therapeutic PSCs. Secondly, aminoglycosides are cleared through the kidneys, and clearance is impaired in neonates, particularly in preterm neonates.[71,72] Therefore, neonates are in need of larger time intervals between doses. Several studies have found that multiple doses per day regimens are inferior to one-dose daily regimens in achieving therapeutic PSCs and TSCs in neonates.[69,73-75]

Over the last 20 years, larger doses given once daily have become widely established in aminoglycoside treatment in neoantes.[73] However the dosing regimen of aminoglycosides vary greatly.[68-70,72] To achieve satisfactory PSCs and TSCs, a dosing regimen has to account for varying GAs and postnatal ages. This often leads to complicated dosing-regimens with increased risk of erroneous administration.[76] Most current dosing regimens recommend 4-5 mg/kg at intervals between 24-48 hours.[68,69,73] While the relationship between aminoglycosides and toxicity has been reported in adults,[77] this relationship is seldom found in neonates.[69] In the age of extended-interval dosing regimens, some experts believe that aminoglycoside toxicity has become largely theoretical in the neonatal population.[78,79]

1.3.2 Beta-lactams

Beta-lactams are a major class of antibiotics consisting of several sub-groups such as penicillins, cephalosporins, and carbapenems.[80] Alexander Fleming famously discovered penicillin in 1928, but despite its age, penicillin G (benzylpenicillin), along with ampicillin and cefotaxime, remain among the most commonly used antibiotics in NICUs.[80] Beta-lactams contain a beta-lactam ring and achieve their bactericidal effect through inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall by binding to penicillin-binding proteins.[81] This leads to a futile cycle of peptidoglycan synthesis and degradation that depletes cellular resources and leads to cell death.[81]

Benzylpenicillin is a narrow-spectrum antibiotic that provides coverage against GBS,[82] and in combination with an aminoglycoside provides coverage against most sepsis pathogens in neonates.[83] Ampicillin and other aminopenicillins have relatively similar

uses as benzylpenicillin, with an added effect against Gram-negative bacteria due to their amino-group.[84] Benzylpenicillin and ampicillin are, however, susceptible to the beta-lactamase enzyme commonly found on the cell surface of *Staphylococci,*[85] a common causative agent of both EOS and LOS.[4,5,25] Cloxacillin and Flucloxacillin are beta-lactamase stabile penicillins and consequently provide coverage against *S. aureus.*[86] However, their use is impaired by high resistance rates in many countries.[87]

Cephalosporins are broad-spectrum antibiotics often used in the treatment of neonatal infections.[58] Cefotaxime, a third-generation cephalosporin, provides coverage against most Gram-negative bacteria and some Gram-positive bacteria, including GBS.[86,88] All isolated pathogen from EOS-cases, and most LOS pathogen, were susceptible to the combination of cefotaxime and gentamicin in an English national-surveillance study.[86] In addition, cefotaxime effectively penetrates the blood-brain barrier and is a good option for the treatment of neonatal meningitis.[56,89] However, cephalosporins, in particular third-generation compounds, are associated with an increased selection of antibiotic resistant bacteria.[90]

Though toxicity is rare with beta-lactams, amoxicillin and ceftriaxone are suspected of toxicity.[91,92] Mrvos *et al.* associated amoxicillin with nephrotoxicity and while this was not dose-dependent,[91] PSCs < 140 mg/L have been proposed as a target for amoxicillin therapy, but beta-lactam PSCs are rarely measured.[93] Ceftriaxone is a competitive inhibitor of bilirubin's binding to albumin, which may place the neonate at risk of bilirubin encephalopathy.[94] Additionally, co-administration with ceftriaxone and intravenous calcium has been associated with an increased risk of embolism.[95]

In contrast to aminoglycosides, beta-lactam's bactericidal effect is dependent on the amount of time the serum concentration exceeds the MIC.[83,92,96] De Hoog *et al.* propose that the time > MIC should be at least 40-50%. Beta-lactams are water-soluble and have a large V_D in neonates.[97] They are eliminated through the kidneys, and half time is increased in neonates, particularly in preterm infants.[97] To maintain a high time > MIC while keeping serum concentrations therapeutic, small doses are given after 8-12 hour intervals. The British National Formulary (BNF) for Children recommend beta-lactam dosing intervals of 12 hours for neonates < 7 days of age and 8 hours for neonates ≥ 7 days of age.[68]

1.3.3 Glycopeptides

Vancomycin is a glycopeptide antibiotic that achieves bactericidal effect by inhibiting cell wall synthesis.[98] An increase in rates of methicillin-resistance among *S. aureus* and CoNS have caused vancomycin to become the first choice therapy for neonatal staphylococcal infections in many countries.[99] Vancomycin is generally effective against *Staphylococci*,[86] but glycopeptides are associated with increased development of resistance.[87] Many authors advise reducing vancomycin usage in neonates.[31]

Particularly feared are the multi-resistant bacteria: vancomycin-resistant *Staphylococci* and vancomycin-resistant *Enterococci*.[100,101] The British National Institute for Health Care and Excellence (NICE) guidelines recommend increasing the dosage of gentamicin to 8 mg/kg instead of using vancomycin for EOS.[56] Lawrence *et al.* attempt to use Cloxacillin whenever susceptibility patterns allow it.[87] In Norway, vancomycin is seldom used as *S.*

aureus is largely susceptible to beta-lactamase stabile penicillins (methicillin) and gentamicin.[85]

There are many unexplained factors in vancomycin pharmacokinetics in neonates, but their efficacy seems dependent on time > MIC.[102] Vancomycin is associated with nephrotoxicity and nephrotoxicity in adults,[103,104] and a study by Vella-Brincat *et al.* found that vancomycin was more associated with hearing loss than gentamicin in neonates.[78] Some authors propose keeping the vancomycin serum concentration between 10 mg/L and 25/30 mg/L,[78,105,106] and others propose keeping TSCs between 5-15 mg/L and PSCs between 30-40 mg/L.[98,107,108] Some authors find measuring PSCs unnecessary to avoid toxicity.[109] To maintain serum concentrations within therapeutic ranges, several authors recommend a high-loading dose followed by continuous infusion of vancomycin.[105,106,110]

As with other antibiotics, vancomycin is water-soluble and cleared through the kidneys. Consequently, neonates have a high V_D and prolonged clearance of vancomycin compared with older children and adults.[98] Due to variable protein-binding capacities for vancomycin and variable kidney functions, V_D and clearance vary greatly among neonates.[78] This lends itself to complicated dosing regimens with a high propensity for prescription errors.[105]

1.3.4 Choice of empiric antibiotic regimens

Most EOS-guidelines recommend the combination of a penicillin and an aminoglycoside as empiric antibiotic treatment. The NICE guidelines and the BNF for children recommend benzylpenicillin and gentamicin as the first-choice antibiotic regimen for EOS.[56] The American Academy of Pediatrics (AAP) guidelines, however, recommend

ampicillin and gentamicin as first line treatment.[57] Both the NICE and the AAP guidelines focus on quickly discontinuing antibiotics when blood-cultures are negative and sepsis is no longer suspected. The BNF for children recommend flucloxacilline to treat LOS.[68]

Ampicillin as IAP for GBS colonization is associated with development of ampicillin-resistant *E. coli* strains,[20,21] and could theoretically cause more resistance than penicillin when used to treat potential EOS. Metsvaaht *et al.* performed a RCT that compared benzylpenicillin and gentamicin with ampicillin and gentamicin.[111] Treatment failure was defined as a need to change antibiotics and/or death occurring within seven days, and the rate of treatment failure was 14% in both regimens. They found no bacteria fully resistant to either treatment regiment except for a few cases of CoNS, but two Gram-negative bacteria were resistant to ampicillin.

De Man *et al.* performed a prospective cross-over interventional study comparing two treatment approaches in two identical NICUs.[90] One NICU used benzylpenicillin and tobramycin as an empiric antibiotic regimen for EOS and flucloxacillin and tobramycin for LOS. The other NICU used amoxicillin with cefotaxime for both EOS and LOS. After six months the empiric regimens were switched. Patients who received amoxicillin and cefotaxime had an 18-fold increase in the relative risk of colonization with resistant Gram-negative bacilli compared with neonates who received benzylpenicillin and tobramycin. Clark *et al.* found an unexplained increase in mortality among patients who received cefotaxime and ampicillin compared with patients who received gentamicin and ampicillin.[112] In light of these studies, it is particularly alarming that 20% of NICUs in the UK and Ireland use a cephalosporin as empiric therapy in spite of national guidelines.[113]

1.3.5 Adverse effects of antibiotic treatment

Antibiotic resistance has been a concern for several years, but recently more focus has been placed on how antibiotic treatment affects the development of gut microbiota in neonates. During birth, the foetus is colonized with bacteria from the mother's vaginal tract.[114] In the gut flora of vaginally delivered full term infants, bacteria like *E. coli*, *Enterobacteria*, *Streptococci*, and *S. aureus* are found on the first days of life.[114,115] As the neonates ages it comes in contact with bacteria from the environment and mother's milk.[116] During the first few months to a year of life, the concentrations of *Bifidobacteria*, *Lactobacilli*, and *Bacteriodes* increase, while the concentrations of *Staphylococci*, *Streptococci*, and *Enterococci* decrease.[115,117] After one year of life, most children's gut microbiota starts to resemble that of an adult.[118]

Several early life events like caesarean section, prematurity, a lack of breast-feeding, and antibiotic treatment can disrupt this process.[119,120] Antibiotic treatment, particularly long-term treatment with broad-spectrum antibiotics, can cause a selection pressure that causes antibiotic susceptible pathogens to die while other pathogens survive.[120] Some bacteria that are harmless in a diverse flora are opportunists that can cause disease when protective bacteria like *Bifidobacteria*, *Bacteriodes*, and *Lactobacilli* are supressed.[121]

Recent studies have increased our knowledge on how the composition of gut flora affects long-term health. Decreased diversity and an unfavourable composition are associated with adverse outcome.[117] Ajslev *et al.* found that antibiotic treatment in infants with normal-weight mothers increased the risk of childhood obesity, while antibiotic treatment had a protective effect against childhood obesity in infants born to over-weight

mothers.[122] Some authors speculate that a disturbance in the gut flora may increase the risk of atopic diseases, allergies, asthma, autoimmune diseases like diabetes-type I, and even autism.[117,123-126] Causality has not been established for any of these diseases, but this topic is of great interest to many authors and is sure to be studied extensively in the future.

NEC is a disease characterized by gut inflammation, which typically affects extremely premature (GA < 28 weeks) and dysmature infants in the first two to three weeks of life.[127,128] The severity of NEC can range from mucosal ulceration to transmural necrosis. The disease is classified according to the Bell's staging criteria with grades from I to III, where grade II patients are in need of medical treatment, and grade III patients are in need of surgery.[129] The pathophysiology is somewhat unclear, but increasing prematurity, enteral feeding, unfavourable composition of the gut microbiota, and gut ischemia leading to inflammation seem to be a part of it.[130] Neonatal antibiotic treatment has been found to increase the risk of NEC,[131,132] while probiotic supplementation has a protective effect.[133,134] The mortality rate is 15-30% in neonates with NEC and it is highest among low BW infants and infants in need of surgery.[128,135]

Fungemia is, as the name implies, a blood-stream infection with fungi, most commonly *Candida albicans*.[25] Fungi are reported to cause from 4% to 12% of LOS cases.[25,27] Prematurity, low birth weight, and use of central venous catheters are important risk factors for fungemia.[136] Previous antibiotic treatment, particularly with broad-spectrum antibiotics like cephalosporins and carbapenems, increases the risk of fungemia by selection pressure.[137] Fluconazole and nystatin appear to be relatively safe and effective as prophylaxis for fungemia, and are recommended to neonates with a GA < 26-28 weeks.

Some authors also recommend empiric antifungal treatment when there are signs of sepsis (LOS) in neonates with a GA < 27 weeks after treatment with cephalosporins or carbapenems.[138] Mortality rates are approximately 30% for VLBW fungemia patients,[25] and fungemia has been reported to cause complications like endocarditis, meningitis, brain parenchyma infection, and renal abcesses.[139]

2 Aims of the thesis

The aim of this thesis is to assess existing literature by performing a systematic-review on how neonatal antibiotic treatment affects the following factors:

- The composition of the infant's gut microbiota
- The risk of developing necrotizing enterocolitis
- The risk of late-onset fungal sepsis

3 Methods

3.1 Search strategy

Relevant original articles were identified through the Embase, Pubmed, and Medline databases. We used no time restrictions, but the search was restricted to human studies written in English. Table I displays the PICO (population, intervention, comparison, and outcome) model we used to develop our search strategy. We used a combination of medical subject heading (MeSH) terms and text searches.

In Medline and Pubmed, we combined the MeSH terms Infant, Newborn and Anti-Bacterial Agents with one of the following outcome terms; (1) Microbiota, (2) Enterocolitis, Necrotizing, or (3) Fungemia. The Embase database uses its own key words, and we combined Newborn and Antibiotic Agent with one of the following outcome terms; (1) Microflora, (2) Necrotizing Enterocolitis, or (3) Fungemia. We searched all three databases in free text and combined (a) Infant, Low Birth Weight or Infant, Postmature or Infant, Premature Or Infant, Newborn with (b) Anti-Bacterial Agents or Antibiotics and (c) one of the following outcome combinations; (1) Microbiota or Microbiome or Microbiomes or Gut Flora, (2) Necrotizing Enterocolitis, or (3) Fungemia or Fungemias or Candidemia. Bibliographies of relevant review articles were manually searched for additional original articles.

3.2 Study selection and data extraction

Studies were eligible for inclusion if they examined neonatal antibiotic treatment as a risk factor for either alteration of microbiota, NEC or fungemia. Neonatal antibiotic

treatment was defined as treatment with intravenous antibiotics after birth and up to the first month of life. There had to be comparisons between either (1) neonates with or without antibiotic treatment, (2) neonates with different antibiotic regimens (broad versus narrow spectrum), and/or (3) neonates with different treatment lengths. Case reports, case series, studies with a non-neonatal or unspecified population, and studies examining enteral or perinatal antibiotic treatment were excluded. Studies with missing abstracts were not analysed and studies with unavailable full text versions were not included. This was a collaboration project and two authors (JWF and Eirin Esaiassen)searched through all abstracts and made independent conclusions on whether studies should be included. In case of disagreements, the supervisor (CK) had the decisive vote.

For each included study we collected data regarding study design, GA, BW, antibiotic intervention, and main outcome. Retrospective studies were defined as studies that assessed exposures after the outcome had occurred, while prospective studies assessed exposures before the outcome. Flow charts that illustrated study inclusion were created in Microsoft Excel 2011.

3.3 Work process

The first two-week period in August 2013 was used to write the study protocol. The search strategy was developed in cooperation with a university librarian, CK, and EE in August 2014. We attempted to perform the search and develop selection criteria in October 2014, but this had to be redone. EE and JWF performed the final search on the 2nd of December 2014. I filtered abstracts until early March 2015. The period from

March-May 2015 was used to write the thesis. CK supervised the study and provided guidance during the revision of the thesis.

4 Results

4.1 Effect of antibiotic treatment on gut microbiota

A total of 14 studies were included.[140-153] Figure 1 demonstrates the findings from the search and the exclusion process. Of the included studies, five (36%) aimed to examine the effect of neonatal antibiotic treatment on gut microbiota. Table 2 presents an overview and the main results of the included studies.

Studies that assessed gut microbiota diversity found decreased diversity among neonates treated with antibiotics.[142,145-148,154] In a cohort of extremely preterm infants, Jacquot *et al.* found that early antibiotic treatment increased gut flora diversity early on, but when faecal samples were taken after six weeks, the diversity was inversely correlated with the length of antibiotic treatment. Two studies found that neonatal antibiotic treatment decreased the total number of bacteria,[146,153] while one study did not find this association.[145]

Several studies have found that antibiotic treatment significantly decreased the concentrations of beneficial bacteria like *Bifidobacteria*, *Bacteriodes*, and *Lactobacilli* in a group of infants with varied GAs.[145,154] Butel *et al.* studied the development of *Bifidobacteria* colonization in a cohort of preterm infants and found no effect from neonatal antibiotic treatment.[143] They did not, however, adjust for confounders or specify the antibiotic regimens or treatment lengths. The findings by Fouhy *et al.* demonstrate that while *Bifidobacteria* have an ability to recover after antibiotic treatment, antibiotic treatment has a long-term effect on the composition of the gut flora.[145]

Neonatal antibiotic treatment has also been found to increase the levels of *Enterobacteria* in stool samples.[145,147] Some studies found a decrease in *Clostridium* colonization after antibiotic treatment,[141,144,150] while others found and increase in *Clostridium* colonization.[148] Blakey *et al.* found that neonatal antibiotic treatment decreased the levels of *Clostridium* in the first eleven days of life, but increased the levels of *Clostridium butyricum* and *perfringens* on day 12-20.[154] Some studies found decreased levels of *Staphylococci* after neonatal antibiotic treatment,[147,151] The findings of Greenwood *et al.* support this,[147] but other studies have found that neonatal antibiotic treatment did not affect the rate of colonization with *Staphylococci* and in some cases even increased the number of *Staphylococci*.[142,154] Greenwood *et al.* found that a short treatment with antibiotics (< 5 days) decreased the prevalence of *Enterococci* in the gut flora, while a prolonged duration of treatment (≥ 5 days) increased the prevalence of *Enterococci*.[147]

4.2 Effect of antibiotic treatment on the risk of necrotizing enterocolitis

A total of twelve studies were included.[132,147,155-164] Figure 2 shows the search results and the exclusion process. All study populations consisted of neonates with varying degrees of prematurity. Three of the twelve included studies (23%) did not aim to examine how neonatal antibiotic treatment affected the risk of developing NEC.[147,157,164] The studies that defined NEC defined it as Bell's stage II or III. Table 3 presents an overview and the main results of the included studies.

Of the six studies that assessed the risk of developing NEC after neonatal antibiotic treatment, three found a significantly increased risk,[155,156,158] two studies did not find a

significant risk increase,[132,161] and one study found a protective effect from antibiotic treatment.[159] Alexander *et al.* found that antibiotic treatment had a protective effect against NEC in neonates with a previous diagnosis of sepsis (OR 0.85; 95% CI 0.78 – 0.94), but increased the risk of NEC in neonates without previous sepsis. The two studies that found an unaffected risk after neonatal antibiotic treatment found an increased risk of a composite outcome that consisted of either NEC and death or NEC, LOS, and death.[132,161]

Most studies that examined different durations of antibiotic treatment found an association between NEC and prolonged treatment,[132,147,156,158,161,164] but some studies had to create a composite outcome of NEC and other outcomes such as death and LOS to find an association.[132,147,161] Two studies did not find an association between antibiotic treatment duration and NEC.[155,163] Three studies examined different types of antibiotic treatment, and all of them found an association between aminoglycoside treatment and NEC.[155,157,160]

4.3 Effect of antibiotic treatment on the risk of fungemia

A total of eleven studies were included.[137,165-174] Figure 3 shows the search results and the exclusion process. Of the eleven included studies, seven (64%) aimed to assess neonatal antibiotic treatment as a risk factor for candidemia or fungemia.[137,165,167,168,171,173,174] Table 4 presents an overview and the main results of the included studies.

Three studies examined the association between fungemia/candidemia and general antibiotic treatment,[137,171,174] but only one found a significant association.[171] Five studies

assessed the association between fungemia/candidemia and the duration of antibiotic treatment,[137,165,168,170,173] but only three studies found a significant association.[165,170,173] Broad-spectrum antibiotic treatment, on the other hand, was significantly associated with fungemia or candidemia in all studies that examined the association.[137,165-168,174]

5 Discussion

5.1 Gut microbiota

Low diversity in the gut microbiota is associated with adverse outcome.[117] All but one of the included studies in the microbiota category found a change in gut microbiota in patients who received neonatal antibiotic treatment. This study, however, only assessed the levels of *Bifidobacteria* in the gut flora and did not study the effect of prolonged treatment or broad-spectrum antibiotics.[143] The findings in the literature indicate that neonatal antibiotic treatment decreases the total bacterial count in the infant gut.[146,153] Studies that study the levels of individual bacteria present disparaging results, but most findings indicate decreased levels of beneficial bacteria like *Bifidobacteria*, *Lactobacilli*, and *Bacteriodes*[141,145,175] and increased levels of *Enterobacteria*.[145,147]

Some of the conflicting results may be explained by poor methodology in many of the studies. Most of the included studies had small study samples. There were many potential confounders, such as different rates of breastfeeding, caesarean sections, maternal factors (life-style, age, weight), GA and BW, and different times and techniques for collecting and analysing faecal samples. Antibiotic treatment may in itself be a marker of prematurity and/or severe disease, which may have an effect on gut microbiota. While all studies included in this category were either cohorts or RCTs, and only one of the included studies was retrospective, most of the included studies failed to account for or adjust for potential confounding.[143,144,146,148-151,153,154,175]

Only two studies compared different treatment regimen,[142,152] and few studies specified which regimens the included neonates received.[145,147,154] Most studies did not examine the

effect of prolonged antibiotic treatment, but those that did found that increased lengths of treatment increased the effect on gut microbiota.[146,147] Being unable to assess the effect of prolonged treatment may be part of the reason that some studies did not find an association between antibiotic treatment and certain changes in the gut flora. In addition, local susceptibility rates are likely to vary, causing antibiotics to affect gut bacteria differently in different studies.

5.2 Necrotizing enterocolitis

Some of the included studies in the NEC category indicate that antibiotic treatment, in particular prolonged treatment, may increase the risk of NEC.[132,147,155,156,158,161,164] However, the findings by Alexander *et al.* indicate that antibiotic treatment may at least partially serve as a marker of severe disease, which in itself may increase the risk of NEC.[155] Their findings also indicate that antibiotics may have a protective effect against NEC in patients with neonatal sepsis. Cotten *et al.* try to account for this potential confounding/interaction by excluding patients with a previous diagnosis of culture-confirmed sepsis and stratifying patients based on the need for mechanical ventilation.[176] Other studies in this category also excluded patients with a previous diagnosis of sepsis from analysis.[132,161] The study by Krediet *et al.* found a protective effect from neonatal antibiotic treatment on NEC, but had a small study sample and did not specify the rate of previous sepsis.[159]

All but three studies in the NEC category were retrospective, which makes it more difficult to measure potential confounders. The majority of the included studies had a small sample size, and this may be part of the reasons that some studies did not find a

significant association between antibiotic treatment and NEC.[132,161-163] Most studies specified the antibiotic agents or regimens that were used, but there were only three studies that compared different antibiotics. Those that did, however, presented results that seem to indicate that aminoglycosides are more associated with NEC than some other types of antibiotics.[155,157,160]

5.3 Fungemia

Broad-spectrum antibiotics were uniformly associated with fungemia, but the results were inconclusive on whether general antibiotic treatment or prolonged antibiotic treatment are associated with fungemia. Studies that tested this association in multivariate regression models, however, found that the association decreased or disappeared.[166-168] This may indicate that other variables, such as infection and/or prematurity, may cause part of the association between broad-spectrum antibiotic treatment and fungemia. However, Cotten *et al.* found that antibiotic treatment was also associated with fungemia in neonates with negative blood-cultures,[137] and many of the included studies tried to adjust for GA and BW.

There were several methodological challenges in these studies. Four studies did not aim to examine risk factors for candidemia or fungemia.[169,170,172,177] This led to a lack of adjustment for potential confounders or interacting factors in the relationship between neonatal antibiotic treatment and fungemia. Additionally, only a few of the studies compared different types of antibiotics,[177] and few of the remaining studies specified which antibiotics that were used.[167] All but two of the included studies were

retrospective, and the majority of them were case-control studies. Lastly, many of the included studies had a small study population.

5.4 General discussion

The primary strength of this study was that the systematic search was thorough by including both MeSH terms and searches in free-text. MeSH-terms are only added in the electronic databases after an extended time period. Consequently, the use of free-text searches enabled us to find the most recently published studies. The fact that we conducted our search in the three largest medical databases ensured that we found a higher number of published studies. By having an additional researcher read through abstracts we decreased the possibility of wrongfully excluding studies.

However, our study also has limitations. Performing a systematic review for the first time proved challenging. There are few studies that aim to assess the effects of neonatal antibiotic treatment on the gut microbiotia, the risk of developing NEC or the risk of developing fungemia. Those that did often used retrospective study designs, and often failed to account for confounders. The large variation in study designs makes it difficult to summarize results in a cohesive manner. These factors, combined with the small sample sizes of most of the studies, make it difficult to draw firm conclusions based on the existing literature.

Neonatal antibiotic treatment seems to decrease gut flora diversity and the total bacterial count, but findings on individual bacteria were diverging. The risk of NEC appears to increase after neonatal antibiotic treatment, particularly after prolonged treatment.

Aminoglycosides were more strongly associated with NEC than other antibiotics. Antibiotic treatment appears to have a protective effect against NEC in neonates with infection. Broad-spectrum antibiotic treatment seems to increase the risk of fungemia. The methodological quality was poor in many of the included studies, which made it difficult to draw firm conclusions. In neonatal populations, RCTs can be difficult to perform due to ethical concerns. However, more RCTs, or at least well-designed prospective studies, are needed to evaluate adverse outcome after neonatal antibiotic treatment.

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7 Figures

Figure 1: Search results and inclusion process for studies in the gut microbiota category

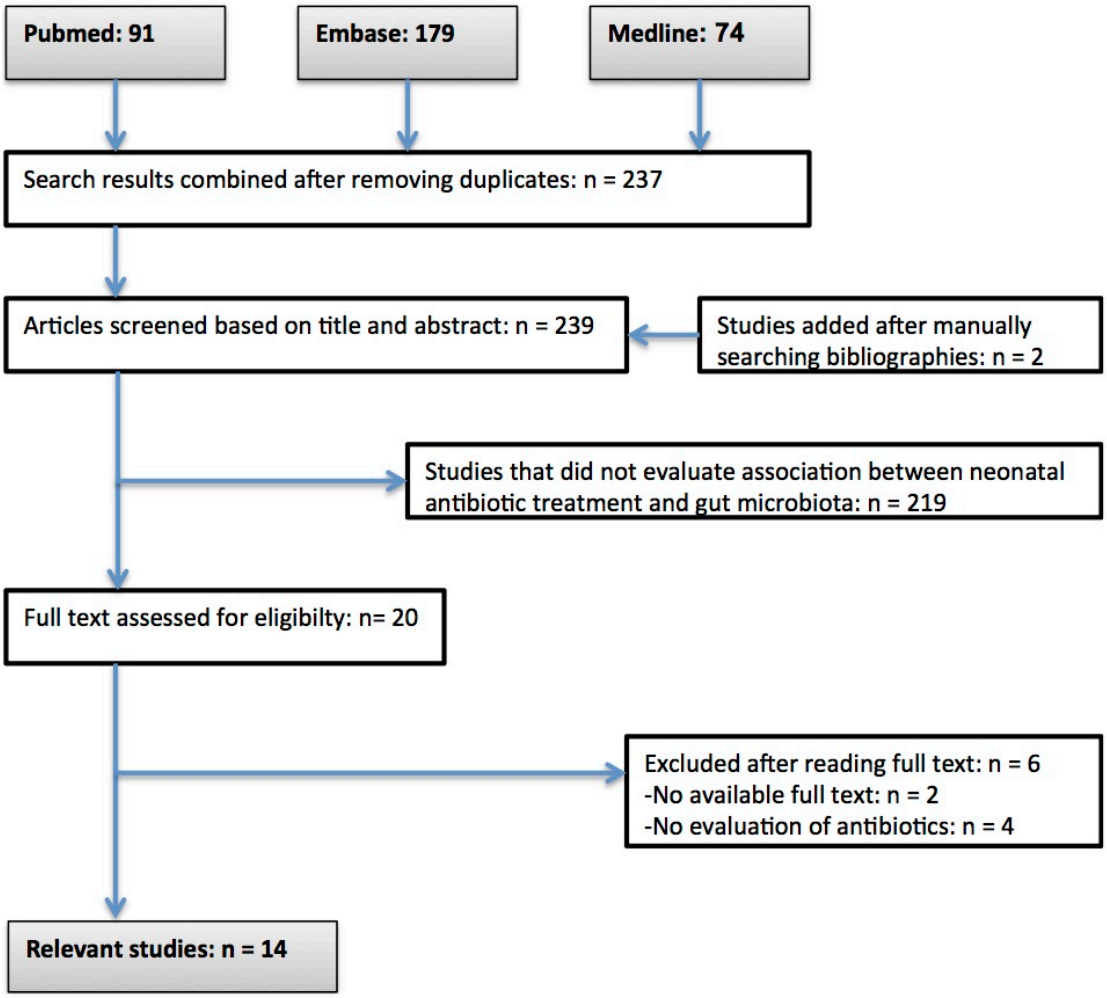

Figure 2: Search results and inclusion process for studies in the NEC category

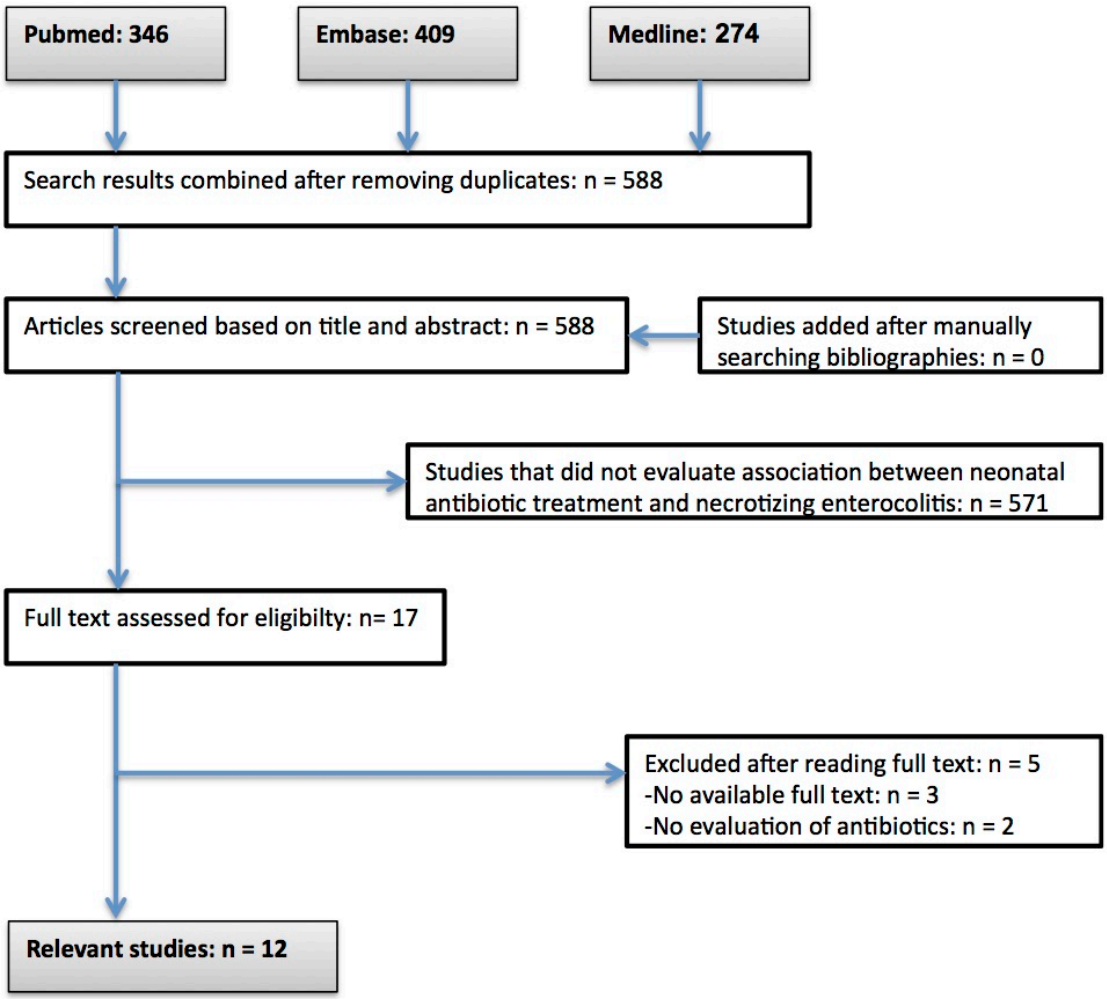

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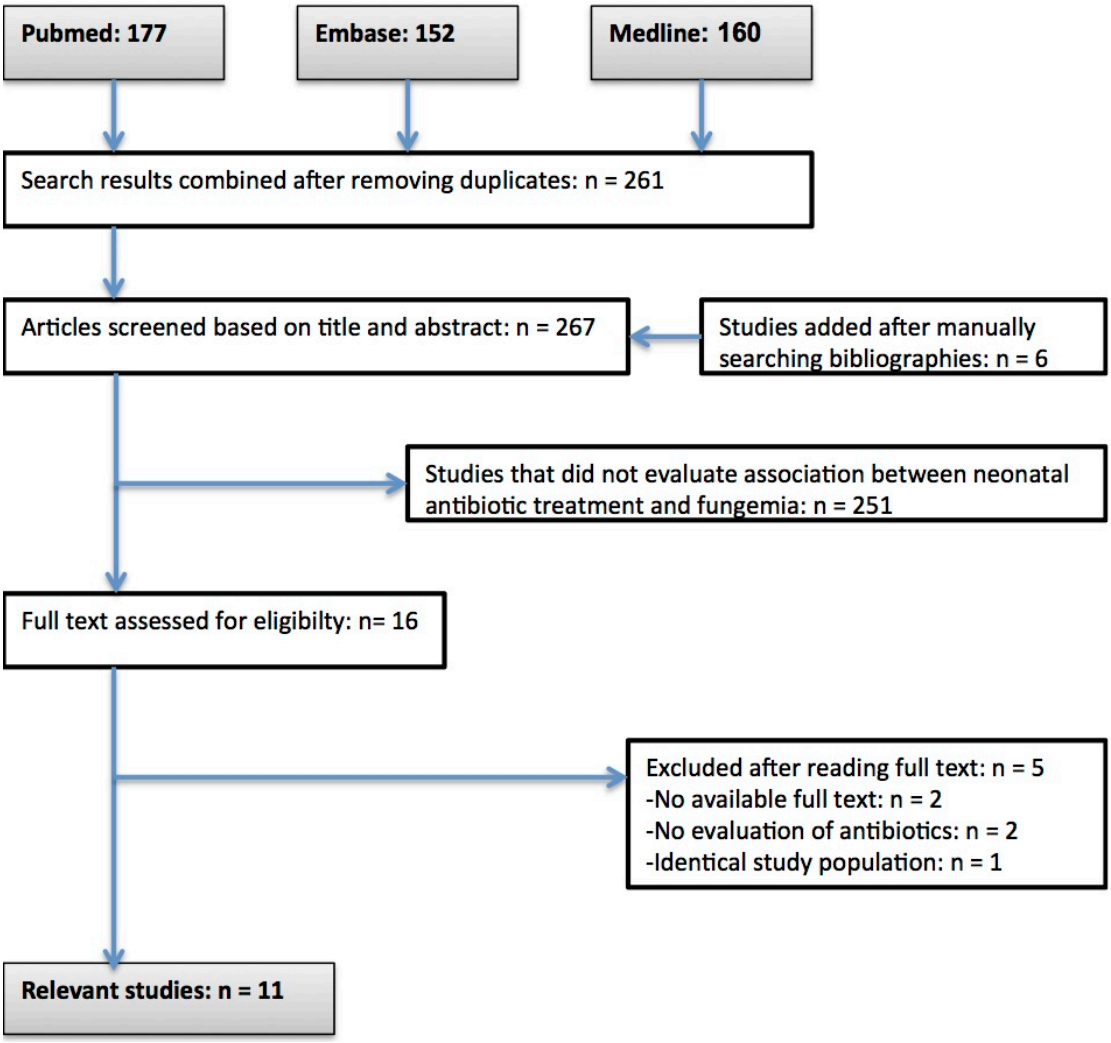
Figure 3: Search results and inclusion process for studies in the fungemia category



8 Tables

Table 1: PICO model displaying search strategy

P (population)	I (Intervention)	C (Comparison)	O (Outcome)
Neonates	Antibiotic exposure • Yes • Long • Broad-spectrum	Antibiotic exposure • No • Short • Narrow-spectrum	Change in gut flora Fungemia Necrotizing enterocolitis