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Tn*916*/Tn*1545* family in oral streptococci, a literature review from 1992 til 2017

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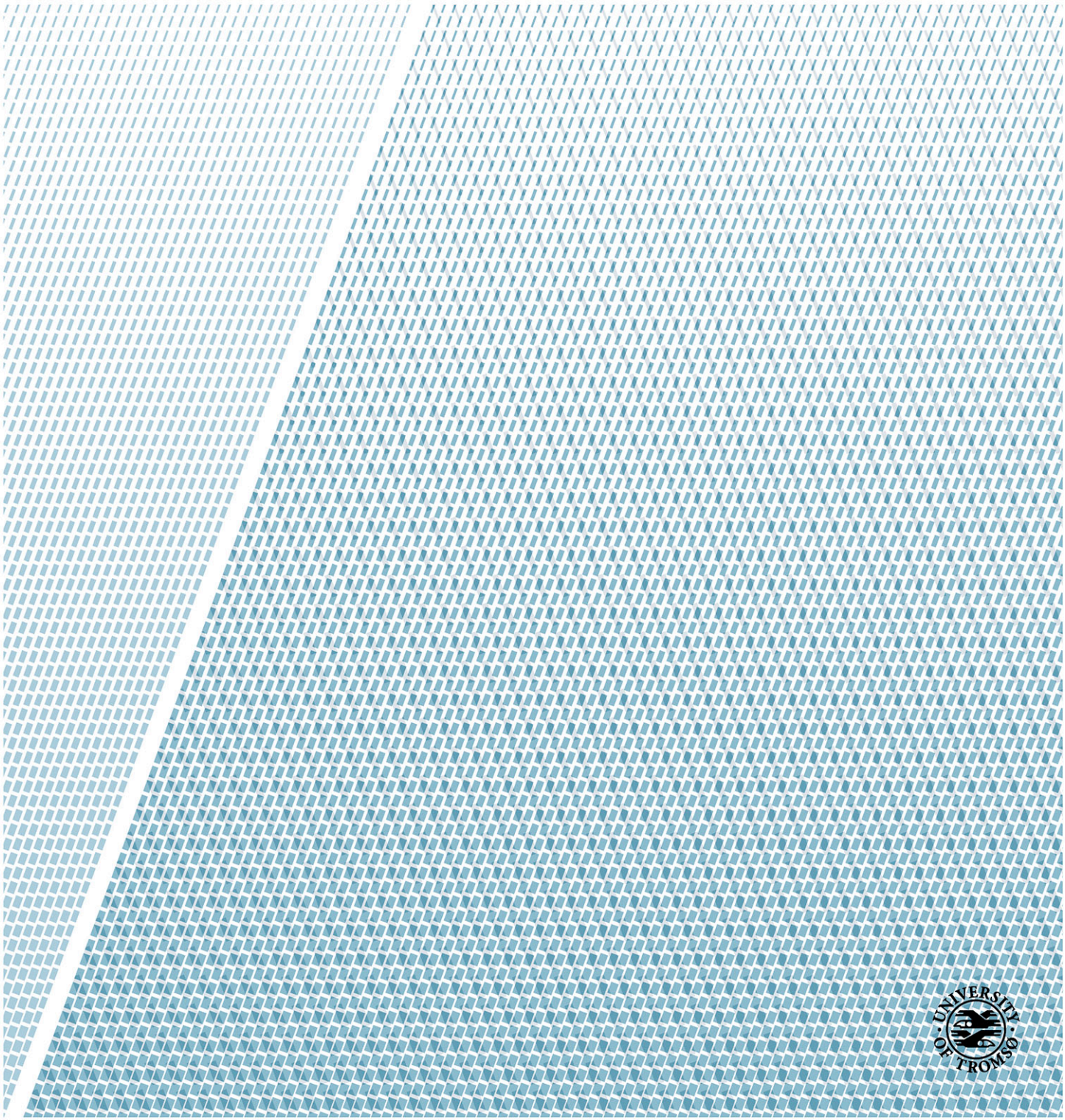

Table of contents

Introduction

In the oral cavity, there is a complex bacterial community that likely consists of thousands of bacterial species who are both indigenous and transient. These bacteria live primarily as complex, polymicrobial biofilms often called dental plaque(1). The close proximity of the bacterial cells within this dental biofilm provides an excellent environment for horizontal gene transfer, which can lead to the spread of antibiotic resistance genes amongst the biofilm inhabitants (1). Several transmissible genetic elements carrying antibiotic resistance genes, including but not limited to, plasmids and conjugative transposons, have been reported from human oral bacteria. In addition, rapidly accumulating genome sequence data are facilitating identification of the oral bacterial MOBILOME (Mobilome is defined as: all mobile genetic elements that carry antibiotic resistance gene).

Indigenous oral bacteria are those whose normal habitat is the oral cavity. However, many bacterial species can be found transiently in the oral cavity. It is usual to isolate bacterial species in the oral cavity that are temporarily colonizing oral surfaces. However, others bacteria succeed in finding a suitable surface for their persistence, i.e. permanent colonization. The ecological characteristics of the different surfaces found in the oral cavity, each with different key ecological factors such as adhesion ligands, pH, nutrients, redox potential, oxygen tension, and temperature, make it a unique microbial habitat in the human body (1).

Antibiotic resistance genes and acquisition of mobile genetic elements

Clinically isolated oral streptococci have shown resistance to a panel of antibiotics, and healthcare workers all over the world face this problem on a daily basis. Antibiotic resistance genes found in oral bacteria encode for multi-drug efflux pumps and resistance genes to aminoglycosides, β-lactams, bacitracin, bacteriocins, macrolides and tetracycline (2). Mobile genetic elements (MGE) that carry antibiotic resistance genes in oral bacteria can be plasmids, phages, transposons and integrons. Several studies have demonstrated the transfer of antibiotic resistance genes by the various MGE in oral bacteria (3, 4).

Relevant facts

Use of antibiotics

Antibiotics are generally considered adjunctive therapy for urgency endodontic treatment. Their value cannot be underestimated, especially when drainage cannot be achieved or the infection shows signs of local extension or systemic involvement. Beta- lactam, tetracyclin and macroloide antibiotics have been prescribed in endodontics, especially for the treatment of acute apical abscesses associated with systemic involvement, spreading infections, abscesses in medically comprised patiens who are at increased risk of a nonoral secondary infection after becteremia, propfylaxis for medically comprised patients during routine endodontic therapy, and replantation of avulsed teeth (5).

Antibiotic resistance

Antibiotic resistance is a socioeconomical and clinical health problem that is here on permanent basis. Over the past years, the use and misuse of antimicrobials has increased the number and types of resistant microorganisms. Consequently many infectios diseases may one day become untreatable due to the development of pan-resistance. With the growth of global trade and travel, reisistant micoorganisms can spread promptly to any part of the world. Overuse, underuse and misuse of medicines contribute to the development of drug resistance. Sub- therapeutic doses of antibiotics used in animal- rearing can result in resistant microorganisms, which can spread to humans (6).

Resistance can be innat or acquired. Some bacterial species show a high intrinsic resistance to a number of antibiotics whereas others are normally highly antibiotic susceptible. Acquired resistance is resistance that originally was not present within the bacteria, but has been evolved through genetic alterations in the genome, or by horizontal transfer of resistance genes located on various types of mobile genetic elements (MGE) (7).

Today there are no examples of antibacterial agents that have not been developed antibiotic resistance against. Therefore, although there is hope for novel classes of antibiotics to be developed, bacteria will certainly evolve resistance against them too. Therefor it makes sense to do evertyhing possible to prolong the life span of the already excisting antibiotics (8-10). By knowing how reisistance is spread between species and how it evolves in the population, steps can be introduced to prevent or at least delay the growth of antibiotic resistance.

We only have to go 20-30 years back to a situation where there was no penicillin resistance amongst pneumococci. Antibiotic resistance in bacteria is a result of classical Darwinian selection, caused by applying antibiotics to people with bacterial infections.

Mobile genetic elements (MGE)

Mobile genetic elements are sequences of genetic material that can change places on the chromosome, be exchanged between chromosomes, between bacterias and between different bacterial species. They encode enzyms and other proteins necessary to facilitate the movement of genetic material between bacterial chromosomes (11). Transposition of mobile genetic elements can radically alter genome structure and sequence. In doing so, they can alter gene expression and cellular function. The play a critical role in the spread of virulence factors, and therefore can lead to antibiotic resistance. MGE could be transposons, plasmids, bacteriophages and integrones (12).

Plasmids

Plasmids are extra chromosomal genetic material found in many bacterial cells. They have a circular shape. Ususally plasmids do confer a spesific property to the cell, like toxin production, antibiotic resistance, factor production etc. They can also be responsible for transporting of bacterial genes and antibiotic resistance from one cell to another (13).

Plasmids are used in genetic engineering to generate recombinant DNAs and as a mechanism to transfer genes between organisms. This is done by splicing genes into the ring formed plasmid, which after will be able to transport the gene into a bacterial cell, where the gene will be put in function.

Plasmids differ from chromosomes by being non- essential to bacteria. Although plasmids are self- replicating molecules that reside within host cell, they are not considering part of the bacterial genome. This is becuase the actual plasmid can be transported from one bacterial species to another, and also becuase plasmids are not nescessary for the bacteral vital conditions.

Plasmids are replicons, which means they contain their own origin of replication and are in charge of their own replication. Plasmids normally time their replication with the host cell replication, so that each daughter cell receives a copy of the plasmid.
Plasmids do rely on the host cell to provide energy and raw materials, similar to viruses, but plasmids do not damage the host cell.

In oral streptococci, several plasmids have been discovered. For example the beta plasmids from Streptococcus faecialis strain DS5. This plasmid codes for erythromycin and lincomycin resistance, and was introduced for group F streptococci by transformation.

Transposons
A transposon is a moving genetic element, and can move from one place to another within the same genome (14). A transposon is a part of the host cell DNA, and was first thought not to have its own center of replication like the plasmid. Today this is shown not to be true. Evidence has been found for Tn*916* to replicate by itself (15).
Transposons are categorized into classes according to their mechanism. Mechanisms can be either "copy and paste" (class I) or "cut and paste" (class II). Class I, also called retrotransposons, copy themselves in two stages; first from DNA to RNA by transcription, then from RNA to DNA by reverse transpcription. The DNA copy is then inserted into the genome in a new position. Retrotransposons behave very similarly to retroviruses such as HIV.
Class II, also called DNA transposons, do not copy themselves through transcription/reverse trancription. They "cut and paste" genetic material from one place in the chromosome to another (16). Together with plasmids, transposons are able to harbour antibiotic resistance genes.

Transposons are considred mutagenes (mutagen: factor that increases the mutation frequency) and can damage the host cell genome in different ways:

- A transposon that inserts itself into a functional gene will most likely disable that gene.
- After a transposon leaves a gene, the resulting gap will probably not be repaired correctly.
- Multiple copies of the same sequence, can hinder precise chromosomal pairing during mitosis and meiosis, resulting in unequal crossovers, one of the main reasons for chromosome duplication.

- Transpsons can carry accessory genes such as antibiotic resistance genes.

Transposons are found in many forms of life. They might have arisen independently, or perhaps just once and then spread to other kingdoms by horizontal gene transfer (17).

Integrones

Integrones are repeated sequences of DNA benefitted by bacteria to absorb foreign genes from other cells, especially antibiotic resistance genes (18). An integrase gene codes for an intregrase protein, which is responsible for putting the gene into the genome of the bacteria.

The integron consists of

- a promotor, necessary for efficient transcription and expression of gene cassettes present in the integron
- an *intl* gene encoding an integrase
- A recombination site *attl*

At least six classes of integrons have been determined according to their intl gene sequences. The gene casettes are incorporated in the integrons. The casettes contain genes (500- 1000 bp) and a recombination site. Integrons express gene casettes and are capable of exchanging them. Many gene cassettes encode antibiotic resistant determinants. Integrons seem to have a major role in the spread of multidrug resistance in bacterial strains.

Phages

A bacteriophage, known as a *phage*, is a virus that infects and replicates within a bacterium. Bacteriophages are composed of proteins that encapsulate DNA or RNA genome, and may have relatively simple or elaborate structures. Their genome size vary, and may contain everything from four to several hundreds of genes (19).

Phages replicate within the bacterium following the injection of their genome into its cytoplasm. Bacteriophages are ubiquitous viruses, found wherever bacteria exist. It is estimated there are more than 10^{31} bacteriophages on the planet.

Phages can transfer antibiotic resistance between bacterial species (20).

Tn*916*/Tn*1545* family of conjugative transposons

The transposons Tn916 and Tn1545 were both discovered in the 1970s when tetracycline resistance was transferred from a resistant to a susceptible strain of *Enterococcus faecalis* in

the absence of a detectable plasmid. They are both members of the large family of related conjugative transposons known as the Tn*916*/Tn*1545* family, which are found in an extremely diverse range of bacteria (11).

The Tn*916*/Tn*1545* family is the most widespread of the conjugative transposons. With the huge increase in bacterial genomic sequence data available due to the widespread use of next generation sequencing, more putative conjugative transposons belonging to the Tn*916*/Tn*1545* family are being reported. Almost all of the transposons belonging to the Tn*916*/Tn*1545* family encode tetracyclin resistance, but increasingly resistance to other antimicrobials is also detected (11).

Subsequently a variety of Tn*916*/Tn*1545* like elements have been described with elements containing different recombinases and resistance genes.

Tn*916* is the prototype of this family of conjugative transposons. The genetic structure of Tn*916* is modular. These elements comprise four functional modules: conjugation, regulation, recombination and accessory genes, the latter are often antibiotic resistance genes. Most Tn*916*/Tn*1545* like elements possess the tetracycline resistance gene *tet*(M), located within the regulatory module. Some contain macrolide resistance genes *erm*(B) (ex. Tn*1545*, Tn*6002* and Tn*6003*) and *mef*(A) (ex. Tn*2009* and Tn*2017*), as well as kanamycin (*aphA-3*) (Tn*1545*) and mercury (*mer*) (Tn*6009*) resistance genes. Tn*916*-like elements have been found in >30 bacterial genera and are common among the human oral commensal bacteria, which act as a reservoir for resistance genes.

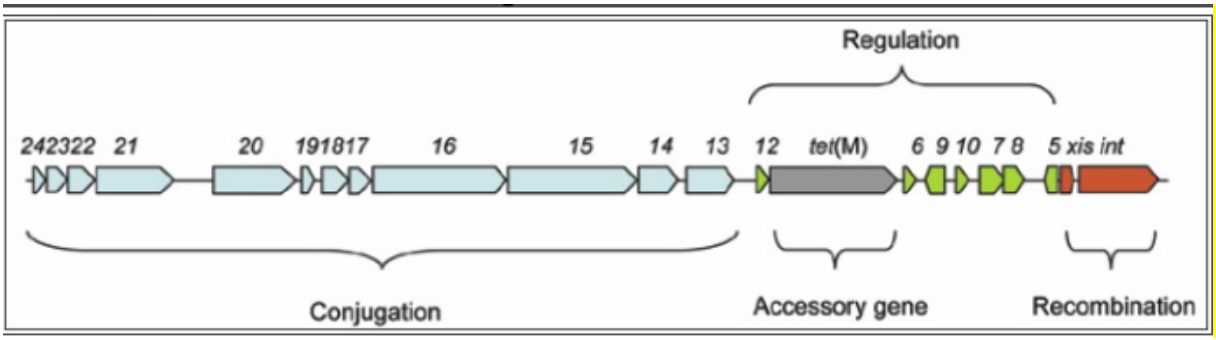

Figure 1. The structure of *tn916* is shown.

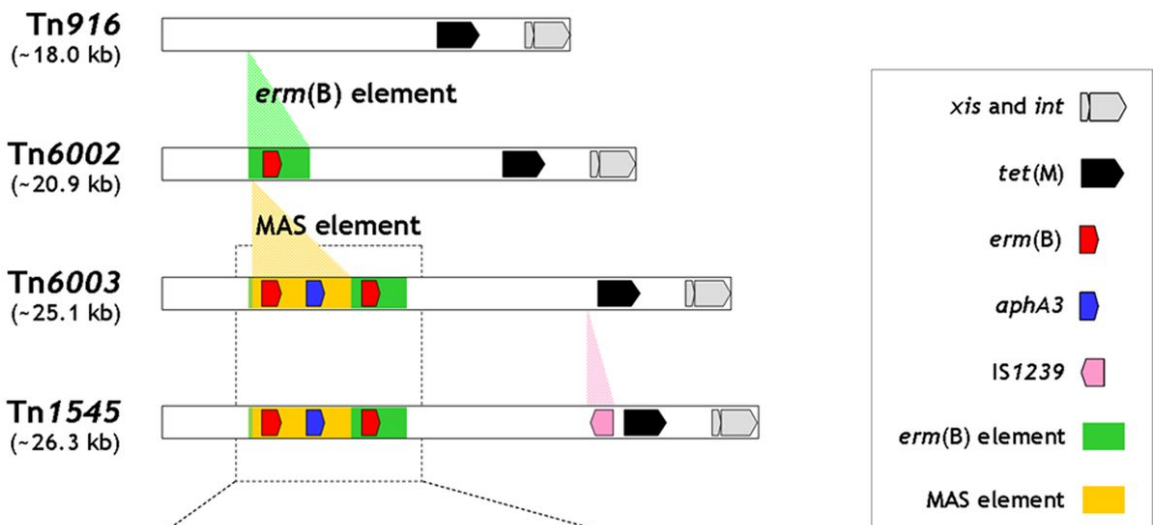

Figure 2. The simple structure of various member of the Tn*916*/Tn*1545* family is shown.

In the current master project, an account of all published articles in the last 25 years about Tn*916*/Tn*1545* like elements with clinically relevant antibiotic resistance in oral streptococci, will be systematically reviewed.

Material and Methods

Criteria to select published articles:

In the current study, the pubmed database (www.pubmed.com) was used to retrive all published articles that meet our criteria. The criteria we set for searching the database were: any article published in the last 25 years regarding Tn*916*/Tn*1545* like elements in oral streoptococci. In addition, one fundamental article which reported about these elements in oral streptococci from 1984, was also included.
Search words: Tn916 and resistance and oral streptococci and mobile genetic elements.

In the current master project, an account of all published articles in the last 25 years about mobile genetic elements with clinically relevant antibiotic resistances in oral streptococci, were systematically reviewed with a set of predetermined questions. These questions were:

What were the objectives of the study?
Which streptococci strains were tested in the study?
Which methods were used?
How many resistant streptococci found in the study?
Does tn916 or any other mobile genetic elements exist?
Are the mobile genetic elements transferable?

Results

After typing in the predetermined search words in Pubmed, eleven articles were listed. Three of them were excluded because they were literature studies or because of no access to them. The included articles were one from 2016, one from 2015, one from 2012, one from 2011, one from 2007, one from 2001, one from 1992 and one from 1984.

The articles

Disseminated tetracycline resistance in oral streptococci: implication of a conjugative transposon

This article is from 1984 and written by four authors; DL Hartley, KR Jones, JA Tobian, DJ LeBlanc and FL Macrina (21).

What was the objective of this study?

The aim of this study was to check oral streptococci and their hybridisation with TCR (tetracyclin resistance determinant from S.mutans U202). This is to see if the bacterial species are resistant to tetracyclin. The isolated bacteria were obtained from patients treated with tetracyclin, before doing the study.

Which streptococci strains **were tested in this study?**

Eleven oral streptococci (including *Streptococcus sanguis* I, *S.sanguis* II, *Streptococcus mitis*, and *Streptococcus salivarius*) from seven patients were examined.

Which methods were used?

The authors used the specific Tetracyclin resistance DNA sequence (cloned Tetracyclin resistance determinant from S. mutans U202) as a molecular probe in studying the dissemination of TCR among oral streptococcal species, isolated from patients treated with tetracycline. They were grown on brain heart agar. Southern blot analysis and antibiotic susceptibility testing were used.

How many % resistant oral streptococci and type of antibiotic?

All of the eleven strains studied were resistant to clinically significant levels of tetracyclin. Five strains (*S. salivarius* I1, *S. mitis* 118, *S. sanguis* II F206, *S. sanguis* I C219, and *S. mitis* P107) displayed constitutive expression, whereas the rest displayed inducible expression.

Which genes were dominant among the resistant species?

It has previously been shown that the TCR determinant cloned from *S. mutans* U202 belongs to the *tet*(M) gene. The authors conclude that the Tcr determinants of the strains studied here represent the *tet*(M) gene.

Does Tn*916* or other mobile genetic elements excist? Which elements?

The authors believe that the transferable TCR element present in *S. Sanguis* I 1141 is a conjugative transposon. Data suggest that this resistance transfer element occupies a chromosomal location in streptococcal cells and that it strongly resembles the conjugative transposon Tn*916* in its behavior.
This study presents physical evidence that a related DNA sequence specifying TCR is disseminated in oral streptococcal strains.

Are the mobile genetic elements transferable?

Yes, the transposon detected is able to transfer to new bacterial cells. Data indicate that at least one strain (*S.sanguis*11141) was able to transfer its TCR phenotype to other streptococci in the absence of detectable plasmid DNA. This transfer could be made to enteric streptococci and to oral streptococci.

Natural occurrence of structures in oral streptococci and enterococci with DNA homology to Tn916

This study is from 1992 and written by Bentorcha, Clermont, de Cespèdès G, Horaud T. from Laboratoire des Staphylococcus et der Streptocoques, Institut Pasteur, Paris, France (22).

What was the objective of this study?

The aim of the study was to trace the dispersion of genetic elements related to Tn*916* in oral streptococci and enterococci and to screen for the existence of elements presenting homology with Tn*916* but having an internal structure different from that of Tn*916*.

Which streptococci strains **were tested in this study?**

Seventeen oral streptococci and 18 enterococci were tested for the presence of DNA sequences homologous to the conjugative transposon Tn*916*, encoding tetracycline resistance.

Which methods were used?

The oral streptococci were collected from cerebrospinal fluids, teeth, sinuses and vaginas between 1979 and 1988. The enterococci were isolated from blood and pus between 1960 and 1987. Methods used were DNA isolation, digestion by restriction enzymes, agarose gel electrophoresis, DNA blotting and DNA-DNA- hybridization.

How many % resistant streptococci and type of antibiotic?

All the strains were resistant to tetracyclines, including minocycline, and most of them were resistant to other antibiotics too.

Which genes were dominant among the resistant species?

In most of the strains studied that carried Tn*916*/Tn*1545* like elements, the *tet*(M) determinant was located on a 4.8-kbHincII fragment, as it is in Tn*916*, and more rarely *tet*(M) was located on a slightly smaller fragment. *Tet*(O) was also detected in other streptococci species.

Does Tn*916* or other mobile genetic elements excist? Which elements?

Tn/Tn*1545* like structures were found on the chromosomes of 11 oral streptococci out of the 17 investigated streptococci.

Are the mobile genetic elements transferable?

No detectable transfer of the TCR marker in the 17 oral streptococci.

Transfer of Tn916-like elements in microcosm dental plaques

This article is from 2001 and written by Roberts AP, Cheah G, Ready D, Pratten J, Wilson M, Mullany P from Department of Micobiology, Eastman Dental Institute for Oral Health Care Sciences, University College London (4).

What was the objective of this study?

The aim of this study was to see if Tn*916* like elements was transfered between streptococcal bacteria in a constant- depth film fermentor.

Which streptococci strains **were tested in this study?**

Microcosm dental plaques were grown from an inoculum of human saliva in a constant-depth film fermentor. The inoculum contained four tetracycline-resistant streptococcal species , each of which contained a Tn*916*-like element. They were *S. salivarius, S. mitis, S. oralis* and *S. gordonii.*

Which methods were used?

All strains were grown on brain heart infusion agar and added antibiotics, rifampin and tetracyclin. Transconjugants were selected on media containing tetracycline and rifampin, so only the resistant species were selected and investigated further.

Filter mating experiments were used for transferring elements from one strain to another.

Constant depth film fermentor was used. This method is necessary to grow organisms in such a manner as to mimic their physiological growth as a biofilm state in vivo.

To determine if a Tn*916*-like element was present, a Southern blot was performed on *Hin*cII-digested genomic DNA

How many % resistant streptococci and type of antibiotic?

This study was performed to illustrate transferability and not prevelance of resistance.

Which genes were dominant among the resistant species?

Tn*916*-like elements are present within the oral microflora, and in this paper the authors show that they could transfer *tet*(M) not only in filter-mating experiments but also in a model oral biofilm.

Does Tn*916* or other mobile genetic elements excist? Which elements?

Yes, Tn*916* element is invistigated in this study.

Are the mobile genetic elements transferable?

Yes, they are transferable. This study was the first to demonstrate the transfer of *tet*(M) within a model of oral biofilm, an important finding because the oral cavity is one of the most

colonized environments within humans and oral bacteria have the opportunity to come into contact with bacteria that pass through the oral cavity; also, oral bacteria can be readily transferred from one human to another. This work demonstrates that oral streptococci are responsible for harboring and disseminating these promiscuous mobile elements to other oral microflora.

Demonstration of in vivo transfer of doxycycline resistance mediated by a novel transposon

This article is from 2007 and written by Warburton PJ, Palmer RM, Munson MA, Wade WG from King`s College London, Dental Institute (16).

What was the objective of this study?

The aim of this study was to investigate the transfer of bacterial doxycycline resistance between oral bacteria in subjects receiving systemic doxycycline for the treatment of periodontitis.

Which streptococci strains **were tested in this study?**

Streptococci from subgingival plaque obtained from two patients with periodontitis.
In the first object, a strain of Streptococcus sanguinis resistant to doxycycline, was a minor component of the pre- treatment streptococcal flora but dominated post- treatment. In another subject, a strain of *Streptocuccus cristatus*, which was sensitive to doxycycline before treatment, was found to have acquired novel conjugative transposon during treatment, rendering it resistant to doxycycline and erythromycin.

Which methods were used?

Bacteria from the subgingival plaque were cultured before and after treatment with systemic doxycyclin, genotyped and investigated for the presence of antimicrobial resistance determinants and conjugative transposons. Molecular methods used were repetitive extragenic palindromic (REP) gene amplification, PCR amplification, conjugal-mating experiments and southern blot hybridization.

How many % resistant streptococci and type of antibiotic?

Type of antibiotic: doxycyclin.

In subject A: proportion anaerobic organisms resistant to doxycyclin rose from 2.4% before treatment, to 51.9% after treatment. In subject B: fell from 18.8% before treatment to 2.9% after treatment. Another interesting result in this study, was that the number of Streptococci in subject B increased from 45 til 89 after doxycyclin treatment. The number of streptococci in subject A were constant before and after treatment.

Which genes were dominant among the resistant species?

In subject A: REP-PCR genotyping showed that the post treatment resistant *S. Sanguinis* belonged to the same clonal type as the resistant *S. Sanguinis* strains seen pre-treatment, therefore the authors conclude that the resistance is due to clonal expansion of an already resistant bacterial population. The amount of resistant *S.sanguinis* before treatment was 6 out of total 8 detected, and the amount after treatment was 98 out of 98 detected (100%).

In subject B: After treatment, five bacterial species resistant to doxycycline were detected on TYC plates (*S. Australis, S.Cristatus, S. Infantis, S. Oralis and S. Parasanguinis)* but only one of these, *S. cristatus*, was also found before treatment, although it was then only doxycycline-sensitive. REP-PCR genotyping showed that the pre-treatment sensitive strains belonged to the same clonal type as the post treatment resistant strains.

PCR for *tet*(M), *int* -Tn and *xis* -Tn in *S. Cristatus* demonstrated that the resistant strains of *S. Cristatus* after treatment had acquired the *tet*(M) resistance determinant and integration/ excision genes typically found in the Tn*916* family of conjugative transposons. In post-treatment, *S. Cristatus* was demonstrated to harbour erm(B) by PCR.

Analysis showed that *S.cristatus* harbored a conjugative novel transposon, and that this was responsible for the conjugal transfer of doxycycline resistance. Sequencing of the transposon revealed it to be a complex transposon made up of Tn*916* and a fragment of DNA encoding five genes of unknown source.

Does Tn*916* or other mobile genetic elements excist? Which elements?

A conjugavtice novel transposon was found in *S. Cristatus,* Tn*6002*, and was resposible for the conjugal transfer of antibiotic resistance in subject B.

This study has demonstrated the transfer of antimicrobial resistance from one oral streptococcal species to another oral streptococcal during antimicrobial treatment of periodontitis. This confirms that the administration of antimicrobial agents for common chronic bacterial infections, such as periodontitis, can contribute to increased levels of bacterial resistance to antimicrobials.

Are the mobile genetic elements transferable?

Yes. This is the first direct demonstration of transfer of antimicrobial resistance carried on a conjugative transposon between oral bacteria during systemic antimicrobial treatment of periodontitis in humans.

Impact of minocycline ointment for periodontal treatment of oral bacteria

This article was written in 2011 by Nakao, Takigawa S, Sugano N, Koshi R, Ito K, Watanabe H, Senpuku H from National Institute of Infectional Diseases, Department of Bacteriology, Japan (23).

What was the objective of this study?

In this study, the objective was to find out if use of minocycline ointment results in more or less tetracyclin resistant oral streptococci.

Which streptococci strains **were tested in this study?**

Supragingival plaque samples from 41 adults with periodontal disease, who had not taken any antibiotics for 6 months, were collected. The streptococci investigated in this study were *S. mitis, S. salivarius, S. sanguinis and S. oralis.*

Which methods were used?

Isolation of the bacterial species, cultivation on agar plates, PCR and southern blotting are the methods used in this study.

How many % resistant streptococci and type of antibiotic?

Before minocyclin ointment treatment, the presentage of antibiotic resistant bacteria were 11,9%. After minocyclin ointment treatment, the presentage was 34,2%. The antibiotic used in this study was minocyclin, a broad spectra antibiotic belonging to the tetracyclin class.

Which genes were dominant among the resistant species?

49 tetracyclin resistant oral streptococci from 16 individuals possessed *tet*(M) gene.

Does Tn*916* or other mobile genetic elements excist? Which elements?

The study identified *tet*(M) on the Tn*916*-like elements as the gene responsible for tetracycline-resistance.

Are the mobile genetic elements transferable?

The authors concluded that genetic transfer is also possible for Tn*916* found in this study.

Tn916-like elements from human, oral, commensal streptococci possess a variety of antibiotic and antiseptic resistance genes

This article is from 2012 and written by Ciric L, Allatif M, Sharma P, Patel R, song X, Mullany P, Roberts AP from University College London, Department of Microbial Diseases (24).

What was the objective of this study?

The objective of this study was to map the content of antibiotic resistance genes in human oral streptococci, and their content of mobile genetic elements.

Which streptococci strains **were tested in this study?**

48 minocycline-resistant oral streptococci cultured from pooled saliva, from healthy human volunteers. The streptococci species investigated were *S. oralis, S. mitis, S. sanuguinis, S.gordonii.*

Which methods were used?

The species identity of all the streptococci was determined by amplifying and partially sequencing the superoxide dismutase gene, *sod*(A). The *sod*(A) sequences of all isolates were aligned and a comparison was made. A total of 25 different *Streptococcus* sp. were isolated. All 48 isolates were analysed by performing long polymerase chain reactions (PCRs) designed to amplify a large range of Tn*916*-like transposons (including Tn*916*, Tn*6002*, Tn*6003*, Tn*1545*, Tn*3872*, Tn*2009*, Tn*2010*, Tn*2017*, Tn*6084* and Tn*6079*) followed by digestion with *Hin*cII and sequence analysis.

How many % resistant streptococci and type of antibiotic?

The article does not inform about the prevelence of antibiotic resistant streptococci out of the total investigated species.

Which genes were dominant among the resistant species?

Tet(M) and *erm*(B) were the genes dominant among the resistant species.

Does Tn916 or other mobile genetic elements excist? Which elements?

Tn*3872* was found in 19 isolates (39.6%). Tn*916* [encoding tetracycline resistance via *tet*(M)] was found in 15 isolates (31.3%). Tn*6002* [encoding macrolide resistance via *erm*(B)] was found in 14 isolates (29.2%) and Tn*6087* was found in 1 isolate (2.1%).

Are the mobile genetic elements transferable?

All elements were screened for their ability to excise from the host genome by PCR amplification of the joint of the left and right ends of the element, which are coupled when a circular DNA molecule is formed. In total, 32 (66.7%) of the 48 elements representing all four types of Tn916-like element were capable of excision from the host chromosomes, the first step in conjugative transfer.

Resistance Genes and Genetic Elements Associated with Antibiotic Resistance in Clinical and Commensal Isolates of Streptococcus salivarius

This article is from 2015 and written by France; Chaffanel F, Charron Bourgoin F, Libante V, Leblond- Bourget N, Payot S from Faculté des Sciences et Technologies Universitè de Lorraine (25).

What was the objective of this study?

The objective with this study was to map clinical and commensal isolates susceptibilities to different antibiotics (tetracyclines, macrolides, lincosamides, aminoglycosides, phenicol

antibiotics), the presence of selected resistance genes (*tet*(M), *tet*(O), *erm*(A), *erm*(B), *mef*(A/E), and *cat*(Q) and associated genetic elements.

Which streptococci strains were tested in this study?

92 clinical and 120 oral/digestive commensal streptococcus salivarius strains were collected and analyzed by Multilocus Sequence Typing (MLST).

Which methods were used?

Selected strains (92 clinical and 46 commensal strains) were then examined for their susceptibilities to tetracyclines, macrolides, lincosamides, aminoglycosides, and phenicol antibiotics by multilocus sequence typing (MLST). The presence of resistance genes *tet*(M), *tet*(O), *erm*(A), *erm*(B), *mef*(A/E), and *cat*(Q) and associated genetic elements was investigated by PCR, as was the genetic linkage of resistance genes.

How many % resistant streptococci and type of antibiotic?

The results indicated that only 41% of the clinical isolates and 24% of the commensal isolates were susceptible to all the antimicrobials tested.

High rates of erythromycin resistance were found for both clinical isolates (56%; $n = 52$) and commensal isolates (76%; $n = 35$). The second-highest resistance rates were those against tetracycline: 28% ($n = 26$) for the clinical strains and 17% ($n = 8$) for the commensal strains. Two clinical strains (L20 and N11) and two commensal strains (F3-10 and F4-20) were resistant both to chloramphenicol and to erythromycin.

Which genes were dominant among the resistant species?

Erythromycin resistant species:

Among the 52 clinical strains that were resistant to erythromycin, 21 harbored only an *erm*(B) gene, 29 carried only a *mef*(A/E) gene, and 2 carried both *erm*(B) and *mef*(A/E) genes . Most of the erythromycin-resistant commensal strains harbored a *mef*(A/E) gene ($n = 32$), and only three carried an *erm*(B) gene.

Tetracyclin resistant species:

28% ($n = 26$) clinical strains and 17% ($n = 8$) commensal strains were resistant to tetracyclin. Most of the tetracycline-resistant strains carried a *tet*(M) gene ($n = 32$). No *tet*(M) or *tet*(O)

tetracycline resistance determinant was detected in 2 strains. Three clinical strains (L25, L31, and L57) carried a *tet*(M) gene but were susceptible to tetracycline.

Cloramphenicol, erythromycin and kanamycin resistant species:

Two clinical strains (L20 and N11) and two commensal strains (F3-10 and F4-20) were resistant both to chloramphenicol and to erythromycin. These four strains were found to carry a *mef*(A/E) and a *catQ* gene. In addition, only two clinical strains (T00 and T80) were resistant to kanamycin.

Does Tn*916* or other mobile genetic elements excist? Which elements?

In this study, they searched for four types of genetic elements:

(i) the integrative and conjugative element ICE*St3*

(ii) the integrative and conjugative element Tn*916* and its variants carrying either an *erm*(B) (Tn*3872* and Tn*6002*) or a *mef*(A/E) gene (Tn*2009*)

(iii) the MEGA element, which carries a *mef*(A/E) gene

(iv) the IQ element, recently identified in viridans group streptococci

More than one third of the clinical strains (n = 36) and half of the commensal strains (n = 24) were positive for the relaxase gene of ICE*St3.* Among these strains, 23 clinical and 15 commensal strains were also positive for the integrase gene of ICE*St3.*

A total of 31 clinical strains and 30 commensal strains gave a positive signal for the MEGA element, and all were also positive for the *mef*(A/E) gene. Two commensal strains (F3-10 and F4-20) carried a *mef*(A/E) gene but did not harbor a MEGA element. These strains were also resistant to chloramphenicol. They found that strain F4-20 carries a *catQ* resistance gene, which colocalizes with a *mef*(E) gene on an IQ-like element of 12,038 bp. This element differs from the IQ element described for *S. pneumoniae* strain 529 in that it carries a *mef*(E) gene instead of a *mef*(I) gene.Forty-one clinical strains (44%) and 11 commensal strains (24%) carried the relaxase gene of Tn*916.* Among these, 23 clinical and 6 commensal strains were also positive for the *tet*(M) gene. A genetic linkage between the *erm*(B) gene and Tn*916* genes was detected for 21 clinical strains and 3 commensal saliva strains. Two strains (L25 and L31) carried a Tn*3872* element with a silent *tet*(M) gene. One clinical strain (T00) gave positive signals for both Tn*3872* and Tn*6002* elements.

Thirty-two strains (13 clinical and 19 commensal) harbored both MEGA and ICE*St3* elements; 22 strains (12 clinical and 10 commensal) carried both MEGA and

Tn*916* elements; and 8 strains (2 clinical and 6 commensal) carried all three of these genetic elements.

Are the mobile genetic elements transferable?

A genetic linkage between a macrolide resistance gene and genes of Tn*916* was detected in 23 clinicalstrains and 5 commensal strains, with a predominance of Tn*3872* elements (n = 13), followed by Tn*6002* (n = 11) and Tn*2009* (n = 4) elements. Four strains harboring a *mef*(A/E) gene were also resistant to chloramphenicol and carried a catQ gene. Sequencing of the genome of one of these strains revealed that these genes colocalized on an IQ-like element, as already described for other viridans group streptococci. ICESt3-related elements were also detected in half of the isolates. This work highlights the potential role of *S. salivarius* in the spread of antibiotic resistance genes both in the oral sphere and in the gut.

Antibiotic Resistance Patterns and Related Mobile Genetic Elements of Pneumococci and β-Hemolytic Streptococci in Thai Healthy Children

This article is from 2016 and written by Tantivitayakul P, Lapirattanakul J, Vichayanrat T, Muadchiengka T from faculty of dentistry at Mahidol University in Bangkok, Thailand (26).

What was the objective of this study?

To investigate carriage rate, antibiotic resistance and related mobile genetic elements of pneumococci and BHS from school-children.

Which streptococci strains were tested in this study?

This article consider *Streptococcus pneumoniae* and beta- hemolytic streptococcus (BHS), who were collected from 220 school children.

Which methods were used?

Pneumococci and BHS were collected and testet for antibiotic susceptibility pattern by disc diffusion. Antibiotic resistance genes and related genetic elements were detected by PCR with specific primers.

How many % resistant streptococci and type of antibiotic?

Results indicated that fifty-four BHS isolates were resistant to erythromycin (28 %), tetracycline (52 %), or clindamycin (13 %). All isolates tested were 100 % sensitive to penicillin and levofloxacin.

Which genes were dominant among the resistant species?

The dominant erythromycin resistance genes in BHS were *mef*E and *erm*B, while the most common tetracycline resistance gene in this population was *tet*M.

Does Tn*916* or other mobile genetic elements excist? Which elements?

Almost all erythromycin- and tetracycline-resistant streptococci (97 %) contained various genetic elements, including mega elements and six different transposon types (Tn*2009*, Tn*2017*, Tn*917*, Tn*3872*, Tn*6002* and Tn*916*).

Are the mobile genetic elements transferable?

This article does not prove that the MGEs are transferable, but it says that "carriages of BHS with multidrug resistance in children might be important reservoirs of antibiotic-resistance genes carried by transposons. Tn*916*-like elements could lead to dissemination of the antibiotic resistance genes among genus streptococcus in human oral cavity and nasopharynx".

Discussion and conclusions

The time line of 25 years plus one relevant article from 1984, presents a development in technology and method to investigate antibiotic resistance provided by MGE. Knowledge and terminology about antibiotic resistance and mobile genetic elements have undergone some changes during this time and it will continue to change.

In the article from 2016, the tetracyclin resistance among BHS from 220 school children were 28% resistant to erythromycin and 52% were resistant to tetracyclin. In the article from 2015, erythromycin resistance among 46 commensal streptococcus salivarius were 76% and tetracylin resistance among them were 17%. The purpose of both studies was to map isolates susceptibilities to different antibiotics. The variation in results may be due to the minimal difference in amount of species isolated, 54 vs 46, or due to differences in populations.

In the articles from 2011 and 2007, isolates were first exposed for antibiotics, doxycyclin and minocyclin, and then showed increased antibiotic resistance for tetracyclins. In the 2007 article, the increase in resistance was due to transfer of bacterial doxycycline resistance between oral bacteria. In the article from 2011, resistance in oral streptococci to antibiotic increased from 11,9% to 34,2%. This was shown to be a result of acquired resistance due to change in genetic material (subject B), and clonal expansion (subjectA). This provide an evidence for the development and raise of antibiotic resistance after being exposed to antibiotic treatments.

Most of the articles show association between Tn*916*, *Tet*(M) and tetracyclin resistance. There is also shown collaboration between *mef*(E), *erm*(B) and erythromycin resistance. Association of chloramphenicol /erythromycin resistance and *mef*(A/E) and a *catQ* gene, is also shown in the studied articles.

The different studies investigated different bacterial isolates, number of strain analysed and have different methods for analysis. This can be problematic when comparing the results to each other and trying to make a conclusion. Some studies, for example "*Antibiotic Resistance Patterns and Related Mobile Genetic Elements of Pneumococci and β-Hemolytic Streptococci in Thai Healthy Children(2016)"* investigated isolates after testing them for antibiotic susceptibility, while others for example "*Tn916-like elements from human, oral, commensal streptococci possess a variety of antibiotic and antiseptic resistance genes, 2012"* only have included antibiotic resistant oral streptococci in their studies. In the first example we therefore get the amount of antibiotic resistant streptococci out of total investigated species, while in the second example we are not

informed about the original amount of streptococci species invistigated. Therefore we lack information about the prevalence of antibiotic resistant among streptococci out of total investigated species in these cases, and cannot come up with a percentage of resistant isolates.

Unfortunatly not all studies investigated the same oral streptococci types, used the same isolation methods, compared them to the same types of antibiotic and used the same methods for investigating content of genes and mobile genetic elements. For example in the article from 2015, isolates were investigated for their susceptibilites against tetracyclines, macrolides, lincosamides, aminoglycosides and phenicol antibiotics, while in the study from 2007 and 2011 isolates are tested for their way of reacting to minoxyclin and doxycyclin expose. This results in variaton of approach and results revealing different type of antibiotc susceptibilities. The articles from 2001 and 1992 have similar approaching methods by both investigating dispersion and contents of *Tn916*- like structures in oral streptococci.

On the other hand, while having articles and studies dealing with a broad set of antibiotics, isolates, approaching methods etc, we get more broad understanding and information of the complexity that the antibiotic resistance among oral streptococci and is in relation to MGE, specifically to Tn*916*/Tn*1545.*

In conclusion, antibiotic resistance in oral streptococci provided by MGE like Tn*916*/Tn*1545* family is an acknowledged problem in the literature. The propagation and expansion of these elements within and between bacterial species is aided by the use of antibiotic. Rational use of antibiotics is therefore an important element to help reduce the problem of antibiotic resistance.

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