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ORIGINAL ARTICLE

Resistance to the tetracyclines and macrolide-lincosamide-streptogramin group of antibiotics and its genetic linkage – a review

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Abstract

An excessive use of antimicrobial agents poses a risk for the selection of resistant bacteria. Of particular interest are antibiotics that have large consumption rates in both veterinary and human medicine, such as the tetracyclines and macrolide-lincosamide-streptogramin (MLS) group of antibiotics. A high load of these agents increases the risk of transmission of resistant bacteria and/or resistance determinants to humans, leading to a subsequent therapeutic failure. An increasing incidence of bacteria resistant to both tetracyclines and MLS antibiotics has been recently observed. This review summarizes the current knowledge on different tetracycline and MLS resistance genes that can be linked together on transposable elements.

Key words

antibiotics, genetic determinants of resistance, transposons, transmission of resistance

INTRODUCTION AND BACKGROUND

An excessive use of antimicrobial agents poses a risk for the selection of resistant bacteria, which could be either causative agents of a specific disease or a reservoir of genetic determinants of resistance. The latest surveillance report on antimicrobial consumption in the community (i.e. outside hospitals) lists macrolides and tetracyclines as the third and fourth most commonly used subgroups of antibacterials in Europe [1]. Even more pronounced, however, is their use in veterinary medicine, where tetracyclines are the most frequently sold therapeutic antibiotics (37 %), and macrolides account for 8% of the total sales, and are the fourth most frequently sold antibiotic class for veterinary use within Europe [2]. Because of their wide usage in veterinary medicine, their presence in the environment and agriculture is ubiquitous. Table 1 lists the tetracycline and macrolide-lincosamine-streptomycin (MLS) antibiotics that have been approved for human or veterinary use.

Tetracyclines are broad spectrum antibiotics discovered in the late 1940s. They block the attachment of charged aminoacyl-tRNA to the ribosomal acceptor (A) site, and so interfere with the protein synthesis by preventing the introduction of new amino acids to the nascent peptide chain. They are effective against a wide range of gram-positive and gram negative bacteria, chlamydiae, mycoplasmata, rickettsiae and protozoan parasites [3, 4]. Due to their low cost, they have been widely used in veterinary medicine for the treatment of various infections, such as colibacillosis, chronic respiratory disease, enteritis and many others [3]. In Europe, tetracyclines belong to the most widely used veterinary antibiotics, ranging from 12.4 up to 102.8 mg/kg of produced meat [5]. Moreover, tetracyclines have also been used in aquaculture [6] and agriculture [7].

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Table 1. List of approved tetracyclines, macrolides, lincosamides and streptogramins for human or veterinary use in European Union (2011_Adriaenssens, 1999_EMEA).
http://www.chemeurope.com/en/encyclopedia/ATC_code_J01.html#J01AA_Tetracyclines

Tetracyclines	Macrolides	Lincosamides	Streptogramins
Chlorotetracycline[b]	Azithromycin[c]	Clindamycin[c]	Pristinamycin[c]
Clomocycline[c]	Clarithromycin[c]	Lincomycin[b]	Quinupristin/ Dalfopristin[c]
Demeclocycline[c]	Dirithromycin[c]	Pirlimycin[a]	Virginiamycin[a]
Doxocycline[b]	Erythromycin[b]		
Lymecycline[c]	Flurithromycin[c]		
Metacycline[c]	Gamithromycin[a]		
Minocycline[c]	Josamycin[b]		
Oxytetracycline[a]	Kitasamycin[a]		
Penimepicycline[c]	Midecamycin[c]		
Rolitetracycline[c]	Miocamycin[c]		
Tetracycline[a]	Oleandomycin[b]		
	Rokitamycin[c]		
Glycylcyclines[c]*	Roxithromycin[c]		
	Spiramycin[b]		
	Telithromycin[c]		
	Tildipirosin[a]		
	Tilmicosin[a]		
	Tulathromycin[a]		
	Troleandomycin[c]		
	Tylosin[a]		
	Tylvalosin[a]		

* A new class of antibiotics derived from tetracycline, with tigecycline as the only glycylcycline antibiotic approved for antibiotic use by now
[a] veterinary use only
[b] veterinary and human use
[c] human use only

To-date, 59 tetracycline resistance genes (*tet* genes) have been described. They can mediate resistance to tetracyclines by three different mechanisms: ribosomal protection, efflux and enzymatic inactivation of the active compound [8, 9].

MLS antibiotics are three chemically distinct, but functionally similar antibiotic classes. Macrolides with mycarose sugars on the fifth carbon in the lactone ring, lincosamides and streptogramin A inhibit the peptidyl transferase reaction [10], whereas macrolides of the erythromycin group prevent the early events of peptide elongation, and streptogramin B blocks the exit tunnel through which the nascent peptide chains exit the ribosome, resulting in the release of incomplete peptides [10, 11].The first described macrolide erythromycin has a moderately broad spectrum of activity, while newer semi-synthetic derivatives (e.g. clarithromycin and azithromycin) have a broader spectrum and are used in human medicine for the treatment of upper and lower respiratory tract infections, infections of the skin and soft tissue, sexually transmitted diseases, community-acquired pneumonia and atypical *Mycobacterium* infections. Together with lincosamides, macrolides are also used for the treatment of group B streptocoocal infections, or for the intrapartum prevention of *Streptococcus agalactiae* neonatal infections in penicillin hypersensitive patients [12, 13]. In combination with fluoroquinolones, erythromycin is commonly used in the therapy of severe infections caused by *Campylobacter* spp. [5]. In veterinary medicine, macrolides (e.g. tylosin) are recommended for the treatment of respiratory infections in cattle, swine and poultry. Further indications include treatment of proliferative enteropathy, enteritis and arthritis in swine, necrotic enteritis in poultry, and mastitis in cattle caused by Gram-positive bacteria.

A total of 92 genes that confer resistance to MLS antibiotics have been described to-date [14]. They can be roughly divided into three groups, depending on the mechanisms by which they confer resistance to one or all of these groups of antibiotics. Three main mechanisms of resistance to MLS antibiotics have been described: methylation of rRNA (target modification), active efflux and inactivation of the antibiotic. Target modification is achieved *via* the action of the protein product of one of more than 42 different *erm* (erythromycin rRNAmethylase) genes. They confer crossresistance between macrolides, lincosamides and streptogramin B (so-called MLS_B resistance) and evoke most concerns. Active efflux and inactivating enzymes represent two additional mechanisms of resistance that are targeted only to particular antibiotics or antibiotic classes. For example, *mef* genes encode for macrolide efflux, *msr* genes for efflux of macrolides and streptogramin B, and the *lsa* gene for efflux of lincosamides and streptogramin A.

There is a vast body of information overseen by traditional microbiologist on the non-antimicrobial use of tetracyclines and macrolides in clinic and research, as recently reviewed by Aminov [15]. This type of therapy often includes low-dose, long-term exposure to antibiotics, which promotes dissemination of antibiotic resistances among commensal and pathogenic microbiota. Some progress has been made in developing compounds that retain their immunomodulatory activities, while abolishing antimicrobial activities [15], but continuous efforts are necessary in order to completely circumvent the selective pressure exhibited on the microbiota through this type of therapy.

The occurrence of bacteria resistant to both tetracyclines and MLS antibiotics has been observed [16, 17, 18]. This review will summarize the current knowledge on the topic of genetic linkage of different tetracycline and MLS resistance genes, and possible risks coupled with it for public health.

TETRACYCLINE AND MLS RESISTANCE GENES AND TRANSPOSONS

Identification of antibiotic resistance genes provides valuable information; however, knowledge about their association with mobile genetic elements is crucial for assessment of the risk for acquisition and dissemination of antimicrobial resistance. Transposable elements are by definition 'specific DNA segments that can repeatedly insert into one or more sites in one or more genomes; [19]. They can be distributed on both chromosomes and plasmids, and are able to interact by recombination between elements and/or by transposition into other elements, forming all kinds of novel chimeric structures [20, 21]. Their complex nomenclature has been revised, and since 2008 all newly-discovered, fully sequenced and/or functional autonomously transposable elements that show <100 % sequence identity with the closest relative, were designated with a new Tn number. The acronyms ICE (integrative conjugative elements) and IME (integrative mobilizable element) are retained, but now interchangeable with CTn or MTn for conjugative transposon or mobilizable transposon, respectively, if conjugation or mobilization can be proved [22]. The first conjugative transposon identified in bacteria was Tn*916* from *E. faecalis*[23], and was carrying the tetracycline resistance. Since then, a vast amount of different transposable elements have been identified, including those that carried several resistance and virulence genes [24]. Under the pressure of any of antibiotics to which the element confers resistance, the whole transposon is retained and, accordingly, multiple antibiotic resistance genes remain in the population. They can persist for decades in animal husbandry due to co-selection, fitness and other phenomena [25]. An overview of transposons and selected mobile genetic elements conferring resistance to tetracyclines and/or MLS antibiotics discussed in this review is given in Table 2 and Figure 1.

There is a variety of transposons conferring tetracycline resistance. They are most often associated with tetracycline resistance, carry the *tet*(M) gene and belong to the Tn*916* transposon family. An excellent review on this topic was recently published and is recommended for more information on the subject [19]; however the main focus of this study are transposons carrying both resistance determinants, against tetracycline and MLS antibiotics, some of which belong to the Tn*916* transposon family and will be discussed in detail. Elements associated with MLS resistance are the Tn*917* transposon that carries the *erm*(B) gene [26] or MEGA element (macrolide efflux genetic assembly, 5.5kb) that carries the *mef*(E)-*msr*(D)operon [27]. The Tn*916* transposon family has a broad host range and transfer readily to a wide variety of Gram-positive and Gram-negative bacteria [19]. The integrase (*int*) and excisase (*xis*) genes used for identification of this group of transposons are indistinguishable by PCR methods from the *int* and *xis* genes of the Tn*1545* transposon [28]. They differ by only one nucleotide over approximately 2 kb. Moreover, the *tet*(M)genes from these two different transposons exhibit 94.5 % nucleotide identity. Nevertheless,

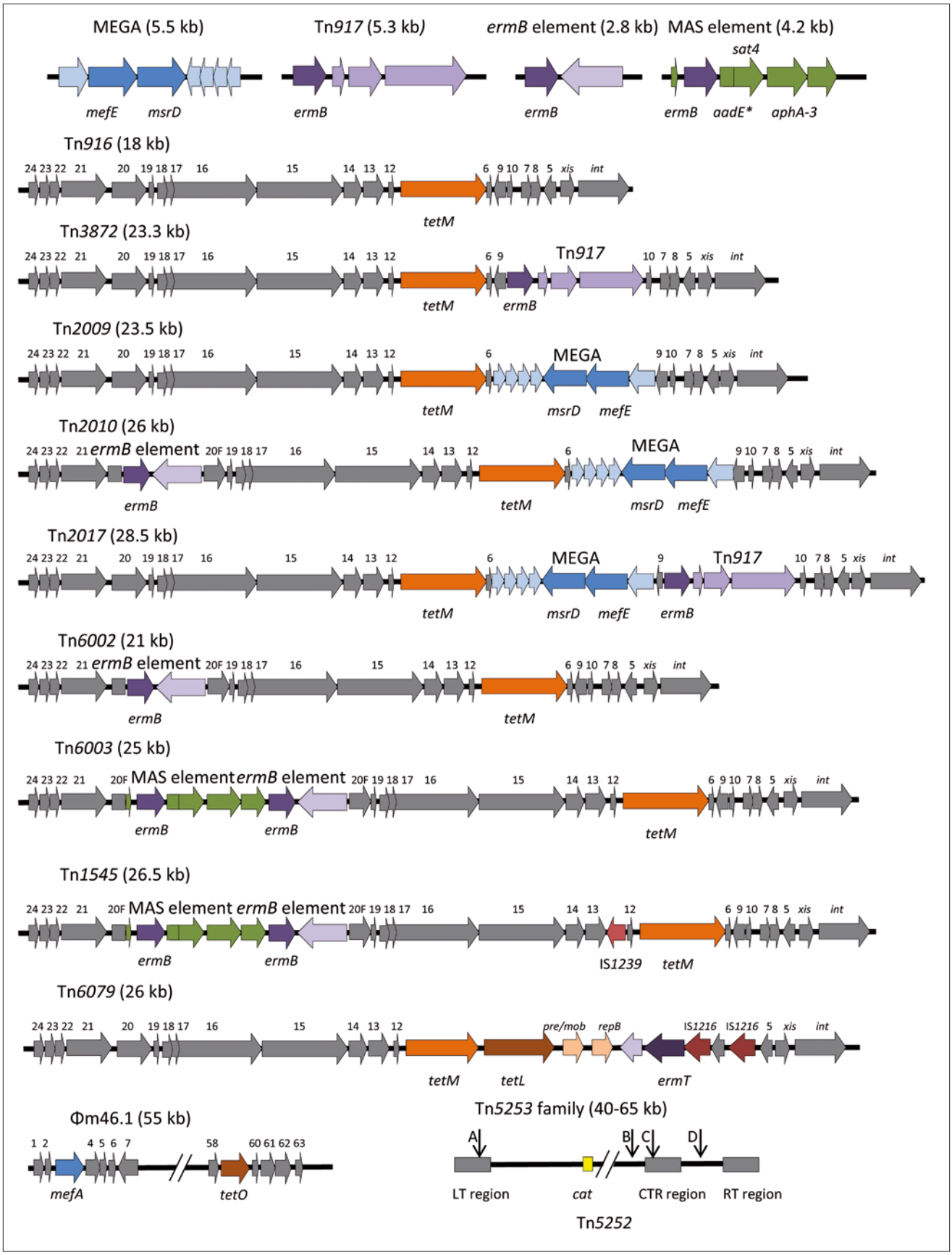

Figure 1. Schematic drawing of transposons and selected mobile genetic elements conferring resistance to tetracyclines and/or MLS antibiotics discussed in this review. Tn5253 transposon family is shown as a Tn5252 element (LT, left terminal region; CTR, conjugal-transfer related region; RT, right terminal region) with indicated (arrows) insertion sites of Tn5251 or other Tn916-like transposons (A, Tn6002; B, Tn5251/Tn916 or SpnRi3ermB-like element; C, Tn916 or Tn6002; D, Tn916, Tn6002, Tn2009 or Tn3872)

Table 2. Summary of transposons conferring resistance to tetracycline and/or MLS discussed in this review

Transposon	tet gen	Comments	Size	Reference
Tn916*	tet(M)		18 kb	(Franke and Clewell, 1981)
Tn917		erm(B)	7 kb	(Shaw and Clewell, 1985)
MEGA		mef(A)-msr(D) operon	5.5 kb	(Del Grosso et al. 2006)
Tn1545*	tet(M)	MAS and erm(B) elements inserted into Tn916-like structure	26.5 kb	(Cochetti et al. 2008)
CTnDOT	tet(Q) or tet(X)	erm(F), erm(B) and aad	65-100 kb	(Gupta et al. 2003; Shoemaker et al. 2001)
Tn2009*	tet(M)	MEGA element inserted into Tn916-like structure	23.5 kb	(Del Grosso et al. 2004)
Tn2010*	tet(M)	erm(B) element inserted into Tn2009	26 kb	(Del Grosso et al. 2006; Li et al. 2011)
Tn2017*	tet(M)	Tn917 inserted into Tn2009	28.5 kb	(Del Grosso et al. 2009)
Tn3872*	tet(M)	Tn917 inserted into Tn916	24 kb	(Cochetti et al. 2007; McDougal et al. 1998)
Tn5253	tet(M)	Tn916-like transposon inserted into Tn5252 carrying the cat gene	40-65 kb	(Ayoubi et al. 1991; Mingoia et al. 2011)
Tn6002*	tet(M)	erm(B) element inserted in Tn916	21 kb	(Warburton et al. 2007)
Tn6003*	tet(M)	MAS element inserted into Tn6002	25 kb	(Cochetti et al. 2007)
Tn5385	tet(M)	tet(M) (Tn5381); aac(6')-aph(2'') (Tn4001); erm(B) and merRAB (Tn5384); bla (Tn552); aadE	65 kb	(Rice and Carias, 1998)
Tn6079*	tet(M) and tet(L)	Tn916/Tn1545-like transposon carrying the erm(T) gene	26 kb	(de Vries et al. 2011)
ICESp2905	tet(O)	erm(A) (ICESp2907 integrated into ICESp2906)	65 kb	(Brenciani et al. 2011; Giovanetti et al. 2012)

* Tn*916*-like elements

Tn*916* and Tn*1545* differ in size (18 vs. 25.2kb) and genetic content. In addition to the *tet*(M) gene, Tn*1545* also harbours other determinants of resistance, i.e. the MAS (macrolide-aminoglycoside-streptothricin) element and the *erm*(B) element [29]. Thus the co-selection of tetracycline and MLS_B resistance may occur *via* the Tn*1545* transposon, and might be underestimated if isolates were screened only with *int/xis* specific primers [18, 30]. A new Tn*6079* conjugative transposon of the Tn*916*/Tn*1545*-like family that harbours two tetracycline resistance genes [*tet*(M)and *tet*(L)], and one gene conferring MLS_B resistance [*erm*(T)] was recently identified in samples from an infant faecal fosmid library [31]. The presence of genetic determinants of resistance to both tetracyclines and MLS antibiotics was also described for other large transposons, such as the CTnDOT family, Tn*2009*, Tn*2010*, Tn*2017*, Tn*3872*, Tn*5253*, Tn*6058*, Tn*5385*, Tn*6002* and Tn*6003* that arose as a combination of smaller transposons [20, 27, 32, 33, 34].

CTnDOT family transposons are large conjugative transposons from *Bacteriodes* spp. that carry resistance genes against tetracycline (*tet*(X), *tet*(Q)) and MLS antibiotics (*erm*(B), *erm*(F), *erm*(G)) [35]. Their excision and conjugative transfer is dependent on tetracycline, although CTnDOTpositive*Bacteriodetes* spp. are not always tetracycline resistant due to their anaerobic nature and the fact that tetracycline resistance mediated by the *tet*(X) gene is oxygen dependent [4, 34], they can serve as resistance reservoir for other pathogenic bacteria. Dissemination of *tet*(X) resistance gene is of special concern because it confers resistance also against third generation tetracycline tigecycline[36]. Although the use of this antibiotic is strictly regulated, *tet*(X) has already been observed among pathogenic bacteria [37], and sequence similarity of flanking regions around *tet*(X) suggest that its spread is most likely due to horizontal transfer of transposons from the CTnDOT family [38].

Tn*3872* is a composite element resulting from the insertion of the *erm*(B)-containing Tn*917* transposon into *orf9* of Tn*916*. The association between *mefEmsrD* and *tet*(M) was found in Tn*2009* transposons that have the MEGA element inserted into a Tn*916*-like structure [20]. In addition to that, Tn*2010* and Tn*2017* carry the *erm*(B) gene due to the insertion of the *erm*(B) element or Tn*917*, respectively, into a Tn*2009* like structure [27, 39]. Tn*6002* is an element resulting from the insertion of an *erm*(B)-containing DNA fragment (the *erm*(B) element) into Tn*916*[33, 40]. Tn*6003* carries determinants of resistance to aminoglycosides and streptothricin due to the MAS element, which can circularise, excise and turn back Tn*6003* into Tn*6002*[32, 41].

Tn*5253*-like transposons are large composite transposons that consist of a Tn*916*-like element (originally designated as Tn*5251*) conferring tetracycline/MLS resistance, which is inserted into the Tn*5252* element that harbours the *cat* (chloramphenicol) resistance gene [42]. Members of this diverse transposon family are often referred to as ICE elements, followed by an acronym of the species where they were discovered, and a unique number (i.e. ICE*Sp*2905) [43]. Tn*5385* is a composite 65 kb large transposon comprising several smaller mobile elements, including an 18-kb conjugative transposon (Tn*5381*) conferring resistance to tetracycline [*tet*(M)], a 26-kb transposon (Tn*5384*) conferring MLS_B resistance (originally named *erm*(AM)], but now renamed as *erm*(B), as well as resistance to gentamicin (*aac(6')-aph(2'')*) and mercuric chloride (*merRAB*), and a Tn*552*-like staphylococcal beta-lactamase transposon conferring resistance to penicillins (*bla*). The transposon further confers resistance to streptomycin *via* the *aadE* gene [29]. ICE*Sp2905* is a widespread *erm*(A)- and *tet*(O)-carrying genetic element of *S. pyogenes*, resulting from one ICE (ICE*Sp2907*) being integrated into another ICE (ICE*Sp2906*) of clostridial origin [44]. Originally, the MLS resistance determinant was named *erm*(TR), but it has been renamed to *erm*(A).

Giovanetti et al. [45] genotyped clinical isolates of *S.pyogenes,* and in addition to the *erm*(B) and *tet*(M) genes, they identified a new genetic linkage between the *tet*(O) and *erm*(A) or *mef*(A) genes. The authors demonstrated that the *tet*(O) gene moved in conjugation experiments with and without the *erm*(A) gene, but always with the *mef*(A) gene.

This association of *tet*(O) and *mef*(A) is due to the prophage Φm46.1 identified in *S. pyogenes*[46]. The association of *tet*(O) with the *erm*(B) gene was further described by Martel et al. [47]. All in all, as more sequence information is revealed, and new functional metagenomics approaches are applied in the research of the resistome, it is reasonable to expect that new transposable elements will continue to be discovered. Nevertheless, their functionality has to be proved in order to be designated by a new Tn number [22, 31].

TRANSMISSION OF TETRACYCLINE AND MLS RESISTANCE

In vitro transfer of antimicrobial resistance has been studied by many authors. Vignaroli et al. [48] investigated isolates of enterococci co-resistant to tetracycline and erythromycin originating from meat and faeces of chickens and pigs. They found that under *in vitro* conditions the isolates from faeces more readily transferred the resistance to enterococci of human origin. Jasni et al. [49] demonstrated reciprocal genetic exchange between *E. faecalis* and *C. difficile*, where the Tn*5397* transposon carrying the *tet*(M) gene was incorporated at a single specific target site. Wasels et al. [50] demonstrated the transfer of Tn*6194* that carries the *erm*(B) gene between *C. difficile* and *E. faecalis*. Florez et al. [51] identified a Tn*916*-like transposon carrying the *tet*(M) gene in plasmids from two *Lactococcus lactis* strains isolated from raw milk starter-free cheese. Conjugation experiments have shown that only the transposon, but not the whole plasmid, could have been transferred from *L. lactis* to *E. faecalis*. Enterococci originating from a total production chain of swine meat commodities were shown to successfully transfer the Tn*916*/*1545* transposon family carrying the *tet*(M) gene to other enterococci and *L. innocua* in both filter mating experiments and mating trials performed in meat matrices [52]. Transfer of tetracycline and erythromycin resistances from a human *E. faecalis* isolate was also demonstrated in a sausage fermentation model, even without antibiotic pressure and as early as within two days of fermentation [53]. In this model, transconjugant bacterial strains were identified among enterococci, pediococci, lactobacilli and staphylococci. In another study, a higher transfer rate for both vancomycin and tetracycline resistance was observed in fermented sausages compared to cheese [54]. Transfer of tetracycline resistance (i.e. the *tet*(M) gene) from *Lactobacillus* isolates originating from fermented dry sausages to *E. faecalis* and *L. lactis* was demonstrated in conjugation experiments by Gevers et al. [55]. Although the transposon Tn*2009* was originally identified in *S. pneumoniae* as non-mobilizable[20], in a later study, however, its conjugation was proved between a plenty of various bacteria, such as *Acinetobacter junii*, *Citrobacter* spp., *E. coli*, *Enterobacter cloacae*, *Klebsiella* spp., *Pantoeaagglomerans*, *Proteus* spp., *Pseudomonas* spp., *Ralstoniapickettii*,*Stenotrophomonasmalto philia*, *E. faecalis*, *Neisseria mucosa* and *Neisseria perflava*[56]. Even the large Tn*5253* transposon or its related variants were conjugally transferred under laboratory conditions from *S. pneumoniae* to *S. pyogenes* and other streptococci, as documented in previous reports [42, 57].

Transfer of antimicrobial resistance, however, is highly influenced by the complexity of microflora and its interactions with the host. Moreover, it is presumed that *in vitro* models underestimate the potential risk for resistance transfer compared to *in vivo* models [58, 59]. Therefore, to appropriately assess the risk for horizontal spread of antimicrobial resistance, many authors have recently performed experimental studies under *in vivo* conditions. Moubareck et al. [60] carried out experiments on gnotobiotic mice, and demonstrated the transfer of *vanA* (vancomycin resistance) and *erm*(B) among enterococci colonising the intestine. Although tylosin did not significantly increase the transfer of vancomycin resistance under *in vitro* conditions, it significantly increased intestinal colonization of the treated mice by vancomycin-transconjugants. A similar observation was reported by Doucet-Populaire et al. [61], who found that administration of tetracycline to gnotobiotic mice increased by 20-fold the transfer of *Tn1545* from *E.faecalis* to *L. monocytogenes*. On a model of gnotobiotic mice, Jacobsen et al. [62] demonstrated that the *tet*(M) and *erm*(B) genes may transfer from *Lactobacillus plantarum* originating from fermented sausages to human isolates of *E. faecalis*. This finding supports the hypothesis that microorganisms of food origin may represent an important source of genetic determinants of resistance for microflora colonising human intestines. However, it should be kept in mind that experiments in gnotobiotic animals are performed under artificial conditions, i.e. in the presence of relatively high numbers of potential recipients and in the absence of competitive microflora. A recent *in vivo* study performed on commercial chickens has shown that the spread of a plasmid-carried *erm*(B) gene occurs not only within the faecal microbiota under both specific (tylosin and lincomycin) and non-specific (chlortetracycline) antibiotic pressure, but also in the absence of any antimicrobial pressure [63]. Moreover, all isolates of enterococci and streptococci that acquired the *erm*(B) gene were also resistant to tetracycline, which indicates that tetracycline may co-select for MLS_B resistance.

De Leener et al. [64] compared enterococci of human and porcine origin for their resistance profile against erythromycin and tetracycline. The *erm*(B) gene was found in 85% of porcine isolates and in all human isolates. The *tet*(M) gene was found in 98 and 89% of the *erm*(B) positive isolates from pigs and humans, respectively. Among the *erm*(B)/*tet*(M) positive isolates, 77 and 70% isolates of porcine and human origin, respectively, carried a transposon of the Tn*916*/Tn*1545* family. The authors therefore suggested either a mutual spread of resistant enterococci between humans and pigs, or the existence of a common reservoir of resistant enterococci. The hypothesis that animals can be a source of resistant enterococci for humans could be supported by the findings of Sorensen et al. [65]. In the latter study, the survival of animal derived enterococcal strains in the human intestine was investigated in 18 volunteers. Transient intestinal carriage of resistant *E. faecium* of animal origin was detected up to 14 days after ingestion, suggesting that enterococci of animal origin can survive the gastric passage and multiply in the human intestine. This transient colonisation increases the risk for transfer of resistance determinants within human intestinal microbiota. De Vries et al. [31] investigated the diversity of microorganisms conferring tetracycline resistance in faecal samples from a healthy mother-infant pair one month after childbirth, and evaluated potential horizontal transfer of tetracycline resistance genes. For that purpose, faecal fosmid libraries were functionally screened for tetracycline resistance genes. In the mother library, identical tetracycline resistance gene

sequences (predominantly *tet*(O), followed by *tet*(W) and *tet*(X)) were present in different bacterial families and even phyla, which may indicate horizontal transfer within the maternal gastrointestinal tract. Although *tet* genes other than those observed in the mother dominated in the infant library, *tet*(O) and *tet*(W) could also be detected in the infant faecal samples. Moreover, *tet*(M), *tet*(L) and *erm*(T) were identified within a novel composite transposon Tn*6079* in the infant library, which indicates a potential for the joint spread of tetracycline and erythromycin resistance within the infant's gut.

CONCLUSIONS

The antibiotic classes discussed in this review have been used for over 50 years, and still today have large consumption rates in human, but even more in veterinary medicine. They are listed by the World Health Organization as highly or even critically important in the case of macrolides. Rates of bacteria resistant to these classes of antibiotics depend on the source of isolation and history of antibiotic usage. Co-resistance between tetracyclines and MLS antibiotics is often due to the occurrence of genetic determinants for tetracycline and MLS resistance on very promiscuous transposons that can interact, recombine and form all sorts of novel chimeric elements. This type of genetic linkage on transposons is of a very great concern. It increases the risk for transfer of resistances to pathogenic species, and enables the co-selection of all genes and their retention in the population, leading to therapeutic failure and severe consequences.

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