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# Resistance of Staphylococci to Macrolides-Lincosamides-Streptogramins B (MLS<sub>B</sub>): Epidemiology and Mechanisms of Resistance

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## Abstract

A total of 92 genes that confer resistance to MLS antibiotics have been described to date. 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 through the action of the protein product of one of more than 42 different *erm* (erythromycin rRNA methylase) genes. They confer cross resistance between macrolides, lincosamides and streptogramin B (so-called MLS<sub>B</sub> resistance) and evoke most concerns. Active efflux and inactivating enzymes (M and L) represent two additional mechanisms of resistance that are targeted only to particular antibiotics or antibiotic classes. Based on the mechanisms of resistance, various resistant phenotypes are expressed. The most prevalent phenotypes are MLS<sub>B</sub> (constitutive or inducible), which is associated with the presence mainly of *ermA* and *ermC* genes, followed by the MS<sub>B</sub> phenotype due to the presence of *msrA* gene. In livestock *S. aureus* strains, such as CC 398, other genes such as *ermT*, *lnuA*, *lsaE* and *mphC* genes are detected.

**Keywords:** staphylococci, MLS<sub>B</sub>, resistance, genes

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## 1. Introduction

Resistance to macrolides-lincosamides and streptogramins B (MLS<sub>B</sub> antibiotics) is associated with three main mechanisms: (1) methylation of rRNA (target modification), (2) active efflux and (3) enzymatic inactivation. Till date, a total of 92 genes, conferring resistance to MLS<sub>B</sub> antibiotics, have been described. The most common genes are *erm*, which encode rRNA

methylases, resulting in the target modification of these antimicrobial agents. More than 42 different *erm* genes have been described to date; bacteria, that carry *erm* genes, express cross-resistance to all these classes of antimicrobial agents. On contrary, genes encoding pumps for active efflux (*msrA* and *lsa*) or enzymes for drug inactivation (*lnu* and *mphC*) confer resistance only to particular antibiotics. Based on the mechanisms of resistance, various resistant phenotypes are expressed. The most prevalent phenotypes are MLS<sub>B</sub> (constitutive or inducible), which, in staphylococci, are associated with the presence mainly of *ermA* and *ermC* genes, followed by the MS<sub>B</sub> phenotype due to the presence of *msrA* gene. In livestock *S. aureus* strains, such as CC 398, other genes such as *ermT*, *lnuA*, *lsaE* and *mphC* genes are detected [1–4].

The macrolide group of antibiotics includes natural members, prodrugs and semisynthetic derivatives. The chemical structure of macrolides is characterized by a large lactone ring containing from 12 to 16 atoms to which are attached, via glycosidic bonds, one or more sugars. Erythromycin, whose lactone ring contains 14 atoms, is the oldest molecule (1952), whereas all second-generation macrolides, like roxithromycin and clarithromycin, are hemisynthetic derivatives of erythromycin. Azithromycin is the only macrolide with 15 carbon atoms. Azithromycin, which is produced through the introduction of a nitrogen atom into the macrolide nucleus at C10, exhibits (1) improved penetration into macrophages, fibroblasts and polymorpho-neutrophils, (2) increased accumulation within acidified vacuoles and (3) extended half-life. Additionally, azithromycin shows improved activity against Gram-negative bacteria and other pathogens associated with parasitic infections. Spiramycin and josamycin are macrolides with 16 carbon atoms. All chemical modifications of macrolides were made in order that their properties and action are optimized.

Although the structure of lincosamides is different from the structure of macrolides, they present a similar action spectrum. Lincomycin, which was isolated in 1962, is a fermentation product of *Streptococcus lincolnensis*. Clindamycin (7-chloro-7-deoxy-lincomycin) is a semisynthetic derivative of lincomycin, produced by substitution of the C7 bearing a hydroxyl group with a chlorine atom. Clindamycin exhibits higher antibiotic activity and digestive absorption.

Type-A streptogramin includes cyclic-poly-unsaturated macrolactones: virginiamycin M, pristinamycin IIA and dalfopristin. Type-B streptogramin consists of the cyclic hexadepsipeptide compounds virginiamycin S, pristinamycin IA and quinupristin. Until now, only three streptogramins have been marketed either for treatment or growth promotion: virginiamycin, pristinamycin and quinupristin-dalfopristin. Virginiamycin, a mixture of virginiamycin M (type A streptogramin) and virginiamycin S (type B streptogramin), has been used mainly as growth promoter feed additive in commercial animal farming in the United States and Europe. In contrast, pristinamycin has been used orally and topically in human medicine only in France. Quinupristin-dalfopristin, in a 30:70 mixture (Synercid), was approved in 1999 for the treatment of serious infections caused by multidrug resistant Gram-positive pathogens, including vancomycin-resistant *Enterococcus faecium* and methicillin-resistant staphylococci (MRS).

MLS<sub>B</sub> antibiotics share a similar mode of action because they inhibit protein synthesis by targeting the peptidyl transferase center within the 50S subunit (23 s rRNA) of the bacterial ribosome [5]. We note that the bacterial ribosomes are 70S particles comprising of two subunits, 30s and 50S, which are made of RNAs enveloped by proteins; 50S is composed of 5S, 23S rRNAs and 36 proteins (L1-L36) [6, 7].

Although the peptidyl transferase center is the main target site for many antibiotics, the exact mechanism for its activity is still unclear [8]. Overall, the inhibitory action of antibiotics is not only determined by their interaction with specific nucleotides. MLS<sub>B</sub> could also inhibit peptidyl transferase by interfering with the proper positioning and movement of the tRNAs at the peptidyl transferase cavity [9, 10].

## 2. Antibacterial spectrum of MLS<sub>B</sub>

The spectrum of MLS<sub>B</sub> includes mainly Gram-positive microorganisms (streptococci, staphylococci); however, some of them also have activity against Gram-negative microorganisms (*Bordetella pertussis*, *Campylobacter*, *Helicobacter*, *Legionella*, *Moraxella catarrhalis*), anaerobes, intracellular pathogens (*Chlamydia* and *Rickettsia*) and *Mycobacterium avium* [11, 12].

It is known that some Gram-positive species have intrinsic resistance to some of them. *Enterococcus faecalis*, *E. avium*, *E. gallinarum* and *E. casseliflavus* express resistance to lincosamides. Among staphylococci, *S. cohnii*, *S. xylosus* and *S. sciuri* are also resistant to lincosamides [11, 12].

## 3. Mechanisms of acquisition of resistance to MLS<sub>B</sub>

Staphylococci resist MLS<sub>B</sub> antibiotics in three ways: (1) through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, (2) through efflux of the antibiotic and (3) by drug inactivation. Modification of the ribosomal target confers broad-spectrum resistance to macrolides, lincosamides and streptogramin B, whereas efflux and inactivation affect only some of these molecules [12].

### 3.1. Ribosomal methylation

The most widespread mechanism of resistance to MLS<sub>B</sub> in Gram-positive bacteria, including both *Staphylococcus aureus* and coagulase-negative staphylococci (CNS), is the methylation of ribosomes, which is the target of MLS antibiotics. Methylation of ribosomes leads to resistance to macrolides, lincosamides and streptogramins B (MLS<sub>B</sub> phenotype) [13]. The MLS<sub>B</sub> phenotype is conferred by erythromycin ribosome methylases (Erm), which are encoded by *erm* genes. *erm* genes have been reported in a large number of microorganisms [14].

Erm proteins, encoded by *erm* genes, dimethylate the A2058 residue of 23S rRNA [13], which is located within the conserved domain V of 23S rRNA in the bacterial ribosome. Domain V of the 23S rRNA plays a key role in the binding of MLS<sub>B</sub> antibiotics. Methylation of 23S rRNA impairs binding of macrolides, lincosamides and streptogramins B, which accounts for the cross-resistance to these drugs. A wide range of microorganisms, including Gram-positive bacteria, spirochetes and anaerobes, which are targeted for MLS<sub>B</sub> antibiotics, express Erm methylases.

More than 42 *erm* genes have been reported so far [14]. In bacteria, *erm* genes are usually carried by plasmids and transposons that are able to move independently. Four major classes are detected in microorganisms: *ermA*, *ermB*, *ermC* and *ermF* [13, 14]. *ermA* and *ermC* typically are staphylococcal gene classes.

### 3.2. Antibiotic efflux

In Gram-positive organisms, acquisition of macrolide resistance by active efflux is caused by two classes of pumps, members of the ATP-binding-cassette (ABC) transporter superfamily and of the major facilitator superfamily (MFS). ABC transporters require ATP to function and are usually formed by a channel comprising two membrane-spanning domains and two ATP-binding domains located at the cytosolic surface of the membrane [12].

The first determinant encoding ABC transporter in staphylococci was the plasmid-borne *msr(A)* gene [15]. The *msr(A)* gene encodes an ABC transporter protein with two ATP-binding domains. The nature of the transmembrane component of the MsrA pump remains unknown. In nature, a fully operational efflux pump is a multicomponent system that is composed of proteins encoded by *msr(A)* and chromosomal genes. MsrA pump has specificity for 14- and 15-membered macrolides and type B streptogramins (the MS<sub>B</sub> phenotype) [15]. MS<sub>B</sub> resistance phenotype is inducibly expressed by 14- and 15-membered macrolides, whereas streptogramins B are not inducers. *msrA*-positive strains are fully susceptible to clindamycin, since this antibiotic is neither an inducer nor a substrate for the pump.

However, latter, the combined resistance to lincosamides, pleuromutilins and streptogramin A (S<sub>A</sub>), referred as the PLS<sub>A</sub> phenotype, was found to be associated with the presence of the ARE subfamily of class 2 ATP-binding cassette (ABC) ATPases, a class of ABC proteins made up of two homologous ABC ATPase domains separated by a flexible linker without any identifiable transmembrane domains [16–18]. The flexible linker between each ATPase domain is presumed to be the drug-binding region of the ARE proteins. The *vga*-, *lsa*- and *sal*-like genes, encoding ABC transporters of the Vga, Lsa, or Sal families confer the PLS<sub>A</sub> resistance phenotype. These genes have been mainly identified in staphylococci causing food-borne diseases [19–26].

### 3.3. Enzymatic inactivation

Enzymatic inactivation confers resistance to structurally related antibiotics only. Esterases and phosphotransferases, encoded by *ere* and *mphC* genes, respectively, confer resistance to erythromycin and other 14- and 15-membered macrolides but not to lincosamides [27–30].

In addition, lincosamide nucleotidyl transferases encoded by *lnu(A)* (formerly *linA*) and *lnu(B)* (formerly *linB*) genes in staphylococci (*S. aureus* and coagulase-negative staphylococci) inactivate lincosamides only [14, 31–33]. In addition, enzymes such as virginiamycin B hydrolase and streptogramin B lactonase, encoded by *vgbA* and *vgbB* genes, which hydrolyze streptogramin B, are rarely found in staphylococci [14, 34, 35].

### 3.4. Uncommon mechanisms of resistance

Ribosomal mutations (A2058G/U or A2059G) of 23S rRNA gene such as mutations in the *rplV* gene, encoding the L22 ribosomal protein have been reported by Prunier et al. [36]. These rare *Staphylococcus aureus* isolates, recovered from patients with cystic fibrosis after long-term treatment with azithromycin, were cross-resistant to azithromycin and erythromycin.



On the other hand, *Staphylococcus epidermidis* isolates, which carried the T2504A mutation of 23S rRNA gene were found to be fully resistant to lincomycin, clindamycin, linezolid and pleuromutilins [37].

#### 4. Resistant phenotypes: expression, detection and interpretation

Depending on the mechanism of resistance and on the carriage of respective genes, staphylococci can express various MLS<sub>B</sub> resistant phenotypes. Briefly, these types are described as follows.

##### 4.1. MLS<sub>B</sub> phenotype (*erm* genotype)

MLS<sub>B</sub> phenotype can be expressed as constitutive or inducible [12]. Isolates with a constitutive MLS<sub>B</sub> phenotype express high level cross-resistance to macrolides, lincosamides and streptogramin B. In fact, clinical methicillin-resistant strains that are constitutively resistant to MLS<sub>B</sub> antibiotics are widespread.

On the other hand, isolates with an inducible MLS<sub>B</sub> phenotype express phenotypically only resistance to macrolides and susceptibility to lincosamides. This phenomenon is explained by the fact that, in constitutive resistance, bacteria produce an active mRNA encoding methylase, whereas in inducible resistance, bacteria produce an inactive mRNA, which is unable to encode ribosome methylases. However, in the presence of a macrolide, which acts like an inducer, the mRNA becomes active [38]. The presence of an inducer leads to rearrangements of mRNA, which allow ribosomes to translate the methylase coding sequence.

Inducible expression of *ermA* or *ermC* genes is characterized by dissociated resistance to MLS<sub>B</sub> antibiotics. Dissociated resistance to MLS<sub>B</sub> antibiotics is due to the differences in the inducing capacity of the antibiotics. For example, 14- and 15-membered ring macrolides, which are inducers, are inactive. Thus, *ermA*- or *ermC*-positive strains are phenotypically resistant to these antibiotics. However, strains remain susceptible to 16-membered ring macrolides, lincosamides, and streptogramins B that are not inducers.

The use of antibiotics being noninducers (such as clindamycin) for treatment of an infection due to a *Staphylococcus aureus* that is inducibly resistant to MLS<sub>B</sub> antibiotics is not devoid of risk. In the presence of these antibiotics, constitutive mutants can be selected *in vitro* at frequencies of  $\sim 10^{-7}$  cfu. Previous reports have demonstrated the risk of selection of constitutive mutants during the course of clindamycin therapy administered to patients with severe infections due to inducibly erythromycin-resistant *S. aureus* [39, 40]. In addition, the risk for selection of a constitutive mutant is higher if, at the site of infection, staphylococcal inoculum is higher.

According to the rules of EUCAST, if a staphylococcal isolate with an inducible MLS<sub>B</sub> phenotype is detected, it must be reported as resistant and considered adding this comment to the report "Clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as constitutive resistance is unlikely to develop during such therapy."

The *ermA* and *ermC* are the most common determinants in staphylococci [41]. The *ermA* genes are mostly spread in methicillin-resistant strains and are borne by transposons related to Tn554, whereas *ermC* genes are mostly responsible for erythromycin resistance in methicillin-susceptible strains and are borne by plasmids. Recently, the *ermT* gene was found to be present in livestock staphylococci [21].

#### 4.2. MS<sub>B</sub>-phenotype (*msrA* genotype)

MS<sub>B</sub> phenotype is associated with resistance only to 14- (clarithromycin, erythromycin, roxithromycin) and 15-membered ring macrolides (azithromycin) and streptogramin B, while 16-membered ring macrolides (josamycin and spiramycin) and lincosamides remain active [12, 15]. The *msrA* resistance determinant was originally detected in *Staphylococcus epidermidis*, and, since then, it has been found in a variety of staphylococcal species, including *S. aureus*. The MS<sub>B</sub> resistance phenotype is inducibly expressed by 14- and 15-membered macrolides. Streptogramins B are not inducers and, therefore, the *msrA*-positive strains are resistant to streptogramins B only after induction. The 16-membered ring macrolides and lincosamides are neither inducers nor substrates for the pump. Thus, *msrA*-positive strains are fully susceptible to these antimicrobials.

Another gene, *msrB* from *Staphylococcus xylosum*, which is nearly identical to the 3' end of *msrA*, has been reclassified as *msrA* [14]. It contains a single ATP-binding domain but also confers an MS<sub>B</sub> phenotype.

Isolates with this phenotype have probably decreased susceptibility to the combination of quinupristin-dalfopristin. Additional tests (see below) are required for its detection.

#### 4.3. M-phenotype (*mphC* genotype)

M-phenotype is associated with the presence of enzymes which inactivate enzymatically only macrolides. Clinical isolates of erythromycin-resistant *S. aureus* and coagulase-negative staphylococci produce phosphotransferases encoded by *mphC* genes [29, 30]. This phenotype must be differentiated from MLS<sub>B</sub>-inducible phenotype and from MS<sub>B</sub> phenotype. Additional tests (see below) are required for its detection.

#### 4.4. PLS<sub>A</sub>-phenotype

PLS<sub>A</sub>-phenotype is associated with resistance to lincosamides, pleuromutilins and streptogramins A, while macrolides and streptogramin B remain active [42]. Various genes such as *vgaA*, *vgaC*, *vgaE*, and *lsaE* have been detected in methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 of swine, cattle and poultry origin and shown to confer this resistance phenotype [43, 44].

#### 4.5. L-phenotype (*lnuB* genotype)

L-phenotype is associated with resistance to lincomycin due to the presence of lincosamide nucleotidyl transferases encoded by *lnuA* and *lnuB* genes. Both *lnu*-like genes confer resistance

to lincomycin. Generally, expression of lincosamide nucleotidyl transferases causes increase of lincomycin MICs by only 1 or 2 dilutions [45]. However, *lnu*-like genes do not confer resistance to clindamycin. Indeed, the bactericidal activity of clindamycin, which is already weak against susceptible strains, is totally abolished [45], but the impact of this alteration on the therapeutic efficacy of clindamycin is unknown. Because of dissociated resistance among lincosamides, the detection of L-phenotype is possible only if lincomycin is used, instead of clindamycin.

Although more than 90 genes conferring resistance to macrolides and lincosamides have been described till date, their presence has not turned out to be a successful story for Gram-positive bacteria. This observation, which is in contrast with the success of emergence of *bla* genes in Gram-negative bacteria, could be explained by: (1) a low-level resistance conferred by these genes or (2) a failure of detection.

#### 4.6. S<sub>B</sub>-phenotype

S<sub>B</sub>-phenotype is expressed by resistance to streptogramin B due to the presence of *vgbA/B* encoding lyases that inactivate the drug. It is very difficult to detect this phenotype since quinupristin is not used alone but combined with dalfopristin. The isolates might express a decreased susceptibility to the combination of quinupristin-dalfopristin .

### 5. Confirmation methods of resistant phenotypes

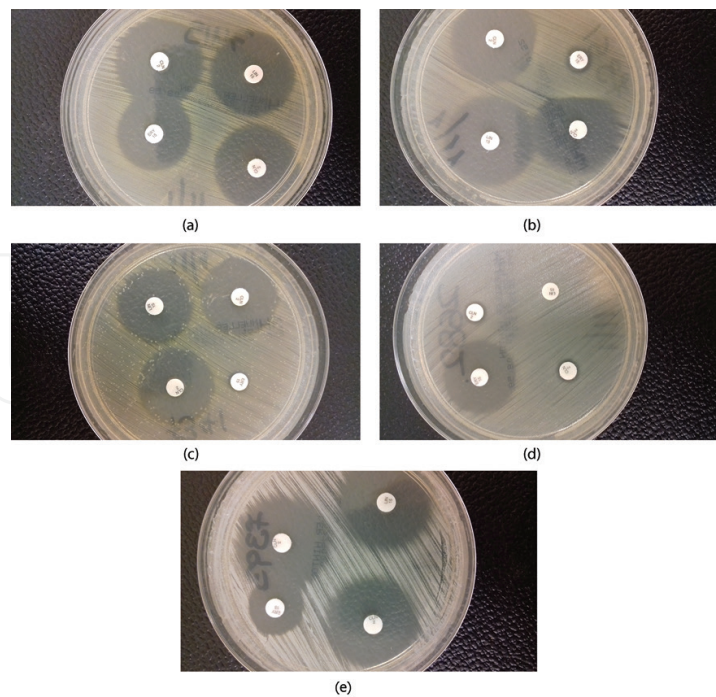
Among the different types of resistant phenotypes, the most common are MLS<sub>B</sub> (constitutive or inducible), MS<sub>B</sub> and M-phenotypes. The clinical microbiology laboratory detects easily and reliably the MLS<sub>B</sub> constitutive phenotype: the isolates are fully resistant to macrolides and lincosamides. However, isolates with MLS<sub>B</sub> inducible, MS<sub>B</sub> and M-phenotypes share the same profile: resistance to macrolides and susceptibility to lincosamides. Therefore, additional test, the double disk diffusion test (D test) is required to be applied.

For the detection of MLS<sub>B</sub> inducible resistance, it is recommended to place the erythromycin and clindamycin disks 12–20 mm apart (edge to edge, D test). In disk-diffusion tests, a D-shaped zone, caused by induction of methylase production by erythromycin, can be observed (**Figure 1**). Nowadays, the automated system Vitek II (BoMerieux) has the possibility to detect it.

However, after a negative D test, the differentiation between MS<sub>B</sub> and M-phenotypes is more complicated and could be based on the MIC values of erythromycin. Isolates with M-phenotype have often lower MIC values to erythromycin, due to the weak activity of hydrolytic enzymes, than isolates with MS<sub>B</sub>-phenotype, which express fully resistance to macrolides. In addition, MS<sub>B</sub>-phenotype affects the susceptibility to quinupristin-dalfopristin, decreasing it slowly.

Finally, it is difficult to discriminate isolates with PLS<sub>A</sub>-phenotype from those with L-phenotype; both share the same profile, including resistance to lincomycin and susceptibility to erythromycin.





**Figure 1.** Expression of various resistant-phenotypes: (a) sensitive; (b)  $MLS_B$ -inducible phenotype; (c)  $MS_B$ -phenotype; (d) L-phenotype and (e) M-phenotype. ERY: erythromycin; CLIN: clindamycin; LIN: lincomycin.

Gene	Primers sequence (5'-3')	PCR fragment size (bp)
<i>ermA</i>	F: TCTAAAAAGCATGTAAAAGAA	645
	R: CTTGATAGTTTATTAATATTAG	
<i>ermB</i>	F: GAAAAGTACTCAACCAAATA	639
	R: AGTAACGGTACTTAAATTGTTTA	
<i>ermC</i>	F: TCAAAACATAATATAGATAAA	642
	R: GCTAATATTGTTTAAATCGTCAAT	
<i>msrA</i>	F: GGCACAATAAGAGTGTTTAAAGG	940
	R: AAGTTATATCATGAATAGATTGTCCTGTT	
<i>msrB</i>	F: TATGATATCCATAATAATTATCCAATC	595
	R: AAGTTATATCATGAATAGATTGTCCTGTT	
<i>lnuA</i>	F: GGTGGCTGGGGGTAGATGTATTAAGTGG	323
	R: GCTTCTTTTGAAATACATGGTATTTTTCGATC	
<i>lnuB</i>	F: CCTACCTATTGTTTGTGGAA	925
	R: ATAACGTTACTCTCCTATTC	
<i>lsaA</i>	F: GGCAATCGCTTGTTTITAGCG	1200
	R: GTGAATCCCATGATGTTGATACC	

MLS: macrolides, lincosamides and streptogramins; PCR: polymerase chain reaction.

**Table 1.** Primer sequences and PCR fragment size of tested MLS resistance genes.

On the other hand, pleuromutilins and streptogramins A are not included in the panel of antibiotics proposed for susceptibility testing. Probably, the values of MICs to clindamycin and quinupristin-dalfopristin, which usually are not affected by L-phenotype, can be used as indicators [46].

Molecular detections of the most common genes involved in MLS<sub>B</sub> resistance are an accurate method for phenotype determination (**Table 1**).

## 6. Historical background

The first report about the activity of erythromycin was confirmed in 1954 by Derek [47]; in 1964, Macleod et al. indicated that lincomycin was effective against *S. aureus* [48]. Inducible resistance to MLS antibiotics was identified in Gram-positive bacteria by Weaver and Pattee shortly after the introduction of erythromycin into clinical practice [49]. One year later, in 1965, Griffith et al. described antagonism between lincomycin and erythromycin [50]. During their study, the authors observed an antagonistic action between lincomycin and erythromycin, when the two drugs were allowed to diffuse into the same area of an agar plate seeded with a strain of *Staphylococcus* which was resistant to erythromycin but sensitive to lincomycin. Since the molecular basis of this mechanism was unknown, the authors explained the phenomenon as the result of an altered metabolism stimulated by erythromycin on erythromycin-resistant staphylococci.

In 1971, Lai et al. demonstrated altered methylation of ribosomal RNA in a erythromycin-resistant *S. aureus* strain, whereas the same study group in 1973, concluded that modification of 23S rRNA, methylation to form dimethyladenine, was responsible for the resistance to lincomycin and spiramycin in *S. aureus* [51]. Subsequently, causation has been attributed to post-transcriptional methylation of A2058 (*Escherichia coli* numbering) at the peptidyl transferase center in domain V of 23S rRNA [52]. The family of enzymes responsible for A2058 has been designed as Erm (erythromycin resistance methylase) with the corresponding genes designed as *erm*. To date, five different methylase genes have been described in staphylococci: *ermA*, *ermB*, *ermC*, *ermF*, *ermY* and *ermT* [21, 53–57].

In 1990, Ross et al. identified *msrA* gene, which encodes an ATP-dependent efflux pump [15]. Esterases encoded by *ereA* and *ereB*, which inactivate erythromycin by hydrolyzing the lactone ring of the macrocyclic nucleus, were identified by Quinissi and Courvalin in 1985 [27]. On the other hand, the nucleotide sequence of *lnuA* gene, which confers resistance only to lincosamides, has been determined by Bisson-Noel and Courvalin, in 1986 [31]. Inactivation of macrolides by phosphotransferases (encoded by *mphC* genes) has also been described by Wondrack et al. in 1996 [29].

To date, a variety of genes (such as *vgaA*, *vgaC*, *vgaE*, *lsaE*, *vgaA*, *lnuA*, *lnuB*, and *mphC*), which are involved in the MLS-resistance expression, have been described and are disseminated among staphylococcal species.

## 7. Epidemiology of MLS<sub>B</sub> resistant staphylococci: recent data

*Staphylococcus aureus* and coagulase negative Staphylococci (CONS) are challenging pathogens causing a variety of infections (minor skin and soft tissue infections, endocarditis,

pneumonia, septicemia, etc.) [58], while the emergence of drug-resistant staphylococci is an important public threat [59]. The isolation frequency of methicillin-resistant *S. aureus* (MRSA) has dramatically increased in the recent years [60]. Thus, these factors have led to a renewed interest in the use of macrolides, lincosamides and streptogramins B (MLS<sub>B</sub>) antibiotics for the treatment of staphylococci-associated infections. From these antibiotics, clindamycin is the preferable agent, because of its excellent pharmacokinetic properties [61]. Additionally, clindamycin is the preferred agent due to its proven efficacy, low cost, the availability of its oral and parenteral forms, tolerability, excellent tissue penetration, its good accumulation in abscesses and because no renal dosing adjustments are required. Clindamycin also inhibits the production of staphylococcal toxin, and can be used as an alternative of penicillin, in patients who are allergic to the latter agent [62]. However, the widespread use of the MLS<sub>B</sub> antibiotics has increased the number of the Staphylococcus isolates which are resistant to them [63].

The rate of MLS<sub>B</sub>-resistant staphylococci varies between countries and species. Unfortunately, in the last decade, data concerning the rate of MLS resistance in staphylococci are limited. Otsuka et al. reported that 97% of MRSA and 34.6% of MSSA were resistant to one or more MLS<sub>B</sub> agents in a study conducted between 2001 and 2006 [64]. Cetin et al. in a large collection of staphylococci in a Turkish hospital have found that 38.5% were resistant to MLS<sub>B</sub> antibiotics, while Uzun et al. reported that during 2011–2012, 79% isolates were found as erythromycin-resistant in a tertiary hospital in Ismir [65, 66]. In a tertiary Greek hospital, the rate of MLS<sub>B</sub> *S. aureus* reached to 44%, whereas in Cyprus 67.61% of *S. aureus* and 59.4% of the coagulase-negative staphylococci were resistant to erythromycin [67, 68]. On the other hand, high rate of erythromycin-resistant staphylococci was also observed in veterinary [69].

Regarding the distribution of resistant phenotypes, the most common are MLS<sub>B</sub> (constitutive or inducible) followed by MS<sub>B</sub>. In Japan, Otsuka et al. revealed higher incidence of the MLS<sub>B</sub>-inducible phenotype than in Europe, Turkey and the USA [41, 64, 70–73]. Such differences in the incidence of phenotypes might reflect differences in the drug usage, the gene carriage and the clonality of strains.

Totally, 92 genes, which confer resistance to MLS antibiotics, have been described to date. 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. Data from different studies agree that the most prevalent genes are *ermA* and *ermC* followed by *msrA* gene [41, 70–74]. Gatermann et al. have demonstrated that in a large collection of coagulase-negative staphylococci *ermC* gene predominated and was constitutively expressed, whereas in *S. aureus* the *ermA* predominates [65, 75]. In livestock *S. aureus* strains, such as CC 398, other genes such as *ermT*, *lnuB* and *lsa* are detected [76–78]. In contrast, *mphC* gene is frequently found in staphylococci isolated from animals [79, 80].

## 8. Conclusions

Staphylococci and specially *S. aureus* are considered as important pathogen in a wide variety of human and animal infections. The sharp emergence and a spread of methicillin-resistant

staphylococci in the community setting and the occurrence of vancomycin-resistant staphylococci, along with vancomycin-intermediate *S. aureus* are of concern. This phenomenon has led to the development of new antimicrobial compounds. Moreover, traditional antibiotics, such as MLS<sub>B</sub>, should be carefully considered for the treatment of infections caused by multiple drug-resistant staphylococci.

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## References

- [1] Chabbert Y. Antagonisme *in vitro* entre la erythromycin et la spiroamycine. Annales De l'Institut Pasteur. 1956;**90**:787-790
- [2] Garrod LP. The erythromycin group of antibiotics. British Medical Journal. 1957;**II**:57-63
- [3] Jones WF Jr, Nichols RL, Finland M. Development of resistance and cross-resistance *in vitro* to erythromycin, carbomycin, oleandomycin, and streptogramin. Proceedings of the Society for Experimental Biology and Medicine. 1956;**93**:388-393
- [4] McGuire JM, Bunch RL, Anderson RC, Boaz HE, Flynn EH, Powell HM, Smith HW. Ilotycin: A new antibiotic. Antibiotics and Chemotherapy. 1952;**2**:281-283
- [5] Bozdogan B, Appelbaum PC. Macrolide resistance in streptococci and *Haemophilus influenzae*. Clinics in Laboratory Medicine. 2004;**24**:455-475. DOI: 10.1016/j.cll.2004.03.006
- [6] Johnston NJ, Mukhtar TA, Wright GD. Streptogramin antibiotics: Mode of action and resistance. Current Drug Targets. 2002;**3**:335-344
- [7] Cocito C, Di Giambattista M, Nyssen E, Vannuffel P. Inhibition of protein synthesis by streptogramins and related antibiotics. Journal of Antimicrobial Chemotherapy. 1997;**39**(Suppl A):7-13
- [8] Schlünzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, Yonath A, Franceschi F. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature. 2001;**413**:814-821. DOI: 10.1038/35101544
- [9] Franklin TJ, Snow GA. Biochemistry of Antimicrobial Agents. 2nd ed. New York: John Wiley; 1975
- [10] Brisson-Noël A, Trieu-Cuot P, Courvalin P. Mechanism of action of spiramycin and other macrolides. Journal of Antimicrobial Chemotherapy. 1988;**22**(Suppl B):13-23



- [11] Steigbigel NH. Macrolides and clindamycin. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell Douglas and Bennett's Principles and Practice of Infectious Diseases. 4th ed. New York: Churchill Livingstone; 1995. pp. 1719-1727
- [12] Leclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases*. 2002;**34**:482-492. DOI: 10.1086/324626
- [13] Weisblum B. Erythromycin resistance by ribosome modification. *Antimicrobial Agents and Chemotherapy*. 1995;**39**:577-585
- [14] Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrobial Agents and Chemotherapy*. 1999;**43**:2823-2830
- [15] Ross JI, Eady EA, Cove JH, Cunliffe WJ, Baumberg S, Wootton JC. Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. *Molecular Microbiology*. 1990;**4**:1207-1214
- [16] Kerr ID, Reynolds ED, Cove JH. ABC proteins and antibiotic drug resistance: is it all about transport? *Biochemical Society Transactions*. 2005;**33**:1000-1002. DOI: 10.1042/BST20051000
- [17] Jacquet E, Girard JM, Ramaen O, Pamard O, Lévaïque H, Betton JM, Dassa E, Chesneau O. ATP hydrolysis and pristinamycin IIA inhibition of the *Staphylococcus aureus* Vga(A), a dual ABC protein involved in streptogramin A resistance. *The Journal of Biological Chemistry*. 2008;**283**:25332-25339. DOI: 10.1074/jbc.M800418200
- [18] Novotna G, Janata J. A new evolutionary variant of the streptogramin A resistance protein, Vga(A)LC, from *Staphylococcus haemolyticus* with shifted substrate specificity towards lincosamides. *Antimicrobial Agents and Chemotherapy*. 2006;**50**:4070-4076. DOI: 10.1128/AAC.00799-06
- [19] Allignet J, El Solh N. Characterization of a new staphylococcal gene, *vgaB*, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. *Gene*. 1997;**202**:133-138
- [20] Haroche J, Allignet J, Aubert S, Van Den Bogaard AE, El Solh N. *satG*, conferring resistance to streptogramin A, is widely distributed in *Enterococcus faecium* strains but not in staphylococci. *Antimicrobial Agents and Chemotherapy*. 2000;**44**:190-191
- [21] Kadlec K, Schwarz S. Identification of a plasmid-borne resistance gene cluster comprising the resistance genes *erm*(T), *dfrK*, and *tet*(L) in a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrobial Agents and Chemotherapy*. 2010;**54**:915-918. DOI: 10.1128/AAC.01091-09
- [22] Jung YH, Shin ES, Kim O, Yoo JS, Lee KM, Yoo JI, Chung GT, Lee YS. Characterization of two newly identified genes, *vgaD* and *vahH*, [corrected] conferring resistance to



streptogramin A in *Enterococcus faecium*. *Antimicrobial Agents and Chemotherapy*. 2010;**54**:4744-4749. DOI: 10.1128/AAC.00798-09

- [23] Wendlandt S, Lozano C, Kadlec K, Gómez-Sanz E, Zarazaga M, Torres C, Schwarz S. The enterococcal ABC transporter gene *lsa(E)* confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *The Journal of Antimicrobial Chemotherapy*. 2013;**68**:473-475. DOI: 10.1093/jac/dks398
- [24] Lozano C, Aspiroz C, Rezusta A, Gómez-Sanz E, Simon C, Gómez P, Ortega C, Revillo MJ, Zarazaga M, Torres C. Identification of novel *vga(A)*-carrying plasmids and a Tn5406-like transposon in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* of human and animal origin. *International Journal of Antimicrobial Agents*. 2012;**40**:306-312. DOI: 10.1016/j.ijantimicag.2012.06.009
- [25] Hauschild T, Fessler AT, Kadlec K, Billerbeck C, Schwarz S. Detection of the novel *vga(E)* gene in methicillin-resistant *Staphylococcus aureus* CC398 isolates from cattle and poultry. *The Journal of Antimicrobial Chemotherapy*. 2012;**67**:503-504. DOI: 10.1093/jac/dkr446
- [26] Hot C, Berthet N, Chesneau O. Characterization of *sal(A)*, a novel gene responsible for lincosamide and streptogramin A resistance in *Staphylococcus sciuri*. *Antimicrobial Agents and Chemotherapy*. 2014;**58**:3335-3341. DOI: 10.1128/AAC.02797-13
- [27] Ounissi H, Courvalin P. Nucleotide sequence of the gene *ereA* encoding the erythromycin esterase in *Escherichia coli*. *Gene*. 1985;**35**:271-278
- [28] Arthur M, Andremont A, Courvalin P. Distribution of erythromycin esterase and rRNA methylase genes in members of the family *Enterobacteriaceae* highly resistant to erythromycin. *Antimicrobial Agents and Chemotherapy*. 1987;**31**:404-409
- [29] Wondrack L, Massa M, Yang BV, Sutcliffe J. Clinical strain of *Staphylococcus aureus* inactivates and causes efflux of macrolides. *Antimicrobial Agents and Chemotherapy*. 1996;**40**:992-998
- [30] Chesneau O, Tsvetkova K, Courvalin P. Resistance phenotypes conferred by macrolide phosphotransferases. *FEMS Microbiology Letters*. 2007;**269**:317-322. DOI: 10.1111/j.1574-6968.2007.00643.x
- [31] Brisson-Noël A, Delrieu P, Samain D, Courvalin P. Inactivation of lincosaminide antibiotics in *Staphylococcus*. Identification of lincosaminide O-nucleotidyltransferases and comparison of the corresponding resistance genes. *The Journal of Biological Chemistry*. 1988;**263**:15880-15887
- [32] Brisson-Noël A, Courvalin P. Nucleotide sequence of gene *linA* encoding resistance to lincosamide in *Staphylococcus haemolyticus*. *Gene*. 1986;**43**:247-253
- [33] Bozdogan B, Berrezouga L, Kuo M, Yurek D, Farley K, Stockman B, Leclercq R. A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. *Antimicrobial Agents and Chemotherapy*. 1999;**43**:925-929

- [34] Allignet J, Liassine N, El Solh N. Characterization of a staphylococcal plasmid related to pUB110 and carrying two novel genes, *vatC* and *vgbB*, encoding resistance to streptogramins A and B and similar antibiotics. *Antimicrobial Agents and Chemotherapy*. 1998;**42**:1794-1798
- [35] Mukhtar TA, Koteva KP, Hughes DW, Wright GD. Vgb from *Staphylococcus aureus* inactivates streptogramin B antibiotics by an elimination mechanism not hydrolysis. *Biochemistry*. 2001;**40**:8877-8886
- [36] Prunier AL, Malbruny B, Tandé D, Picard B, Leclercq R. Clinical isolates of *Staphylococcus aureus* with ribosomal mutations conferring resistance to macrolides. *Antimicrobial Agents and Chemotherapy*. 2002;**46**:3054-3056
- [37] Liakopoulos A, Neocleous C, Klapsa D, Kanellopoulou M, Spiliopoulou I, Mathiopoulou KD, Papafrangas E, Petinaki E. A T2504A mutation in the 23S rRNA gene responsible for high-level resistance to linezolid of *Staphylococcus epidermidis*. *The Journal of Antimicrobial Chemotherapy*. 2009;**64**:206-207
- [38] Weisblum B. Insights into erythromycin action from studies of its activity as inducer of resistance. *Antimicrobial Agents and Chemotherapy*. 1995;**39**:797-780
- [39] Watanakunakorn C. Clindamycin therapy of *Staphylococcus aureus* endocarditis: Clinical relapse and development of resistance to clindamycin, lincomycin and erythromycin. *The American Journal of Medicine*. 1976;**60**:419-425
- [40] Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *The Journal of Antimicrobial Chemotherapy*. 2001;**48**:315-316
- [41] Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrobial Agents and Chemotherapy*. 1999;**43**:1062-1066
- [42] Malbruny B, Werno AM, Murdoch DR, Leclercq R, Cattoir V. Cross-resistance to lincosamides, streptogramins A, and pleuromutilins due to the *lsa(C)* gene in *Streptococcus agalactiae* UCN70. *Antimicrobial Agents and Chemotherapy*. 2011;**55**:1470-1474
- [43] Deng F, Wang H, Liao Y, Li J, Feßler AT, Michael GB, Schwarz S, Wang Y. Detection and genetic environment of pleuromutilin-lincosamide-streptogramin A resistance genes in staphylococci isolated from pets. *Frontiers in Microbiology*. 2017;**8**:234. DOI: 10.3389/fmicb.2017.00234
- [44] Tessé S, Trueba F, Berthet N, Hot C, Chesneau O. Resistance genes underlying the LSA phenotype of staphylococcal isolates from France. *Antimicrobial Agents and Chemotherapy*. 2013;**57**:4543-4546. DOI: 10.1128/AAC.00259-13
- [45] Leclercq R, Brisson-Noël A, Duval J, Courvalin P. Phenotypic expression and genetic heterogeneity of lincosamide inactivation in *Staphylococcus* spp. *Antimicrobial Agents and Chemotherapy*. 1987;**31**:1887-1891

- [46] Singh KV, Murray BE. Differences in the *Enterococcus faecalis* lsa locus that influence susceptibility to quinupristin-dalfopristin and clindamycin. *Antimicrobial Agents and Chemotherapy*. 2005;**49**:32-39. DOI: 10.1128/AAC.49.1.32-39.2005
- [47] Derek H. Activity of erythromycin against *Staphylococcus aureus*. *British Medical Journal*. 1954;**1**:236-239
- [48] Macleod AJ, Ross HB, Ozere RL, Digout G, Rooyenc V. Lincomycin: A new antibiotic active against staphylococci and other gram-positive cocci: Clinical and laboratory studies. *Canadian Medical Association Journal*. 1964;**91**:1056-1060
- [49] Weaver JR, Pattee PA. Inducible resistance to erythromycin in *Staphylococcus aureus*. *Journal of Bacteriology*. 1964;**88**:574-580
- [50] Gtiffith LJ, Ostrander WE, Mullins CG, Beswick DE. Drug antagonism between lincomycin and erythromycin. *Science*. 1965;**147**:746-747
- [51] Lai CJ, Weisblum B. Altered methylation of ribosomal RNA in an erythromycin-resistant strain of *Staphylococcus aureus*. *Proceedings of the National Academy of Sciences of the United States of America*. 1971;**68**:856-860
- [52] Barta A, Steiner G, Brosius J, Noller HF, Kuechler E. Identification of a site on 23S ribosomal RNA located at the peptidyl transferase center. *Proceedings of the National Academy of Sciences of the United States of America*. 1984;**81**:3607-3611
- [53] Murphy E. Nucleotide sequence of *ermA*, a macrolide-lincosamide-streptogramin B determinant in *Staphylococcus aureus*. *Journal of Bacteriology*. 1985;**162**:633-640
- [54] Wu SW, de Lencastre H, Tomasz A. The *Staphylococcus aureus* transposon Tn551: Complete nucleotide sequence and transcriptional analysis of the expression of the erythromycin resistance gene. *Microbial Drug Resistance*. 1999;**5**:1-7
- [55] Projan SJ, Monod M, Narayanan CS, Dubnau D. Replication properties of pIM13, a naturally occurring plasmid found in *Bacillus subtilis*, and of its close relative pE5, a plasmid native to *Staphylococcus aureus*. *Journal of Bacteriology*. 1987;**169**:5131-5139
- [56] Chung WO, Werckenthin C, Schwarz S, Roberts MC. Host range of the *ermF* rRNA methylase gene in bacteria of human and animal origin. *The Journal of Antimicrobial Chemotherapy*. 1999;**43**:5-14
- [57] Matsuoka M, Inoue M, Nakajima Y, Endo Y. New *erm* gene in *Staphylococcus aureus* clinical isolates. *Antimicrobial Agents and Chemotherapy*. 2002;**46**:211-215
- [58] Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disc diffusion method for the detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase negative *Staphylococcus*. *Journal of Clinical Microbiology*. 2003;**41**:4740-4744
- [59] Mallick SK, Basak S, Bose S. Inducible clindamycin resistance in *Staphylococcus aureus*. A therapeutic challenge. *Journal of Clinical and Diagnostic Research*. 2009;**3**:1513-1518

- [60] Rajaduraiipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. The prevalence and the antimicrobial susceptibility pattern of the methicillin resistant *Staphylococcus aureus*: A multicentre study. *Indian Journal of Medical Microbiology*. 2006;**24**:34-38
- [61] Vivek JS, Rajesh GN, Mukesh S, Manpreet K, Misra RN, Matnani GB, Ujagare MT, Saikat B, Kumar A. The prevalence of inducible clindamycin resistance among community- and hospital-associated *Staphylococcus aureus* isolates in a tertiary care hospital in India. *Biomedical Research*. 2011;**22**:465-469
- [62] Kasten MJ. Clindamycin, metronidazole, and chloramphenicol. *Mayo Clinic Proceedings*. 1999;**74**:825-833
- [63] Saiman L, O'Keefe M, Graham PL III, Wu F, Said-Salim B, Kreiswirth B, LaSala A, Schlievert PM, Della-Latta P. The hospital transmission of community-acquired methicillin resistant *Staphylococcus aureus* among postpartum women. *Clinical Infectious Diseases*. 2003;**37**:1313-1319
- [64] Otsuka T, Zaraket H, Takano T, Saito K, Dohmae S, Higuchi W, Yamamoto T. Macrolide-lincosamide-streptogramin B resistance phenotypes and genotypes among *Staphylococcus aureus* clinical isolates in Japan. *Clinical Microbiology and Infection*. 2007;**13**:325-327. DOI: 10.1111/j.1469-0691.2006.01632.x
- [65] Cetin ES, Gunes H, Kaya S, Aridogan BC, Demirci M. Macrolide-lincosamide-streptogramin B resistance phenotypes in clinical staphylococcal isolates. *International Journal of Antimicrobial Agents*. 2008;**31**:364-368. DOI: 10.1016/j.ijantimicag.2007.11.014
- [66] Uzun B, Güngör S, Pektaş B, Aksoy Gökmen A, Yula E, Koçal F, Kaya S. Macrolide-lincosamide-streptogramin B (MLSB) resistance phenotypes in clinical *Staphylococcus* isolates and investigation of telithromycin activity. *Mikrobiyoloji Bülteni*. 2014;**48**:469-476
- [67] Vallianou N, Evangelopoulos A, Hadjisoteriou M, Avlami A, Petrikkos G. Prevalence of macrolide, lincosamide, and streptogramin resistance among staphylococci in a tertiary care hospital in Athens, Greece. *Journal of Chemotherapy*. 2015;**27**:319-323. DOI: 10.1179/1973947814Y.0000000205
- [68] Petrikkos G, Vallianou N, Evangelopoulos A, Gourni M, Bagatzouni D, Syriopoulou V, Daikos GL. Prevalence of macrolide resistance genes among staphylococci in Cyprus. *Journal of Chemotherapy*. 2006;**18**:480-484. DOI: 10.1179/joc.2006.18.5.480
- [69] Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. *Veterinary Microbiology*. 2007;**121**:307-315. DOI: 10.1016/j.vetmic.2006.12.007
- [70] Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Köhrer K, Verhoef J, Fluit AC. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. *The Journal of Antimicrobial Chemotherapy*. 2000;**45**:891-894



- [71] Azap OK, Arslan H, Timurkaynak F, Yapar G, Oruc E, Gagir U. Incidence of inducible clindamycin resistance in staphylococci: First results from Turkey. *Clinical Microbiology and Infection*. 2005;**11**:582-584. DOI: 10.1111/j.1469-0691.2005.01174.x
- [72] Schmitz FJ, Petridou J, Fluit AC, Hadding U, Peters G, von Eiff C. Distribution of macrolide-resistance genes in *Staphylococcus aureus* blood-culture isolates from fifteen German university hospitals. M.A.R.S. Study Group. Multicentre Study on Antibiotic Resistance in Staphylococci. *European Journal of Clinical Microbiology & Infectious Diseases*. 2000;**19**:385-387
- [73] Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *Journal of Clinical Microbiology*. 2004;**42**:2777-2779. DOI: 10.1128/JCM.42.6.2777-2779.2004
- [74] Spiliopoulou I, Petinaki E, Papandreou P, Dimitracopoulos G. *erm(C)* is the predominant genetic determinant for the expression of resistance to macrolides among methicillin-resistant *Staphylococcus aureus* clinical isolates in Greece. *The Journal of Antimicrobial Chemotherapy*. 2004;**53**:814-817. DOI: 10.1093/jac/dkh197
- [75] Gatermann SG, Koschinski T, Friedrich S. Distribution and expression of macrolide resistance genes in coagulase-negative staphylococci. *Clinical Microbiology and Infection*. 2007;**13**:777-781. DOI: 10.1111/j.1469-0691.2007.01749.x
- [76] Lozano C, Aspiroz C, Ara M, Gómez-Sanz E, Zarazaga M, Torres C. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in a farmer with skin lesions and in pigs of his farm: Clonal relationship and detection of *lnu(A)* gene. *Clinical Microbiology and Infection*. 2011;**17**:923-927. DOI: 10.1111/j.1469-0691.2010.03437.x
- [77] Sarrou S, Liakopoulos A, Chasioti M, Foka A, Fthenakis G, Billinis C, Spyrou V, Pantelidi K, Roussaki-Schulze A, Lachanas V, Makaritsis K, Skoulakis C, Daikos GL, Dalekos G, Spiliopoulou I, Petinaki E. Dissemination of methicillin-susceptible CC398 *Staphylococcus aureus* strains in a rural Greek area. *PLoS One*. 2015;**10**:e0122761. DOI: 10.1371/journal.pone.0122761
- [78] Sarrou S, Liakopoulos A, Tsoumani K, Sagri E, Mathiopoulos KD, Tzouvelekis LS, Miriagou V, Petinaki E. Characterization of a novel *lsa(E)*- and *lnu(B)*-carrying structure located in the chromosome of a *Staphylococcus aureus* sequence type 398 strain. *Antimicrobial Agents and Chemotherapy*. 2015;**60**:1164-1166. DOI: 10.1128/AAC.01178-15
- [79] Lüthje P, Schwarz S. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. *The Journal of Antimicrobial Chemotherapy*. 2006;**57**:966-969. DOI: 10.1093/jac/dkl061
- [80] Li L, Feng W, Zhang Z, Xue H, Zhao X. Macrolide-lincosamide-streptogramin resistance phenotypes and genotypes of coagulase-positive *Staphylococcus aureus* and coagulase-negative staphylococcal isolates from bovine mastitis. *BMC Veterinary Research*. 2015;**11**:168. DOI: 10.1186/s12917-015-0492-8



