Chapter 7

Resistance of Staphylococci to Macrolides-Lincosamides-Streptogramins B (MLS_B): Epidemiology and Mechanisms of Resistance

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Additional information is available at the end of the chapter

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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_B 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_B (constitutive or inducible), which is associated with the presence mainly of *ermA* and *ermC* genes, followed by the MS_B 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_B, resistance, genes

1. Introduction

Resistance to macrolides-lincosamides and streptogramins B (MLS_B 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_B 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_B (constitutive or inducible), which, in staphylococci, are associated with the presence mainly of *ermA* and *ermC* genes, followed by the MS_B 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. Qiunupristin-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_B 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].

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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_B 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_B

The spectrum of MLS_B 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_B

Staphylococci resist MLS_B 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_B 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_B phenotype) [13]. The MLS_B 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_B 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_B 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_B phenotype) [15]. MS_B 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_A), referred as the PLS_A 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_A 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.

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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_B resistant phenotypes. Briefly, these types are described as follows.

4.1. MLS_B phenotype (*erm* genotype)

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

On the other hand, isolates with an inducible MLS_B 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_B antibiotics. Dissociated resistance to MLS_B 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_B antibiotics is not devoid of risk. In the presence of these antibiotics, constitutive mutants can be selected *in vitro* at frequencies of ~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_B 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 Tn*554*, 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_B-phenotype (*msrA* genotype)

MS_B 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_B 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 xylosus*, 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_B 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_B-inducible phenotype and from MS_B phenotype. Additional tests (see below) are required for its detection.

4.4. PLS_A-phenotype

PLS_A-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_B-phenotype

S_B-phenotype is expressed by resistance to streptogramin B due to the presence of *vgb*A/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_B (constitutive or inducible), MS_B and M-phenotypes. The clinical microbiology laboratory detects easily and reliably the MLS_B constitutive phenotype: the isolates are fully resistant to macrolides and lincosamides. However, isolates with MLS_B inducible, MS_B 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_B 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_B 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_B-phenotype, which express fully resistance to macrolides. In addition, MS_B-phenotype affects the susceptibility to quinupristin-dalfopristin, decreasing it slowly.

Finally, it is difficult to discriminate isolates with PLS_A-phenotype from those with L-phenotype; both share the same profile, including resistance to lincomycin and susceptibility to erythromycin.

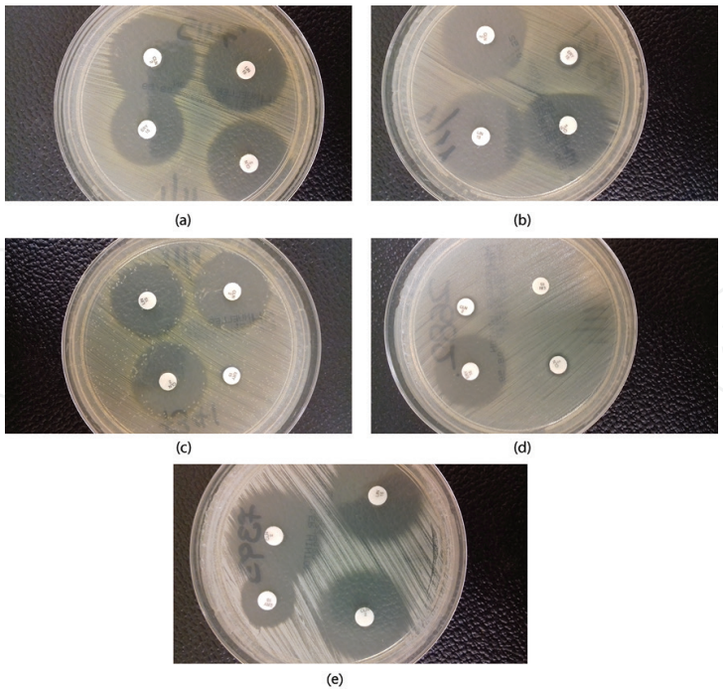

Figure 1. Expression of various resistant-phenotypes: (a) sensitive; (b) MLS_B-inducible phenotype; (c) MS_B-phenotype; (d) L-phenotype and (e) M-phenotype. ERY: erythromycin; CLIN: clindamycin; LIN: lincomycin.

Gene	Primers sequence (5′–3′)	PCR fragment size (bp)
*erm*A	F: TCTAAAAAGCATGTAAAAGAA	645
	R: CTTCGATAGTTTATTAATATTAG	
*erm*B	F: GAAAAGTACTCAACCAAATA	639
	R: AGTAACGGTACTTAAATTGTTTA	
*erm*C	F: TCAAAACATAATATAGATAAA	642
	R: GCTAATATTGTTTAAATCGTCAAT	
*msr*A	F: GGCACAATAAGAGTGTTTAAAGG	940
	R: AAGTTATATCATGAATAGATTGTCCTGTT	
*msr*B	F: TATGATATCCATAATAATTATCCAATC	595
	R: AAGTTATATCATGAATAGATTGTCCTGTT	
*lnu*A	F: GGTGGCTGGGGGGTAGATGTATTAACTGG	323
	R: GCTTCTTTTGAAATACATGGTATTTTTCGATC	
*lnu*B	F: CCTACCTATTGTTTGTGGAA	925
	R: ATAACGTTACTCTCCTATTC	
*lsa*A	F: GGCAATCGCTTGTGTTTTAGCG	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.

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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_B 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 (**e**rythromycin **r**esistance **m**ethylase) 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_B 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_B) 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_B antibiotics has increased the number of the Staphylococcus isolates which are resistant to them [63].

The rate of MLS_B-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_B 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_B 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_B *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_B (constitutive or inducible) followed by MS_B. In Japan, Otsuka et al. revealed higher incidence of the MLS_B-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_B, should be carefully considered for the treatment of infections caused by multiple drug-resistant staphylococci.

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