Mechanisms of Resistance to Antimicrobial Drugs in Pathogenic Gram-Positive Cocci

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Abstract: Many species of Gram-positive cocci are pathogenic. The most important are staphylococci, streptococci, and enterococci. Widespread usage of antibiotics was the main cause for the appearance and spread of resistance to almost all antimicrobials. The occurrence, mechanisms, and genetic background of resistance to antimicrobial drugs other than beta-lactams and glycopeptides among pathogenic staphylococci, streptococci, and enterococci are discussed in the text. Well-established agents (suchas macrolides, lincosamides, streptogramins, aminoglycosides, quinolones, mupirocin, chloramphenicol) as well as new agents (linezolid, daptomycin, quinupristine/dalfopristine, ratapamulin, tigecycline, iclaprim and new generations of quinolones) are considered.

Keywords: *Staphylococcus aureus*, *Streptococcus pneumoniae, Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, antibiotic resistance, plasmids, transposons.

MACROLIDES, KETOLIDES, LINCOSAMIDES AND STREPTOGRAMINS

All macrolides, lincosamides, and streptogramins B (MLS-B) display the same mechanism of action against bacterial cells. The target for the antibiotics is the four-nucleodide ribosomal rRNA (in the peptidyltransferase region) inside domain V of 23S rRNA, in 50S subunit of ribosomes [1, 2]. Ketolides and only a few macrolides, including tylosin, come into contact with domain II of the rRNA and do so *via* interactions with nucleotides G748 and A752. Mechanisms of the resistance to the group are various and not identical in different species. Table **1** presents criteria of susceptibility of Gram-positive cocci to MLS-B according to CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) [3, 4].

Resistance to MLS-B in *S. aureus*

The most common mechanism of resistance to MLS-B depends on a modification of the antibiotic's target. The modification is performed by enzymes, adenyl-N-methyltransferases Erm (erythromycin ribosome methylation) that methylate adenine 2058, leading to resistance to all MLS-B. Some strains synthesize Erm methylases constitutively, and then they are resistant to all MLS-B or inducibly. Strains inducibly resistant to MLS-B-type agents require preexposure to inducer-type MLS-B for expression of resistance. Once induced, these strains would be cross-resistant to all MLS-B agents. Good inducers are macrolides containing a 14-membered ring (except ketolides, M_{14} e.g. erythromycin, clarithromycin, oleandomycin) or 15-membered ring (M_{15}, e.g. azithromycin). The introduction by the CLSI of an additional test to distinguish the inducible and constitutive resistance to MLS-B in streptococci and staphylococci ("D-test") was aimed at exclusion of clindamycin therapy in patients infected by bacteria with inducible type resistance. Even though bacteria are sensitive to clindamycin, they can easily become resistant during treatment. The inducible resistance to MLS-B in *S. aureus* is most frequently determined by *ermA* or *ermC* genes. The frequency of constitutive mutants formation from inducible genes *ermA* was specified as 10^{-6} -10^{-8}, and in the case of inducible genes *ermC*, the frequency is usually much higher. The causes of the constitutive variants formation are deletions, duplications, insertions, and relatively rarely, point mutations in the 200 bp region preceding the 5' end of *erm* gene. The differences between *ermA* and *ermC* genes in the frequency of forming constitutive variants are most probably associated with their different locations and copy numbers. The e*rmA* gene, that can be found in a chromosome of *S. aureus* as a part of Tn*554* (*ermA-spc*) transposon, possess one site with high preference and another with 1000 times lower preference to integration with a chromosome. Most frequently it occurs in one copy per chromosome as a part of Tn*554*. In several MRSA strains, the second copy of Tn*554* can be found in cassettes SCC*mec* type II or III. The e*rmC* gene is usually localized in 2.5-5.0 kb plasmids (e.g. pE194, pWBG738). Small plasmids occur in multiply copies or are parts of large conjugative plasmids, e.g. pUSA03 (37 kb), where they can be found altogether with the *ileS* gene (resistance to mupirocin) [5, 6].

Regulation of the expression of *ermA* and *ermC* genes takes place on the level of translation. The *ermA* genes are preceded in the polycistronic RNA molecule by two genes

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Table 1. Breakpoints Used to Define Susceptibility and Resistance to Antibacterials in 2009 (MIC, mgL^{-1})

Drugs	*S. aureus*		*S. pneumoniae*		*S. pyogenes, S. agalactiae*		*Enterococcus spp.*	
	CLSI*	EUCAST**	CLSI*	EUCAST**	CLSI*	EUCAST**	CLSI*	EUCAST**
	S/R	S/R	S/R	S/R	S/R	S/R	S/R	S/R
Erythromycin	≤0.5/≥8	<1/>2	≤0.25/≥1	≤0.25/>0.5	≤0.25/≥1	≤0.25/>0.5	≤0.5/≥8	-/-
Clarithromycin	≤2/≥8	<1/>2	≤0.25/≥1	≤0.25/>0.5	≤0.25/≥1	≤0.25/>0.5	-/-	-/-
Azithromycin	≤2/≥8	<1/>2	≤0.5/≥2	≤0.25/>0.5	≤0.5/≥2	≤0.25/>0.5	-/-	-/-
Telithromycin	≤1/≥4	-/-	≤1/≥4	≤0.25/>0.5	-/-	≤0.25/>0.5	-/-	-/-
Clindamycin	≤0.5/≥4	<0.25/>0.5	≤0.25/≥1	≤0.5/>0.5	≤0.25/≥1	≤0.5/>0.5	-/-	-/-
Quinupristin/ dalfopristin	≤1/≥4	<1/>2	≤1/≥4	-/-	≤1/≥4	-/-	≤1/≥4	<1/>4
Linezolid	≤4/-	<4/>4	≤2/-	≤4/>4	≤2/-	2≤/>4	≤2/≥8	<4/>4
Ciprofloxacin	≤1/≥4	<1/>1	-/-	≤0.125/>2	-/-	-/-	≤1/≥4	-/-
Levofloxacin	≤1/≥4	<1/>2	≤2/≥8	≤2/>2	≤2/≥8	≤1/>2	≤2/≥8	-/-
Ofloxacin	≤1/≥4	<1/>1	≤2/≥8	≤0.125/>4	≤2/≥8	-/-	-/-	-/-
Moxifloxacin	≤0.5/≥2	<0.5/>1	≤1/≥4	≤0.5/>0.5	-/-	≤0.5/>1	-/-	-/-
Norfloxacin	≤4/≥16	-/-	-/-	-/-	-/-	-/-	≤4/≥16	-/-
Gemifloxacin	-/-	-/-	≤0.125/≥0.5	-/-	-/-	-/-	-/-	-/-
Gatifloxacin	≤0.5/≥2	-/-	≤1/≥4	-/-	≤1/≥4	-/-	≤2/≥8	-/-
Grepafloxacin	≤1/≥4	-/-	≤0.5/≥2	-/-	≤0.5/≥2	-/-	-/-	-/-
Tetracycline	≤4/≥16	<1/>2	≤2/≥8	≤1/>2	≤2/≥8	≤1/>2	≤4/≥16	-/-
Doxycycline	≤4/≥16	<1/>2	-/-	≤1/>2	-/-	≤1/>2	≤4/≥16	-/-
Minocycline	≤4/≥16	<0.5/>1	-/-	≤0.5/>1	-/-	≤0.5/>1	≤4/≥16	-/-
Tigecycline	-/-	<0.5/>0.5	-/-	-/-	-/-	0.25/>0.5	-/-	<0.25/>0.5
Daptomycin	≤1/-	<1/>1	-/-	-/-	≤1/-	≤1/>1	≤4/-	-/-
Chloramphenicol	≤8/≥32	<8/>8	≤4/≥8	≤8/>8	≤4/≥16	≤8/>8	≤8/≥32	-/-
Rifampicin	≤1/≥4	<0.06/>0.5	≤1/≥4	≤0.06/>0.5	-/-	≤0.06/>0.5	≤1/≥4	-/-
Trimethoprim	≤8/≥16	<2/>4	-/-	-/-	-/-	-/-	-/-	<0.032/>1
Trimethoprim/ sulfamethoxazol	≤2/38/ ≥4/76	<2/>4	≤0.5/9.5/ ≥4/76	≤1/>2	-/-	≤1/>2	-/-	<0.032/>1
Gentamicin	≤4/≥16	<1/>1	-/-	-/-	-/-	-/-	-/-	-/-
Tobramycin	≤4/≥16	<1/>1	-/-	-/-	-/-	-/-	-/-	-/-
Amikacin	≤16/≥64	<8/>16	-/-	-/-	-/-	-/-	-/-	-/-
Kanamycin	≤16/≥64	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Netilmycin	≤8/≥32	<1/>1	-/-	-/-	-/-	-/-	-/-	-/-

Abbreviations: R, resistance; S, susceptibility, *Clinical and Laboratory Standards Institute; **European Committee on Antimicrobial Susceptibility Testing.

encoding leader peptides *pepL* and *pep1*, whereas *ermC* is preceded by one gene - *pep*. The hairpin structure formed by *pep* rRNA disables ribosome access to RBS (ribosome binding site) in the *erm* gene. Therefore, Erm methylase synthesis doesn't occur without this inducer. When the inducer particle (M_{14-15}) is binding to the ribosome, the translation of the

Table 2. Resistance to Macrolides, Lincosamides, Streptogramins and Linezolid in Gram-Positive Cocci

Mechanism of resistance	Localization of gene	Resistance	Reference
23S rRNA adenine-metyltransferase			
ErmA[1,5,6]	chr:Tn*554*[1]	$M_{14\text{-}15}$[*], $MKLS_B$[**]	[13, 14, 27, 29
ErmA(TR)[2,3]	Tn*1802*[2]	$M_{14\text{-}15}$[*], MLS_B[**]	[25, 35]
ErmB[1,2,3,4,5,6]	pI258:Tn*551*[1**], Tn*917*[1,2,3], Tn*3872*[2**], Tn*3701*[2**], Tn*3872*[2,3], Tn*2010*[2**], Tn*3701*[3**], Tn*3703*[3**], Tn*3951*[2**], Tn*1545*[2,5,6**], Tn*6002*[2**], Tn*6003*[2**], p:Tn*917*-like[3,4**], Tn*1116*[3**]	$M_{14\text{-}15}$[*], MLS_B[**]	[4, 11, 14, 15, 25, 28, 33, 35, 38]
ErmC[1]	pE194[1*], WBG738[1*], pKH19[1**], pKH20[1**], pUSA03[1**]	$M_{14\text{-}15}$[*], $MKLS_B$[**]	[13]
ErmF[1]	nd	MLS_B	[16]
ErmGM (ErmGA, ErmY)[1]	pMS97[1]	MLS_B	[17]
Cfr[1]	p004-737X[1]	L, Ph, Lz, R	[9]
Efflux			
MefA (MFS)[1,2,3,5]	mega-like[1], mega[2],Tn*1270.1*[2,5], Tn*1207.3*[3], Phi10394.4[3]	$M_{14\text{-}15}$	[16, 25, 33, 35, 36]
MefE[2,3,6]	Tn*916*-like[2], Tn*2009*[2], Tn*2010*[2]	$M_{14\text{-}15}$	[31, 33, 34, 37]
MefI[2]	Tn*916*-like[2]	$M_{14\text{-}15}$	[34]
MefO[3]	nd	$M_{14\text{-}15}$	[37]
MsrA[1] (ABC)	pUL5050[1*],pMS97[1**], pSR1[1**]	M_{14}[*], $M_{14}KS_B$[**]	[14]
MsrB (ABC)[1]	plasmids	M_{14}[*], $M_{14}S_B$[**]	[14]
MsrSA (ABC)[1]	pEP2104[1*], pMC38[1**]	$M_{14\text{-}15}$[*], $M_{14\text{-}15}S_B$[**]	[18]
MsrC[6]	nd	$M_{14\text{-}15}S_B$	[30]
MsrD[2]	nd	$M_{14\text{-}15}S_B$	
Lsa (ABC)[5]	chr	LS_BS_A,Q/D	[26]
Enzymatic inactivation			
EreA[1]	nd	M_{14} M_{16}	[19]
EreB[1]	nd	M_{14} M_{16}	[19]
LinA1 (LnuA1)[1]	pBMSa1[1]	Lincomycin only	[20]
LinB (LnuB)[6]	nd	L	[32]
VgbA[1]	p	S_B	[21]
VgbB[1]	p	S_B	[22]
MphBM[1]	pMS97[1], pSR1[1]	M	[18]
Mutation of gene coding ribosom protein and 23S rRNA			
rplD (L22)[1,2]	chr	EKQ/D[1], MS[2], MKS[2]	[22, 25]
rplV(L4)[1,2]	chr	ESp[1], MKLS[2]	[23, 25]
rrl (23S rRNA)[2]	chr	$M_{16}S$[2], ML[2]	[25]

[1]*S. aureus*; [2]*S. pneumoniae*; [3]*S. pyogenes*; [4]*S. agalactiae*; [5]*E. faecalis*; [6]*E. faecium*; [*]inducible expression; [**]constitutive expression, M, macrolides (M_{14}, Erythromycin, Clarithromycin, Dirythromycin; M_{15}, Azithromycin (azalide); M_{16}, Spiramycin), K, ketolides (Telithromycin); L, lincosamides (Lincomycin, Clindamycin); S, Streptogramins; S_B, Streptogramin B; S_A, Streptogramin A; E, Erythromycin; Q/D-Quinopristine/Dalfopristine; Sp, Spiramycin; R, Rapamulin (Pleuromutilins); Ph, chloramphenicol; Lz, linezolid; chr, chromosome; p, plasmid; tn, transposon; nd, no data.

leader peptide is interrupted and ribosome release doesn't occur. That causes permanent opening of the hairpin rRNA structure and enables the ribosome to access the RBS for gene *erm* and translation [6-8].

The isolated *S. aureus* strains present several other mechanisms of resistance, such as synthesis of methylases ErmGM (ErmY), ErmB (frequent in VRSA) or ErmF, presence of membrane proteins causing active efflux of antibiotic such as MsrA (frequent in VRSA), MsrB (resistance to M_{14}S-B), MsrSA (resistance to $M_{14\text{-}15}$S-B) and MefA (resistance to M_{14}), synthesis of methyltransferases Cfr (resistance to lincosamides) [9-11] and the synthesis of enzymes that inactivate some MLS-B such as esterases EreA, EreB (inactivate M_{14} and M_{16}); nucleotidyltransferases LinA, LinA1 or LinA' (resistance to lincomycin only, clindamycin is modified but remains active), liases VgbA and VgbB (inactivate S-B) and macrolide phosphotransferase MphBM [6, 12]. The resistance can be also a result of mutation inside chromosomal genes encoding ribosomal proteins L22 (in *S. aureus*, resistance to erythromycin, telithromycin, quinupristin and quinupristin/dalfopristin) and L4 (in *S. aureus* resistance to erythromycin and spiramycin) [6, 12]. The most important types of resistance to MLS-B in *S. aureus* are presented in Table **2**.

Resistance to MLS-B in *S. pneumoniae*

Resistance to macrolides in *S. pneumoniae* occurs with different frequences in different countries [24]. According to data from 2006, the highest rate of resistance or intermediate sensitivity were in Malta (47%), Luxembourg and France (36%), Italy (32%), Belgium and Cyprus (31%) and the lowest in Lithuania (<1%), Czech Republic, Estonia and Latvia (3%), Sweden (5%) and Denmark (6%) [24]. Other data from a global international surveillance project (PROTEKT, 1993-2004) showed an increase in the global rate of macrolide resistance, from 31.0% in 1999 to 36.3% in 2003. This surveillance also demonstrated important differences between countries. In Europe in 2003-2004, the highest rates of macrolide resistance were observed in France, 53.1% (90% ErmB), Greece 49.4% (66.2% MefA), Italy 45.6% (55.8% ErmB), Hungary 43.6% (82.4% ErmB), Spain 33.5% (88.3% ErmB), and 30.7% (91.5% ErmB) in Belgium, whereas the lowest rates were found in the Czech Republic (2.9%), Russia (4.1%), and Portugal (6.1%) [25]. In America in 2003-2004, the prevalence of resistance to macrolides was the highest in the United States, 34.7% (55.2% MefA), Venezuela 33.3% (36.6% ErmB), Peru 27.8% (53.3% ErmB), Mexico 27.6% (72.4% MefA), Canada 20.1% (57.7% MefA) with the lowest in Colombia (0%). Most alarming are the resistance rates found in East Asian countries, from 80.6% (53% ErmB) in Hong Kong, 81.6% (76.5% ErmB) in China to 81.9% (58% ErmB) in Japan and 97.6% (68.3% ErmB) in Taiwan [25]. Co-resistance to macrolides and beta-lactam antibiotics is a frequent finding among pneumococci of serotypes 6A, 6B, 14, 15A, 19F, 19A, 23F, and 23A.

Resistance to macrolides in *S. pneumoniae* is mediated in most strains by two main mechanisms, target site modification or an efflux pump. The target site modification by methylases is encoded most commony by the *erm*(B) gene and is related to the MLS_B phenotype (macrolide-lincosamide-streptogramin B resistance). The second mechanism is an efflux pump encoded by *mef* genes and related to the M phenotype (resistance to 14- and 15-membered ring macrolides). Other, less common mechanisms include mutations in the 23S rRNA gene and/or alterations in ribosomal proteins L4 and L22. The most important mechanisms of the resistance in *S. pneumoniae* to macrolides are presented in Table **2**.

The MLS_B phenotype dominate in the majority of European countries, whereas the M phenotype is more frequent in Argentina, Australia, Canada, Mexico, Saudi Arabia, United States, Greece, Austria, England, and Germany. In pneumococci and related streptococci, frequent association of erythromycin and tetracycline resistance is due to transposons such as Tn*1545*, Tn*3872*, and Tn*6002*, resulting from the insertion of the *erm*(B) gene into the Tn*916* family of conjugative transposons that harbour the *tet*(M) gene. The efflux pump mechanism in pneumococci is codified by three subclasses of *mef*(A) genes, including *mef*(E), *mef*(A), and the recently described subclass *mef*(I). The *mef*(E) gene is the most frequently found and is carried by the macrolide efflux genetic assembly (mega) element, whereas *mef*(A) is carried by a defective transposon (Tn*1207.1*) [39]. The most important transposons and other mobile genetic elements (according to [1, 15, 31, 40-44]) that can be found in *S. pneumoniae* and other Gram-positive cocci are presented in Table **3**.

Resistance to MLS-B in *S. pyogenes*

High rates of *S. pyogenes* resistance to erythromycin (>50%) have been reported in Taiwan and Japan and lower rates in Canada (2.0%), Turkey (3.8%) and Brazil (0%) [45, 46]. The analysis of Brazilian *S. pyogenes* isolates in 1978-1997 revealed, that MIC_{50}/MIC_{90} rate for erythromycin was 0.06/0.12, for clarithromycin 0.03/0.06 and for clindamycin from 0.06/0.12 to 0.03/0.06 in different years [46]. In Italy the rates of resistance to MLS-B were 38.3%, 22% in UK, 17% in Finland and 10% in Sweden. Erythromycin resistance has also been recorded in the USA (6.1%- 60% phenotype M) [45]. The most important types of macrolide resistance in *S. pyogenes* are presented in Table **2**. Characteristics of transposable elements carrying the resistance genes are listed in Table **3**.

Resistance to MLS-B in Enterococci

The most common mechanism of resistance to MLS-B depends on the target site modification by methylase encoded by the *erm*(B) and rarely *erm*(A) determinant [14, 27]. Frequently, the efflux mechanisms are present, determining resistance to macrolides (MefA, MefE, phenotype M) and streptogramins B (MsrC, phenotype MS) [29]. The chromosomally encoded efflux mechanism determining resistance to lincosamides and streptogramins A and B (Lsa) is present in most strains of *E. faecalis* [26].

STREPTOGRAMINS A AND QUINUPRISTIN/DALFOPRISTIN

A target for streptogramins A can be found in the 23S rRNA of 50S subunit of bacterial ribosomes, but the site differs from that which affects susceptibility to MLS-B agents. Streptogramins A prevent protein biosynthesis by interfering with substrate binding at both acceptor and donor sites of the peptidyl transferase center, thereby inhibiting the

Table 3. Transposons in Gram-Positive Cocci

Transposon	Size (kb)	IS, Tn and Genes of Antibiotic Resistance	Localization	Resistance
Tn*551*[1]	5.3	*erm*(B)	pI258, chr	MLS_B,
Tn*552*[1]	6.5	*blaZ-blaI*	pIP1066	P
Tn*554*[1,5]	6.5	*erm*(A), *spc*	chr, SCC*mec*	MLS_B,Sp
ΨTn*554*[1]	nd	*cadA*	SCC*mec*	Cd
Tn*558*	5.7	*fexA*	p,chr	F
Tn*558v*	4.7	IS*21*-like, f*exA, cfr*	p	$FPhLLzS_A$
Tn*3851*[1]	5.2	IS*256-aac(6')-aph(2")*-IS*256*	chr	A
Tn*3852*[1]	7.3	*blaZ-blaI*	chr	P
Tn*4001*[1,6]	4.7	IS*256-aac(6')-aph(2")*-IS*256*	pSK1, pSK4, chr, SCCmec	A
Tn*4002*[1]	6.6	*blaZ-blaI*	pSK4	P
Tn*4003*[1]	3.6	IS*257-drfA, thyE*-IS*257*	pSK4, pSK1	Tr
Tn*4004*[1]	7.8	IS*257-merA, merB*-IS*257*	pSK74, pI524	Hg
Tn*4201*[1]	7.5	*blaZ-blaI*	pCRG1600	P
Tn*5404*[1]	16.0	*aphA*, IS*1181*	pIP1066	A
Tn*5405*[1]	12.0	IS*1182*, *aphA*, *aadE* (*ant(6)-Ia)*, *sat4*, IS*1182*	chr, p	A, St
Tn*5406* (Tn*3853*)[1]	5.5	*vgaAv*	chr, p	S_A
Tn*5801*-like (Tn*6014*)	nd	*tet*(M)	chr, p	T
Tn*1546*-like[1,]	10.8	*van*RSHAXYZ	p	VanA
Tn*916*[1,2,3,5]	18.0	*tet*(M)	chr	T
Tn*917*[1,2,3]	5.5	*erm*(B)	chr	MLS_B,
Tn*1116*[2,3]	50.0	*tet*(M), *erm*(B)	chr	$TMLS_B$,
Tn*1207.1*[2, 5]	7.2	*mef*(A)	chr	M_{14-15}
Tn*1545*[,2,5,6]	25.3	*erm*(B)-*aadE* (*ant(6)-Ia)-sat4-aphA-3* (*aph(3')-IIIa)*, *erm*(B), IS*1239*, *tet*(M),	chr	$TAStMLS_B$,
Tn*1806*[2]	10.5	*erm*(A-TR), sph	chr	SpM_{14-15}
Tn*2009*[2]	23,5	Tn*916*[*tet*(M)], mega[*mef*(E)]	chr	TM_{14-15}
Tn*2010*[2]	26.0	Tn*916*:*erm*(B), *tet*(M), mega[*mef*(E)]	chr	$TMLS_B$,
Tn*3872*[2,3]	23.3	Tn*916*[*tet*(M)], Tn*917*[*erm*(B)]	chr	$TMLS_B$,
Tn*3951*[2]	50	*cat*, *erm*(B), *tet*(M)	chr	$PhTMLS_B$,
Tn*5251*[2]	18.0	*tet*(M)	chr	T
Tn*5252*[2]	47.5	*cat*	chr	Ph
Tn*5253*[2]	65.0	Tn*5252* [*cat*], Tn*5251*[*tet*(M)]	chr	PhT
Tn*6002*[2]	20.9	*erm*(B), *tet*(M)	chr	$TMLS_B$,
Tn*6003*[2]	25.1	*erm*(B)-*aadE*(*ant(6)-Ia)-sat4-aphA-3* (*aph(3')-IIIa)*, *erm*(B), *tet*(M)	chr	$TAStMLS_B$,
mega[2]	5.4	*mef*(E)	chr	M_{14-15}
5216IQcomplex[2]	nd	*tet*(M)+*mef*(I)+*msrD*+*catQ*	chr	$TPhS_BM_{14-15}$

(Table 3). Contd…..

Transposon	Size (kb)	IS, Tn and Genes of Antibiotic Resistance	Localization	Resistance
Tn*1207.3*[3]	52.4	*mef*(A)	chr	$M_{14\text{-}15}$
Tn*1207.3Φ10394.4*[3]	nd	*mef*(A), *msrD*	chr	$M_{14\text{-}15}$
Tn*3701*[3]	50	Tn*3703*[*tet*(M), *erm*(B)]	chr, p	MLS_B T
Tn*3703*[3]	50.0	*tet*(M), *erm*(B)	chr, p	MLS_B T
Mtn*LNU*[4]	nd	*linA* (*lnuA*)	p	L
Tn*1546*[5,6,]	10.9	*van*RSHAXYZ	pHTβ	VanA
Tn1547[5,]	90-250	*van*RSYWHBX	pIP964	VanB1
Tn*1549*[5,,6]	34.0	*van*RSYWHBX	pMG2200	VanB2
Tn*5382*[5,,6]	27.0	*van*RSYWHBX	chr, p	VanB2
Tn*5397*-like[5,, 6]	15.0	*tet*(M)	chr	T
EfcTn*1*[6]	nd	*tet*(S)	chr	T
VanG2 element[5,]	240	*van*URSYWGXYT$_G$	chr	VanG2

[1]*S. aureus*; [2]*S. pneumoniae*; [3]*S. pyogenes*; [4]*S. agalactiae;* [5]*E. faecalis*; [6]*E. faecium*; F, florfenicol; T, tetracyclines; A, aminoglycosides; P, penicillins; VanA,vancomycin, teicoplanin; VanB, VanG, vancomycin; St, streptothricin; Tr, trimethoprim; other abbreviations as to the Table **2**.

peptidyl transferase reaction directly [47]. Resistance to streptogramin A in *S. aureus* and *Enterococcus* spp. could be caused by production of (1) acetyltransferases encoded by plasmids, encoded by genes *vatA*, *vatB*, *vatC*, *vatD*, and *vatE*, (2) membrane ATP binding proteins responsible for active efflux of streptogramin A, encoded by genes *vgaA*, *vgaB*, and *vgaAv*, encoded by plasmids or transposons (e.g. Tn*5406*), (3) liases encoded by genes *vgbA* and *vgbB* [47], and (4) methyltransferase that modify site of action for streptogramin A [9].

Synergistic action of dalfopristin (streptogramin A-type agent) and quinupristin (streptogramin B-type agent) causes a bactericidal effect in *S. aureus* (including MRSA, VRSA, and VISA). After quinupristin/dalfopristin application, postantibiotic effect (PAE) can occur, i.e., inhibition of bacterial growth by the antibiotics in concentrations lower than MIC. The PAE length for *S. aureus* is 2-8 hours [10]. Resistance to quinupristin/dalfopristin in *S. aureus* is most commonly due to simultaneous action of two mechanisms: constitutive resistance to MLS-B and resistance to streptogramin A. The presence of only one mechanism eliminates bactericidal effect of quinupristin/dalfopristin with remained bacteriostatic action (in routine susceptibility tests, bacteria are sensitive). Apart from that, the mutation in chromosomal gene encoding ribosomal protein L22 in *S. aureus* causes resistance to quinupristin/dalfopristin [10, 12]. In 2008, resistance to quinupristin/dalfopristin in *S. agalactiae* was first described [48]. In *E. faecium*, resistance to quinupristin/dalfopristin sometimes can be due to the presence of *ermB* + *vatE* genes [28].

OXAZOLIDINONES

Linezolid is an oxazolidinone antibiotic that inhibits synthesis of bacterial proteins by binding to 23S rRNA in 50S ribosomal subunit and demonstrates high activity against *S. aureus* (including MSSA, MRSA, VRSA, and VISA), *Enterococcus* spp. (including VRE), *S. pneumoniae* (including PRSP), and other Gram-positive cocci. The linezolid binds to conserved nucleotide A2602, that is a part of domain V of 23S rRNA particle in 50S subunit, and to two proteins of the same subunit: ribosomal protein L27, which N-terminal region adjacent to the active center of peptidyltransferase, and LepA protein [49]. Criteria for resistance to linezolid are presented in Table **1**.

Resistance to linezolid in *S. aureus* could be a result of two mechanisms: mutation in *rrn* gene or expression of *cfr* gene. Mutation in *rrn* gene encoding 23S rRNA in 50S ribosome subunit causes the modification of linezolid target site inside domain V of 23S rRNA, which prevents antibiotic action. Analysis of isolates revealed the presence of a G2576U mutation in the domain V region of the 23S rRNA gene [50] or T2500A mutation in the same gene [51]. In addition, the loss of a single copy of the 23S rRNA gene was found in two of the linezolid resistant isolates. Among four cases of clinical isolates of *S. aureus* resistant to linezolid the MIC of linezolid values were 6 mg L^{-1} (3 strains) and 8 mg L^{-1} (1 strain). Mutations in 23S rRNA genes (G2576U, G2512U, G2513U, C2610G) were also described to be resistant to linezolid in *E. faecalis* (MIC = 128 mg L^{-1}) and *E. faecium* (G2505A) (MIC = 16 mg L^{-1}) [52].

The second mechanism is connected with production of methyltransferase Cfr that causes methylation of 23S rRNA in the A2503 position. The c*fr* gene has been found in the 55 kb plasmid p004-737X (*istAS-istBS-cfr-Δtnp)*. Expression of c*fr* gene causes resistance to linezolid, lincosamides, phenicols (chloramphenicol), streptogramins A, and retapamulin (a member of pleuromutilin class of antibiotics) [9].

The Tolerance to Linezolid in Enterococci

The tolerance to linezolid occurs 93% of Enterococci that produce biofilm (MIC_{90}/MBC_{90}=4/2048 for *E. faecalis*

4/1024 for *E. faecium*) [53]. Biofilm is produced by 57-100% of *E. faecalis* and 16-48% of *E. faecium* strains [54].

FLUOROQUINOLONES

Criteria for fluoroquinolone resistance [3, 4] are given in Table **1**. Resistance to fluoroquinolones is due to mutations in the genes encoding type II topoisomerases: DNA gyrase (*gyrA* and *gyrB* subunits) and topoisomerase IV (*grlA*, and *grlB* in *S. aureus* and *parC* and *parE* in *Streptococcus* spp. and *Enterococcus* spp.). More frequent are mutations in the A subunits of both topoisomerases (*gyrA*, *grlA*, *parC*). A large number of different mutations associated with resistance to fluoroquinolones have been described [1, 31, 55, 56].

In *S. aureus*, the most common mutations were most commonly described in *gyrA* gene (Ser84Leu, Ala,Val, or Lys; Ser85Pro; Glu86Lys, or Gly; Glu88Val, or Lys; Gly106Asp) and in *grlA* gene (Lys23Asn; Val41Gly; Arg43Cys; Ile45Met; Ala48Thr; Ser52Arg; Asp69Tyr; Gly78Cys; Ser80Phe, or Tyr; Ser81Pro; Glu84Lys, Leu, Val, Ala, Gly, or Tyr; His103Tyr; Ala116Glu, or Pro; Pro157Leu; Ala176Thr, or Gly; Asn327Lys). In *S. pneumoniae*, mutations have been described in *gyrA* gene (Ser81Phe, Tyr, or Cys; Ser84Phe, or Tyr; Glu85Lys, Gln, or Gly) and in *parC* gene (Ala63Tyr; Asp78Asn; Ser79Phe, Tyr, or Ala; Asp83Asn, Gly, or Val; Asp85Gly; Lys93Glu; Arg95Cys; His102Tyr; Ala115Pro, or Val; Tyr129Ser; Lys137Asn). In *E. faecalis*, resistance associated mutations have been found in *gyrA* gene (Ser83Arg, Ile, or Asn; Glu87Lys, Gly) and in *parC* gene (Ser80Arg, or Ile; Glu84Ala). In *E. faecium*, described mutations concern *gyrA* gene (Ser83Ala, Leu, Ile, Tyr, or Arg; Glu87Leu, Gly, or Lys) and *parC* gene (Ser80Ile, or Arg; Glu84Lys, or Thr, Ser97Asn). In *S. pyogenes*, mutations in *gyrA* gene (Ser81Phe; Met99Leu) and in *parC* gene (Ser79Tyr) have been detected. The effect of the mutations is production of topoisomerase proteins that are resistant or less sensitive to fluoroquinolones action.

Another mechanism of resistance to fluoroquinolones depends on increased production of chromosome-encoded proteins NorA, NorB and NorC in *S. aureus*, PmrA in *S. pneumoniae* and EmeA in *E. faecalis* that are responsible for active removing (efflux) of fluoroquinolones from the bacterial cell [55, 57].

TETRACYCLINES

Criteria of resistance to tetracyclines are presented in Table **1**. In *S. aureus*, the resistance to tetracyclines can be due to: (1) active efflux by Tet(K), Tet(L), Tet38, and MetA (MATE) proteins, (2) detachment of antibiotic from 30S ribosomal subunit (ribosomal protection) by proteins Tet(M), Tet(O), and Tet(S) or (3) tetracycline modification by NADP-dependent oxidoreductase Tet(U) [1, 11, 42, 57, 58, 59]. The most common are *tet(K)* genes (that do not cause efflux of minocycline and tigecycline) and *tet(M)* (resistance to tetracyclines except tigecycline). The *tet(K)* genes can be found in small plasmids (ok. 4.5 kb), e.g. pT181 (occurs also in SCCmec III), pT127, and pBC16. The *tet(M)* gene occurs in the chromosome (e.g. Tn*5801*). Plasmid genes *tet(S)* and *tet(U)* are characteristic for VRSA [1, 11]. MetA and TetM overproduction can lead to decreased sensitivity or even resistance to tigecycline [59] In *S. pneumoniae* theTet(M) protein encoded by genes situated in transposons is most important [44]. Tet(K) and Tet(L) proteins causing efflux and Tet(M), Tet(O), and Tet(T) proteins responsible for ribosomal protection occur in *S. pyogenes* [42, 60, 61]. In *S. agalactiae* resistance to tetracyclines is caused by Tet(M) and Tet(O) proteins, responsible for ribosomal protection. Resistance to tetracyclines in *S. agalactiae* strains isolated from humans is relatively common (60-100%), while in animal strains the resistance is below 20%. In *E. faecalis* Tet(K) and Tet(L) proteins responsible for efflux and Tet(M) and Tet(S) proteins connected with ribosomal protection can be found. In *E. faecium* besides Tet(K) (efflux), Tet(M) and Tet(S) (ribosomal protection) a third mechanism of tetracycline resistance was described. It depends on inactivation of antibiotic by NADH-dependent oxidoreductase Tet(U) [31, 42].

AMINOGLYCOSIDES

Streptococci and enterococci possess intrinsic resistance to aminoglycosides due to the lack of the antibiotics transport into the bacterial cell. The criteria of resistance to aminoglycosides for *S. aureus* are presented in Table **1**.

The most common mechanism of resistance to aminoglycosides in *S. aureus* is associated with synthesis of transferases AAC(6')-Ie/APH(2")-Ia (resistance to gentamicin, tobramycin, kanamycin and often to amikacin and netilmicin), ANT(4')-Ia (resistance to tobramycin, kanamycin, neomycin, lividomycin), APH(3")-IIIa (resistance to kanamycin, neomycin, lividomycin) and APH(3')-III (resistance to kanamycin, neomycin) encoded by gene *aphA-3* (*aph(3')-IIIa)* occurring in Tn*3851*, Tn*4031* and Tn*5404* [1, 11].

The genes *aacA-aphD* encoding acetyl/phosphotransferase AAC(6')-Ie/APH(2")-Ia can be found in transposons (e.g. Tn*4001*, Tn*4001*-like) localized in large plasmids and in chromosome (e.g. SCC*mec* IVc). Some of Tn*4001*-like transposons keep the promoter of beta-lactamase operon (reduced *blaZ*), which can cause strong induction of resistance to aminoglycosides by beta-lactam antibiotics and antagonism of beta-lactams towards aminoglycosides (e.g. netilmicin) [62]. Some of the point mutations in *aacA-aphD* gene can increase the spectrum of resistance AAC(6')-Ie/APH(2")-Ia e.g. to arbekacin. Gene *aadD* encoding nucleotidyltransferase ANT(4')-Ia can be found in small plasmids (pUB110) as well as in large e.g. conjugative plasmids. The pUB110 copy occurs in some of the SCC*mec*. The *aph(3")-IIIa* gene encoding phosphotransferase APH(3")-IIIa the most often can be found in plasmids. High MIC >1024 mg L^{-1} for lividomycin allows distinguishing phenotypically the gene from others [62].

S. aureus can synthesize transferases, e.g. ANT(6), encoded by *ant(6)* gene in pS194 plasmid, or *ant(6)-Ia* (*aadE*), inactivating streptomycin. Resistance to streptomycin can also be a result of mutation in the gene encoding ribosomal protein S12. Decreased sensitivity or resistance to aminoglycosides can be due to disrupted transport of the antibiotic into the bacterial cell. This type of resistance occurs the most frequently in SCV *S. aureus* (small colony variant) and is caused by mutations in genes encoding transport enzymes[1, 11].

In Enterococci, detection of the high-level aminoglycoside resistance (HLAR) is important (MIC of gentamicin to 8000 mg L^{-1} or streptomycin MIC to 32000 mg L^{-1}). In the absence of HLAR, aminoglycosides can be used in associated therapy with other antibiotics. HLAR- type resistance to gentamicin in *Enterococcus* spp. is due to an enzyme possessing two functions: acetyltransferase/phosphotransferase AAC(6')-Ie-APH(2")-Ia encoded by Tn*5281*-like and phosphotransferase APH(2")-Ib, APH(2")-Ic, APH(2")-Id, or APH(2")-Ie. High-level resistance to amikacin can be caused by nucleotidyltransferase ANT(4')-Ia and phosphotransferase APH(3')-IIIa and HLAR to netilmicin by acetyltransferase AAC(6')-Ii. High level resistance to streptomycin (HLSR) can be en effect of mutation in the genes encoding ribosomal protein S12 or transferase ANT(6')-Ia (*aadE*), AAD(6'), ANT(3")-Ia [1, 11, 31, 63].

RESISTANCE TO OTHER ANTIBIOTICS

Lipopeptides

Daptomycin is a cyclic lipopeptide with bactericidal action on staphylococci and streptococci, including methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE). MIC_{90} of daptomycin against MRSA = 0.5 mg L^{-1}, against vancomycin resistant *E. faecalis* (VR *E. faecalis* = 1.0 mg L^{-1}, VR *E. faecium*: = 4.0 mg L^{-1}. Daptomycin becomes inserted into the bacterial plasma membrane in a calcium-dependent fashion, leading to membrane depolarisation, release of intracellular potassium ions, and rapid cell death [64] The resistance to daptomycin depends on mutations leading to reducing of daptomycin binding to the target place in bacteria or mutations in various genes (e.g. *mprF*) that encode the lysylphosphatidylglycerol synthetase enzyme involved in the synthesis of phosphatidylglicerol. The described resistance in *S. aureus* (MIC 8-32 mg L^{-1}) possibly was a result of the loss of an 81-kDa-membrane protein interacting with daptomycin. Vancomycin intermediate *S. aureus* (VISA) display a reduced susceptibility to daptomycin probably as a result of the thickened cell wall [65, 66].

Phenicols

Resistance to chloramphenicol can be due to the production of chloramphenicol acetyltransferase (CAT) encoded by plasmids or transposons. In *S. aureus* CAT usually is encoded by small plasmids. Other mechanism of resistance described in *S. aureus* is associated with synthesis of methyltransferase 23S rRNA, Cfr, the enzyme described in chapter "resistance to linezolid".

Ansamycines

The most common cause of resistance to rifampicin is a point mutation in the *rpoB* gene encoding B subunit of RNA polymerase.

Diaminopyrimidynes

Trimetoprim and iclaprim, two 2,4 diaminopyrimidynes are inhibitors of bacterial dihydrofolate reductase. Resistance to trimethoprim in staphylococci is usually due to the production of additional, trimethoprim insensitive dihydrofolate reductases (DHFRs), usually encoded by plasmid genes. In *S. aureus*, S1DHFR encoded by *dfrA* (Tn*4003*) gene, S3DHFR encoded by *dfrG* gene [67] and a new DHFR encoded by *dfrK* gene localized in 40 kb plasmid pKKS2187 [58] were described. In *S. haemolyticus* S2DHFR encoded by *dfrD* gene in pABU17 plasmid was described [58]. *S. pneumoniae* resistant to trimetoprim possess mutations in one *dfr* gene (e.g. E20D, I100L, L135F)

The second 2,4 diaminopyrimidyne recently introduced was iclaprim. It is active against numerous Gram-positive bacteria (*S. aureus*, *S. pneumoniae*, and *S. pyogenes*). In *S. aureus*, it is active against trimetoprim resistant strains. In *S. pneumonie*, activity of iclaprim against trimetoprim resistant strains is considerably reduced (MIC_{90} =0.06 mg L^{-1} for trimetoprim sensitive vs. $MIC_{90} \geq 8$ mg L^{-1} for trimetoprim resistant *S. pneumoniae*) [68].

Pseudomonic Acids

In *S. aureus*, the cause of the resistance to mupirocin is a production of modified isoleucyl t-RNA synthetase. The enzymes modified by chromosomal *ileS* gene mutations cause only diminished sensitivity, whereas resistant enzymes encoded by plasmids (e.g. *ileS* located in plasmids pGO400, pJ2947 or pUSA03) determine full resistance, usually with MIC > 500 mg L^{-1} [1, 5].

Pleuromutilins

Recently introduced as a topical antibiotic, retapamulin acts against certain Gram-positive bacteria, including MRSA. Resistance is caused by Cfr 23S rRNA methyltransferase [9].

Beta-Lactam and Glycopeptides

These most frequently used groups of antibiotics were discussed in details in the recently published first part of the paper [69].

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