Mechanisms of the Resistance and Tolerance to Beta-Lactam and Glycopeptide Antibiotics in Pathogenic Gram-Positive Cocci

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Abstract: Beta-lactams are the most frequently used antimicrobials in combating infections. In the case of gram-positive bacteria resistant to beta-lactams, glycopeptides are the first choice. The occurrence, mechanisms and genetic background of the resistance of pathogenic staphylococci, streptococci and enterococci to beta-lactam and glycopeptide antibiotics were discussed. The resistances to well-established antimicrobials, as well as new agents (ceftobiprole, oritavancin, telavancin, dalbavancin) were taken into consideration in the text.

Keywords: *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis, Enterococcus faecium*, antibiotic resistance, SCC*mec*, plasmids, transposons, MRSA, h-VISA, VISA, VRSA, VRE, PRSP.

CRITERIA AND MECHANISMS OF RESISTANCE TO ANTIMICROBIAL AGENTS IN BACTERIA

Among the most important aims of medical bacteriological laboratories is the performance of antimicrobial susceptibility tests. Most conventional tests determine the bacteriostatic concentrations of the investigated agent. The most frequent measure of activity is the MIC value. Generally, bacteria are recognized as resistant if they are able to multiply in the presence of therapeutic concentration of the agent. Specific breakpoints for individual agents are different for different bacterial species. The Clinical Laboratory Standards Institute (CLSI) in USA, European Committee on Antimicrobial Testing (EUCAST), or national committees in some countries regularly update the breakpoints. It is not uncommon for different countries to use different breakpoints for the same antibiotic and the same bacteria.

The criteria of the resistance to beta-lactam antibiotics based on CLSI 2008 [1] and EUCAST 2008 [2] are presented in Table **1**.

Demonstration of the susceptibility of bacterial strain to antimicrobial agent *in vitro* does not always guarantee the success of antimicrobial therapy. One of several possible reasons could be bacterial biofilm formation. Some authors suggest the application of a new method for determining bacterial susceptibility, named MBIC (minimum biofilm inhibitory concentration) [3]. The other phenomenon responsible for failure of antimicrobial therapy is tolerance. Several definitions for tolerance exist, in one bacteria are recognized as tolerant when the ratio of MBC (minimal bactericidal concentration) to MIC of the drug exceeds 32.

The problem of bacterial resistance has been increasing for a long time and has been the subject of many review papers [4-11]. Bacterial mechanisms of resistance were classified into four major groups:

1- The first group of mechanisms depends on bacterial target modification (protein, peptide or rRNA). This modification could be achieved by five mechanisms: (1.1) mutation in the gene encoding the target for antimicrobial e.g. mutations and/or inter or intraspecific recombinations in the genes encoding PBPs (penicillin binding proteins) in penicillin-resistant *Streptococcus pneumoniae* and viridans streptococci, and mutations in genes encoding topoisomerases II and IV determining resistance to quinolones in various bacteria; (1.2) production of specific enzymes which modify the target, e.g. 23S rRNA methylases which are able to determine the resistance to macrolides, azalides, lincosamides, streptogramins, oxazolidinones and chloramphenicol or 16S rRNA methylases which are able to determine resistance to aminoglycosides in Gram-negative bacteria; (1.3) synthesis of a new target, not susceptible to an antimicrobial agent e.g. transpeptidase PBP2a in methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase-negative staphylococci (MR-CNS), or complex systems of Van proteins determining resistance to glycopeptides in vancomycin resistant enterococci (VRE) as well as several strains of vancomycin resistant *S. aureus* (VRSA) recently described in USA; (1.4) increased synthesis of the normal target molecules, e.g. PBP5` in *Enterococcus faecium*; (1.5) protein protection: ribosomal protection determining resistance to tetracyclines in Gram-positive and Gram-negative bacteria or DNA gy-

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Table 1. The Most Common Resistance and Sensitivity in Gram–Positive cocci [2]

Bacteria	Susceptibility	Resistance
S. aureus	vancomycin, linezolid quinupristin/dalfopristin daptomycin, tigecycline	ceftazidime
Coagulase negative staphylococci (CNS)	vancomycin, linezolid quinupristin/dalfopristin daptomycin, tigecycline	ceftazidime
S. saprophyticus	vancomycin, linezolid quinupristin/dalfopristin daptomycin, tigecycline	fosfomycin novobiocin ceftazidime
S. cohnii	vancomycin, linezolid quinupristin/dalfopristin daptomycin, tigecycline	novobiocin ceftazidime
S. xylosus	vancomycin, linezolid quinupristin/dalfopristin daptomycin, tigecycline	novobiocin ceftazidime
S. capitis	vancomycin, linezolid quinupristin/dalfopristin daptomycin, tigecycline	fosfomycin ceftazidime
S. pneumoniae	imipenem, meropenem vancomycin, teicoplanin linezolid, daptomycin, quinupristin/dalfopristin tigecycline, rifampicin	fusidic acid aminoglycosides
Group A (e.g. *S. pyogenes*), B (e.g. *S. agalactiae*), C and G beta-haemolytic streptococci	penicillin, cephalosporins vancomycin, teicoplanin linezolid, daptomycin, quinupristin/dalfopristin tigecycline	fusidic acid aminoglycosides
S. agalactiae	as above	tetracycline > 60%
E. faecalis	linezolid daptomycin tigecycline penicillins (>90%)	fusidic acid, aminoglycosides[1] cephalosporins[2] quinupristin/dalfopristin clindamycin trimehoprim/sulphamethoxazole[3]
E. faecium	quinupristin/dalfopristin linezolid daptomycin tigecycline	fusidic acid, aminoglycosides[1] cephalosporins[2] trimehoprim/sulphamethoxazole[3] penicillins (>95%)
E. gallinarum *E. casseliflavus*	linezolid daptomycin tigecycline	fusidic acid, aminoglycosides[1] cephalosporins[2] quinupristin/dalfopristin clindamycin trimehoprim/sulphamethoxazole[3] vancomycin

[1]Additionally in some enterococcal strains high levels aminoglycoside resistance (HLAR, HLGR, HLSR) occur. Infections with HLAR strains are not susceptible to reatment with aminoglycosides in assotiation with penicillins or glycopeptides. [2] From the cephalosporins group only ceftobiprole can be active against the ampicillin –senisitive strains. [3]The resistance is clinical (the *in vitro* tests can be false negative).

rase protection determining resistance to quinolones in Gram-negative bacteria.

2- The second group of mechanisms depends on synthesis of enzymes hydrolyzing or inactivating antibacterial

drugs (beta-lactamases, erythromycin esterases, chloramphenicol and aminoglycoside transferases in Gram-positive and Gram-negative bacteria, macrolide, lincosamide or streptogramin A transferases, liases streptogramin A or B in Gram-positive bacteria and quinolone acetyltransferase (e.g. AAC(6')-Ib-cr) in Gram-negative bacteria). In Gram-positive bacteria most of the enzymes are secreted outside.

3- Impermeability of the drug or active efflux from the bacterial cell. Active efflux depends on the presence of specific energy-dependent pumps in bacterial membrane, responsible for efflux of antimicrobials. The best known are: ATP binding cassette superfamily (ABC), MFS (major facilitator superfamily, composed of 12 or 14 transmembrane segments; TMS), RND (resistance-nodulation-cell division superfamily, 12 TMS), SMR (small multidrug resistance superfamily, 10 TMS and 4 TMS) or Na^+/H^+ pump dependent: MATE (multidrug and toxic compound extrusion superfamily, 12 TMS). Active efflux is responsible for acquired tetracycline resistance in many species of bacteria as well as the resistance to quinupristin/dalfopristin seen in most *E. faecalis* strains.

4- Lack of penetration in to the bacterial cell determines natural low resistance to aminoglycosides in streptococci and enterococci.

5- Lack of transformation of pro-drug in to it`s active form (nitrofurans, metronidazole).

RESISTANCE TO BETA-LACTAM ANTIBIOTICS

The targets for beta-lactam antibiotics are specific enzymes (transpeptidases, transglycosylases, carboxypeptidases) taking part in synthesis of peptidoglycan, a key element of bacterial cell walls. The proteins inactivated by beta-lactams are specified as PBP (penicillin binding proteins). The inactivation of an important PBP leads to bacterial cell death.

In various bacteria, all four groups of mechanisms of resistance mentioned above were described for beta-lactams. In the case of target modification, two of the five possibilities were observed: (1) The modification of PBP proteins, caused by mutations only or by mutations and recombination of old and acquired genes encoding PBPs; (2) synthesis of new, additional PBP. Both play an important part in Gram-positive bacteria. Other mechanisms include: (3) synthesis of beta-lactamases hydrolyzing beta-lactam antibiotics (4) the lack of antibiotic penetration to the bacterial cell (5) active removing of the antibiotic from the cell. The last two mechanisms were not observed in Gram-positive cocci. The resistance to beta-lactams is important for clinicians in the case of *S. aureus* and in CNS (coagulase negative Staphylococci), *Enterococcus* spp., *S. pneumoniae* and *Streptococcus* spp. from viridans group. There is no described resistance to beta-lactams among beta-haemolysing Streptococci of Lancefield groups A (e.g. *S. pyogenes*), B (e.g. *S. agalactiae*), C, and G.

Staphylococci

The most important resistance found in *S. aureus* is to methicillin, caused by synthesis of new PBP2a protein. Methicillin resistant *S. aureus* (MRSA) are resistant to all beta-lactam antibiotics except new cephalosporin classified as V generation (ceftobiprole). Mutations in genes encoding PBP2 and PBP4 occur rarely and the strains are specified as MODSA (modified *S. aureus*). The most common mechanism is connected with production of a narrow substrate spectrum beta-lactamase (penicillinase), belonging to class A of Ambler's classification. Beta-latamases are frequently synthesized by MSSA (methicillin sensitive *S. aureus*), and by most of the MRSA strains. The enzymes are encoded by *blaI-blaR1-blaZ* operon, which can be found in many plasmids and transposons (e.g. pI258, pII147, Tn*552*, Tn*4002*, Tn*4201*). Staphylococcal beta-lactamases occur in four variants, types A-D [5]. Additionally, a so called BORSA (borderline oxacillin resistant *S. aureus*) strains that synthesize beta-lactamase with slightly extended spectrum and determining MIC of oxacillin in the range 4-8 mg L^{-1} were described. The frequency of occurrence of MRSA strains in different European countries, according to EARSS data from 2006 [12] is presented in Table **3**.

The action of beta-lactam antibiotics is closely associated with PBPs, enzymes taking part in the last stage of peptidoglycan synthesis. MSSA synthesize four PBPs, PBP1 (744 aa, dimerisation domain and transpeptidase domain), PBP2 (727 aa, carboxypeptidase, transglycosylase and transpeptidase domain), PBP3 (691 aa, dimerisation domain and transpeptidase domain) and PBP4 (491 aa, D-alanyl-D-alanine carboxypeptidase). PBP2 is a key *S. aureus* protein and inactivation of its transpeptidase function leads to bacterial cell death [13]. MRSA strains synthesize additionally PBP2a (PBP2A or PBP2'). PBP2a is a 668 aa protein that replace transpeptidase function of PBP2 and other inactivated PBP but not transglycosylase. Beta-lactam antibiotics inactivate PBP2 transpeptidase domain, whereas transglycosylase domain remains active and it cooperates with PBP2a transpeptidase in MRSA [14]. PBP2a acts together with about 40 other enzymes that take part in synthesis of pentaglycine bridges between L-lysine (3 pentapeptide position) of one chain and D-alanine (4 pentapeptide position) of the other peptidoglycan chain. The only accepted beta lactam inactivating PBP2a is ceftobiprole (MIC_{50} = 1, MIC_{90} = 2 for MRSA strains). PBP2a is expressed inducibly or constitutively [5]. It depends on the presence of the operon *mecI-mecR1-mecA* (inducible expression) or *ΔmecR1-mecA* (constitutive expression). Operons are localized in the specific genetic element, a cassette SCC*mec* (staphylococcal chromosomal cassette mec) situated in the chromosome of MRSA. SCC*mec* are rated among genomic islands (GI) [15]. The most important types of SCC*mec* are presented in Table **4** [16-24].

The *mec* genes are transferred to sensitive strains together with the whole SCC*mec* structure. The six main types of SCC*mec* were described and some subtypes inside the main types were specified in previous studies [16, 18, 19]. The described SCC*mec* (types I-VI) had a size between 20.9 and 66.9 kb (21 to 97 orf). The SCC*mec* are usually composed of 5 regions: J1-*ccr*-J2-*mec*-J3.

SCC*mec* types I, II, III, V and VI mainly occur in hospital MRSA strains whereas SCC*mec* IV in community acquired MRSA (CA-MRSA). CA-MRSA strains more often

produce the toxin Panton-Valentine leukocidin (PVL) (40-90% vs. <5% among other MRSA) [22].

The classification of SCC*mec* into main types is based on site-specific cassette chromosome recombinase type, $CcrAB_{1-4}$ (types 1-4), $CcrC_{1-9}$ (type 5) and a class of the *mec* region. The division of the *mec* region into classes depends on the presence of complete *mecI-mecR1* regulatory genes (classes A.1 and A.2) or occurrence of different sized deletions in *mecI-mecR1* (*ΔmecR1* in classes A.3, A.4, B.1, C.1, C.2, C.2b, D.1, E.1). Moreover, in the main classification of SCC*mec*, their neighborhood and distance from complete or reduced (*Δ,Ψ)*, insertion sequences IS*431*, IS*1182* and IS*1272* are taken into consideration [20].

The types are divided into subtypes on the basis of region *mec* subclasses (Table **4**) and the structure of all five regions. The J1 regions of various subtypes among others differ in the presence of the *pls* gene, encoding plasmin sensitive surface protein, and *kdpE* gene, encoding transcriptional regulatory protein. J2 regions differ in the presence of Tn*554* (Tn*554* contains *erm*(A) genes, determining resistance to macrolides, lincosamides and streptogramins B (MLS-B) and *spc* determining resistance to spectinomycin) and the presence of ΨTn*554* determining resistance to cadmium salts. J3 regions differ in the presence of the *hsdM* gene, encoding type I restriction-modification system DNA methylase, downstream constant region (dcs), and the presence of plasmids pUB110 containing *ant(4')* gene, determining resistance to aminoglycosides and *ble* gene determining resistance to bleomycin, the presence of pT181 containing *tet*(K) gene determining resistance to tetracyclines, the presence of pI258 (possible resistance to MLS-B and beta-lactamase production), and the presence of transposons Tn*554* and Tn*4001* (containing (*aac(6')/aph(2")* gene responsible for resistance to aminoglycosides) [21].

Recently, several SCC*mec* with two different *ccr* genes (e.g. *ccrC* and *ccrA3/ccrB3*) were described [23, 24]. Mixed cassettes are a result of recombination between SCC*mec* type III and V.

Penicillin Resistant *S. Pneumoniae* (PRSP)

Resistance of *S. pneumoniae* to penicillin, amoxicillin, and cephalosporins are determined by changes in PBP1a proteins (358 aa, transpeptidase domain), PBP2b (458 aa, transpeptidase domain and dimerisation domain), and PBP2x (578 aa, transpeptidase domain and dimerisation domain). In most European countries, PRSP strains occur with a frequency <1% to 10% (Table **3**) [12]. *S. pneumoniae* is a bacterium that easily transforms foreign DNA in natural conditions. A new DNA (usually originated from oral streptococci) recombines with the homological regions of the bacterial chromosome, and the process leads to extensive changes in the sequences within many genes including that conferring cell wall synthesis. It concerns *pbp1a*, *pbp2b* and *pbp2x* genes. In streptococci, changes connected with recombination can exceed 20% of genes sequence.

Enterococcus

Enterococci are intrinsically resistant to cephalosporins except ceftobiprole (MIC_{50} of ceftobiprole for *Enterococcus faecalis* = 0.5 mg L^{-1} and MIC_{90} = 2 mg L^{-1}). Resistance to penicillins and carbapenems is due to overproduction of PBP5 and/or mutation in the gene encoding PBP5. Resistance to penicillin, ampicillin, carbapenems, and ceftobiprole occurs much more frequently in *E. faecium* (67-100% of strains isolated from human sources) than in *E. faecalis* (1-10% of strains) (Table **3**) [12]. In *E. faecium*, the *pbp5* mutations such as Met485Ala/Thr, Ala499Thr, Glu629Val and Ser insertion after position 466 were described. In *E. faecium* producing six PBPs the mutation in the *pbp5* gene results in resistance to imipenem and sensitivity to ampicillin at the same time [25]. Recently, a low-affinity PBP encoding gene was found in plasmids in *Enterococcus hirae*. Moreover, transfer of PBP5 encoding gene associated with Tn*5382* in *E. faecium* was observed. Some *E. faecalis* strains produce narrow-spectrum beta-lactamase, similar to beta-lactamase A produced by *S. aureus* [5].

TOLERANCE TO BETA-LACTAM ANTIBIOTICS

Several mechanisms of the tolerance to beta-lactams are postulated. The most common is the reduced autolytic activity. In laboratory conditions it was shown that diminished amounts of N-acetylmuramic acid-L-alanine amidase was produced in the case of *S. pneumoniae* whereas in the case of *E. faecium* production of muramidase was reduced. Tolerance to aminopenicillins can occur in about 10-20% of enterococci that are poor biofilm producers and in 93-100% of intensively producing biofilm strains [3]. Biofilm is produced by 57-100% of *E. faecalis* and 16-48% of *E. faecium* strains [26].

Among *S. pyogenes*, isolated cases of strains tolerant to penicillin were described [27, 28]. The altered PBPs are most probably responsible for this occurrence. About 2% of *S. pyogenes* is moderately penicillin-tolerant (MBC/MIC = 16) [29]. There are no described cases of resistance.

RESISTANCE TO GLYCOPEPTIDES

Resistance to glycopeptides with clinical importance occurs in enterococci and staphylococci [6, 30-32]. Laboratory criteria of sensitivity to glycopeptides in these bacteria according to [1, 2] are presented in Table **2**.

Mechanism of action of glycopeptides is connected with binding to the peptidoglycan precursor -N-acetylglucosamine-N-acetylmuramic acid–L-Ala-D-Glu-L-Lys-D-Ala-D-Ala. The process takes place outside the cytoplasmic membrane. Vancomycin forms 5 hydrogen bonds with D-Ala-D-Ala of this precursor.

Enterococci

Resistant bacteria synthesize changed precursors that instead of D-Ala-D-Ala are terminated with D-Ala-D-lactate or D-Ala-D-serine. The synthesis of D-lactate - terminated precursors is determined by operons VanA, VanB, or VanD. For the expression of resistance, the presence of three enzymes is needed: dehydrogenase $VanH_{A, B}$ or $_{D}$, that catalyse synthesis of D-lactate from pyruvate acid, ligases VanA, VanB or VanD that catalyse the formation of an ester bond between D-alanine and D-lactate, and D, D dipeptidase $VanX_{A, B}$ or $_{D}$, that hydrolyse the dipeptide D-Ala-D-Ala synthesized by the bacterial cell.

Table 2. Criteria of the Resistance to Beta-Lactam Antibiotics and Glycopeptides in Gram-Positive Cocci

Bacteria	Drugs	CLSI (2008) MIC* (mg L^{-1})			EUCAST (2008) MIC* (mg L^{-1})		
		S	I	R	S	I	R
S. aureus	Penicillin	≤0.12	nd	≥0.25	≤0.12		>0.12
	Oxacillin**	≤2	nd	≥4	nd	nd	>2
	Cefoxitin**	≤4	nd	≥8	nd	nd	>4
	Ampicillin	≤0.25	nd	≥0.5	nd	nd	nd
	Vancomycin	≤2	4-8	≥16	≤4	8	>8
	Teicoplanin	≤8	16	≥32	≤4	8	>8
CNS***	Oxacillin	≤0.25	nd	≥0.5	nd	nd	>0.25
Enterococcus spp.	Penicillin	≤8	nd	≥16	nd	nd	nd
	Ampicillin	≤8	nd	≥16	≤4	8	>8
	Vancomycin	≤4	8-16	≥32	≤4	8	>8
	Teicoplanin	≤8	16	≥32	≤4	8	>8
S. pneumoniae	Penicillin parenteral (nonmeningitis)	≤2	4	≥8	≤0.06	0.12-2	>2
	Cefotaxime/ceftriaxone (nonmeningitis)	≤1	2	≥4	≤0.5	nd	>2
	Penicillin parenteral (meningitis)	≤0.06	nd	≥0.12	≤0.06	0.12-2	>2
	Cefotaxime/ceftriaxone (meningitis)	≤0.5	1	≥2	≤0.5	nd	>2
	Penicillin (oral penicillin V)	≤0.06	0.12-1	≥2	≤0.06	0.12-2	>2
	Vancomycin	≤1	nd	nd	≤4	nd	>4
S. pyogenes *S.agalactiae*	Penicillin	≤0.12	nd	nd	≤0.25	nd	>0.25
	Ampicillin	≤0.25	nd	nd	nd	nd	nd
	Vancomycin	≤1	nd	nd	≤4	nd	>4

Abbreviations: S, susceptible; I, intermediate; R, resistant; nd, no data. * MIC values are always determined using twofold concentrations of antibiotic ***S. aureus* and *S.lugdunensis*
***except *S.lugdunensis*

Table 3. Antimicrobial Resistance in Chosen European Countries

Country	1999-2005				2006			
	% MRSA	% VREfl	% VREfm	% PRSP	% MRSA	% VREfl	% VREfm	% PRSP
Austria	8-18	<1	<1-7	<1-2	9	<1	<1	<1
Belgium	21-33	<1	<1-14	<1-5	22	<1	4	4
Bulgaria	24-37	<1-2	<1	6-30	28	2	<1	7
Croatia	32-38	<1-3	<1-22	<1-3	36	<1	3	<1
Cyprus	49-64	<1-3	<1-40	<1-31	32	<1	14	31
Czech Republic	4-13	<1-2	2-14	<1-2	12	<1	4	<1

Table 3. Contd….

Country	1999-2005				2006			
	% MRSA	% VREfl	% VREfm	% PRSP	% MRSA	% VREfl	% VREfm	% PRSP
Denmark	<1-2	0	0	<1	2	<1	<1	<1
Estonia	1-5	<1	<1	<1	3	<1	<1	<1
Finland	<1-3	<1	<1-1	<1-2	3	<1	<1	2
France	27-33	<1	<1-5	5-11	27	<1	3	4
Germany	8-21	<1	<1-11	<1-1	20	<1	8	1
Greece	31-50	<1-13	<1-37	-	43	5	42	-
Hungary	5-20	<1	<1	<1-8	25	<1	<1	1
Iceland	<1-3	<1	<1	<1-2	<1	<1	<1	<1
Ireland	39-42	<1-3	11-31	2-5	42	3	36	3
Israel	38-43	<1-1	8-46	5-11	39	<1	28	6
Italy	37-44	<1-3	15-24	<1-5	38	3	18	<1
Latvia	20-25	nd	nd	<1	18	<1	<1	<1
Lithuania	nd	nd	nd	nd	12	<1	<1	<1
Luxembourg	13-21	<1	<1	<1-11	19	<1	<1	5
Malta	36-56	<1	<1	<1-8	67	<1	<1	3
Netherlands	<1-1	<1	<1-2	<1	1	<1	<1	<1
Norway	<1	<1-3	<1	<1	<1	<1	<1	<1
Poland	15-24	<1	<1-5	<1-30	20	<1	<1	<1
Portugal	25-47	3-6	<1-47	<1	48	5	26	<1
Romania	36-72	<1	<1-17	10-23	55	<1	<1	10
Slovenia	10-21	<1	<1	<1-2	7	<1	6	5
Spain	23-28	<1	1-3	7-11	25	<1	3	8
Sweden	<1-1	<1	<1-2	<1	<1	<1	<1	<1
Turkey	35-43	<1-1	3-5	<1-7	36	<1	4	5
UK	33-44	2	33	<1-4	42	1	18	<1

MRSA, methicillin resistant *S. aureus*; VREfl, vancomycin resistant *E. faecalis*; VREfc, vancomycin resistant *E. faecium*; PRSP, penicillin resistant *S. pneumoniae* (resistant if MIC ≥2 mg L^{-1}); nd, no data.

Table 4. The Scheme of the Genomic Organization of SCC*mec* in *Staphylococcus Aureus*

SCC*mec* Type		Size (kb)	Genotype	Strains**
New*	Old			
1B.1.1	I	~34	J1(*pls*)-*ccrA1/B1*-J2-mecB.1-J3(dcs)	NCTC 10422, COL
1B.1.2	IA		J1*(pls)*-*ccrA1/B1*-J2-mecB.1-J3*(*dcs, pUB110)	PER34, HPV1007

Table 4. Contd…

SCC*mec* type		size (kb)	Genotype	Strains**
new*	old			
2A.1	II	~57	J1*(kdpCBE)*-*ccrA2/B2*-J2(Tn*554*)-mecA.1-J3(pUB110, dcs)	N315
2A.1.1	IIa	~57	J1*(kdpCBE)*-*ccrA2/B2*-J2(Tn*554*)-mecA.1-J3*(* pUB110, dcs)	Mu50, MRSA252
2A.1.2	IIb		J1(IIb)-*ccrA2/B2*-J2(Tn*554*)-mecA.1-J3(pUB110, dcs)	
2A.4.1	IIA	~40	J1(IVb)-*ccrA2/B2*-J2(Tn*554)*-mecA.4 -J3(pUB110, dcs)	AR14/0298
2A.1.3.2	IIB	~31	J1(IVb)-*ccrA2/B2*-J2-mecA.1-J3(pUB110, dcs)	
2A.3.3.3	IIC	~33	J1(IVb)-*ccrA2/B2*-J2(Tn*554*)-mecA.3-J3(pUB110-IS*431*-dcs)	
2A.4.4	IID	~33	J1(IVb)-*ccrA2/B2*-J2(Tn*554*)- mecA.4-J3*(*dcs)	AR13/3635.2
2A.3.5	IIE	27	J1(IVb)-*ccrA2/B2*-J2(Tn*554*)- mecA.3-J3(dcs*)*	AR13.1/3320 .2
2B.1.1	IVa	~24	J1(IVa)-*ccrA2/B2*-J2-mecB.1-J3(dcs)	CA05, JCSC:(4744, 1469, 2167), MW2
2B.1.2.1	IVb	~21	J1(IVb)-*ccrA2/B2*-J2-mecB.1- J3(dcs)	8/6-3P(JCSC1978)
2B.1.2.2	IVf	~23	J1(IVb)-*ccrA2/B2*-J2-mecB.1- J3	
2B.1.3.1	IVc	~25	J1(IVc)-*ccrA2/B2*-J2-mecB.1-J3*(Δdcs*, Tn*4001*)	81/108
2B.1.3.2	IVc		J1(IVc)-*ccrA2/B2*-J2-mecB.1-J3(dcs)	2314
2B.1.3.3	IVe	~23	J1(IVc)-*ccrA2/B2*-J2-mecB.1- J3	AR43/3330.1
2B.1.4	IVd		J1(IVd)-*ccrA2/B2*-J2-mecB.1- J3(dcs)	JCSC4469
2B.1.5	IVg		J1(IVg)-*ccrA2/B2*-J2-mecB.1- J3(dcs)	M03-68
2B.1.6	IVh		J1(IVh)-*ccrA2/B2*-J2-mecB.1- J3(dcs)	HGSA: (157, 158, 163, 168), HAR22
3A.1.1	III	~67	J1-*ccrA3/B3*-J2(ΨTn*554*)-mecA.1-J3(pT181, ΔpI258, Tn*554*)	85/2082, 85/3907
3A.1.2	IIIA		J1-*ccrA3/B3*-J2(ΨTn*554*)-mecA.1-J3(ΔpI258, Tn*554*)	HU25
3A.1.3	IIIB		J1-*ccrA3/B3*-J2(ΨTn*554*)-mecA.1-J3	HDG3
3A.1.4	IIIC		J1-*ccrA3/B3*-J2(ΨTn*554*)-mecA.1-J3(pT181)	
4A.1.1	VI	32	J1-*ccrA4/B4*-J2-mecA.1-J3	BK20781
4B.1.1	VI		J1-*ccrA4/B4*-J2-mecB.1- J3(dcs)	HDE288
5_1C.1.1	V	~33	J1*(hsdR)*-*ccrC1*-J2-mecC.1-J3*(hsdM)*	JCSC6082
5_1C.2.1	V	~29	J1*(hsdMSR)*-J2-*ccrC1*-mecC.2-J3	JSGH17, WBG8318
$5_{2/8}$C.2b.1	V***	41	*J1*-ccrC2-*J2*-*mecC2b* –ccrC8-*J3*	ST59

*In SCC*mec* type new nomenclature the class and subclass of SCC*mec* were involved. **complete or partial sequences in GenBank. *** originally authors classified it as SCC*mec* typeVII**.** Abbreviations: J (junkyard) region; *ccr* genes, *ccrA1* and *ccrB1* (in SCC*mec* type I), *ccrA2* and *ccrB2* (in SCC*mec* type II and IV), *ccrA3* and *ccrB3* (in SCC*mec* type III), *ccrA4* and *ccrB4* (in SCC*mec* type VI) and *ccrC* (in SCC*mec* type V); classes of *mec* complexes, mecA.1 (*mecI*-*mecR1*-*mecA*-IS*431mec*), mecA.2 (IS*431*-*mecI*-*mecR1*-*mecA*-IS*431mec*), mecA.3 (IS*1182*-*ΔmecI*-*mecR1*-*mecA*-IS*431mec*), mecA.4 (*ΔmecI*-IS*1182*-*ΔmecI*-*mecR1*-*mecA*-IS*431mec*), mecB.1 (ΔIS*1272*-*ΔmecR1*-*mecA*-IS*431mec*), mecC.1 (IS*431*-*ΔmecR1*-*mecA*-IS*431mec*), mecC.2 (IS*431*-*ΔΔmecR1*-*mecA*-IS*431mec*), mecC.2b (ΔIS*431*-*ΔmecR1*-*mecA*-IS*431mec*), mecD.1 (*ΔmecR1*-*mecA*-IS*431mec*), mecE.1 (*ΔΔmecR1*-*mecA*-IS*431mec*); Other abreviations in text;

The operon VanA (*vanR*$_A$*S*$_A$*H*$_A$*AX*$_A$*Y*$_A$*Z*$_A$) is situated in Tn*1546* and demonstrate inducible expression controlled by two regulatory genes *vanR*$_A$ (regulator) and *vanS*$_A$ (sensor, signal histidine kinase localized in the cytoplasmic membrane). Activation of VanS$_A$ sensor is caused by both vancomycin and teicoplanin. Polymorphisms in the central *vanRSHA* region of Tn*1546* were scarcely detected, while alterations upstream of *vanR* and downstream of *vanA* were frequently identified involving mutations (*vanS* and *vanX*), deletions (*vanY*), insertions: IS*1216V* (14 types), IS*Ef1* (six types, located within *vanX*-*vanY* region at nucleotide 9044), and IS*19* [33]. VRE strains that possess VanA operon are resistant to vancomycin, teicoplanin and dalbavancin but remain sensitive to the new glycopeptides telavancin (MIC 4-8 mg L^{-1}) and oritavancin (MIC 1-4 mg L^{-1}) [6].

Operons VanB (*vanR*$_B$*S*$_B$*Y*$_B$*W*$_B$*H*$_B$*BX*$_B$) and VanB2 (*vanR*$_B$*S*$_B$-*ISEnfa200*-*Y*$_B$*W*$_B$*H*$_B$*BX*$_B$) demonstrate inducible expression and are controlled by two regulatory genes: *vanR*$_B$ (regulator) and *vanS*$_B$ (sensor). Activation of sensor VanS$_B$ is caused only by vancomycin (teicoplanin doesn't induce resistance). The operon VanD (*vanR*$_D$*Y*$_D$*H*$_D$*DX*$_D$) can be found in the chromosome and is expressed constitutively (inactive gene *vanS*$_D$) [6, 30]. VRE strains possessing VanB operon are resistant to vancomycin but remain sensitive to teicoplanin and new glycopeptides: dalbavancin (MIC=1 mg L^{-1}) and oritavancin (MIC=0.25 mg L^{-1}) [6].

The synthesis of the D-serine - ended precursors is determined by operons VanC, VanE, VanG and VanL and is associated with resistance to vancomycin but not to teicoplanin and new agents. Three enzymes are needed for resistance: serine racemase VanT$_{C,E,G\ or\ L}$ that catalyses conversion of L-serine to D-serine, ligase VanC, VanE, VanG or VanL catalysing D- alanine -D-serine synthesis and two domain D,D-dipeptidase/carboxypeptidase VanXY hydrolyzing peptide D-Ala-D-Ala. The operons VanC (*vanS*$_C$*R*$_C$*T*$_C$*XY*$_C$*C)* are localized in chromosomes of *E. gallinarum* (*vanC-1*), *E. casseliflavus* (*vanC-2* and *vanC-4*), and *E. flavescens* (*vanC-3*), and are expressed constitutively or are inducible. The operon is responsible for specific resistance or decreased sensitivity to vancomycin that is not transmittable between species. Operons VanE (*vanEXY*$_E$*T*$_E$*R*$_E$*S*$_E$*)* and VanG (*vanU*$_G$*R*$_G$*S*$_G$*Y*$_G$*W*$_G$*GXY*$_G$*T*$_G$) are situated in the chromosome and demonstrate inducible expression. VanG can be transmitted to other species whereas VanE cannot [30]. The operon VanL (*vanLXY*$_L$*Tm*$_L$*Tr*$_L$*R*$_L$*S*$_L$-IS*Enfa364*) was found in a chromosome of *E. faecalis* strain N06-0364 [34]. Mechanisms of resistance to glycopeptides are presented in Table **5**. The frequency of resistance to glycopeptides among enterococci in Europe is presented in Table **3**.

Resistance to Glycopeptides in *S. aureus*

Strains VRSA (vancomycin resistant *S. aureus*) and much more frequent VISA or GISA (vancomycin or glycopeptide intermediate *S. aureus*) are another serious problem. GISA do not possess genes *van*, known in enterococci, their level of resistance is low (MIC 4 – 8 mg L^{-1}), and mechanism is not resolved. These strains and their precursors are discussed below. A few strains isolated in the USA and in Asia are true VRSA which acquired the *vanA* genes from enterococci [36, 46]. Presently seven strains of VRSA have been described in the USA. The first two of them were described in 2002 and both possessed Tn*1546*, the element carrying operon *vanA* [37]. Michigan strain (MI-VRSA) was resistant to high concentrations of vancomycin and teicoplanin, whereas the second one from Pennsylvania (PE-VRSA) was resistant to vancomycin only. It turned out that the genes, responsible for resistance to vancomycin in MI-VRSA demonstrate considerable stability, while in case of the PE-VRSA strain, the genes were easily lost [35]. MI-VRSA strain has a 7.9 kb plasmid, a derivative of a plasmid similar to pSK41, with a Tn*1546*-like insertion [36, 37]. In 2004, another VRSA strain was isolated in New York. The NY strain presented the lowest resistance and it was shown that VRSA could be easily missed by routine antibiotics sensitivity tests, especially automated systems [47]. On the basis of molecular typing (MLST, multilocus sequence typing) it was found that all VRSA strains belong to the same clonal complex ST5. Moreover, the mutation in *hsdR* gene encoding Sau1 (mod/res, modification -restriction system 1) was found in all VRSA [36]. In some *S. aureus* strains possessing the *vanA* gene (independently of it's expression) other resistance genes probably acquired from *E. faecium* were detected: *erm*(B) (resistance to MLS-B*), aadE (ant(6)-Ia)* (resistance to streptomycin), *sat4* (resistance to streptothricin), *aphA-3* (*aph(3')-IIIa* - resistance to aminoglycosides), *msrA* (the efflux of macrolides and streptogramin B), *aac(6')-aph(2")-Ia* (resistance to aminoglycosides) and *tet*(S) and *tet*(U), (resistance to tetracyclines) [37]. VRSA strains isolated to date are sensitive to the new glycopeptides telavancin and oritavancin, but were resistant to dalbavancin [6].

As mentioned above, the failures of glycopeptide therapy are much more frequently concerned with the *S. aureus* strains that do not posses the *van* genes. Although bacterial resistance is not the only reason for the treatments failure, the role of strains with low level of resistance should be considered. Low-level resistance to glycopeptides is typical for VISA/GISA and their putative heteroresistant precursors h-GISA (h-VISA, hetero VRSA, VHRSA). MIC of teicoplanin for heteroresistant strains is ≤ 4 mg L^{-1} with subpopulations growing in concentrations > 4 mg L^{-1}, MIC of vancomycin is < 4 mg L^{-1} and subpopulations grow at 4-8 mg L^{-1} of vancomycin. There are several proposed mechanisms to explain the decreased susceptibility. The most common hypothesis is that the drug is trapped by increased amounts of peptidoglycan precursors and thickened cell walls. But other changes in cell walls were observed among VISA strains: decreased muropeptide amidation and cross-linking, increased PBP2 and PBP2a, and decreased PBP4 and increased purine biosynthesis. In laboratory strains, mutations in genes *sigB* (sigma factor), *ddh* (D-lactate dehydrogenase), *tca* (bicyclomycin and teicoplanin resistance protein), *vraR* (long-chain-fatty-acid – CoA ligase), *agr* and *sar* (global regulatory systems) [48] were described.

The new glycopeptides have diverse activity on VISA strains. MIC of oritavancin was =1-8 mg L^{-1}, telavancin =2 mg L^{-1} and dalbavancin = 2 mg L^{-1} [49].

Table 5. Mechanisms of Resistance to Glycopeptides in *S. aureus* and Enerococci

Bacteria	Type	Mechanism	MIC (mg L^{-1})		Localization	Reference
			vancomycin	teicoplanin		
S. aureus	VanA	D-Ala-D-Lac	≥256-<4	≥32	Tn1546	[35-37]
	VISA	mutation in *sigB, ddh, tca, vraR, agr,sarA* and other	4-8	8-24	chr	[6]
E. faecalis	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546*,	[30]
	VanB-1	D-Ala-D-Lac	32-≥256	0.125-0.19	chr, p,Tn*1547*,	[38]
	VanB-2	D-Ala-D-Lac	24-≥256	0.094-0.25	Tn*5382*, Tn*1549*	[38]
	VanB-3	D-Ala-D-Lac	≥256	0.25	chr	[38]
	VanD	D-Ala-D-Lac	64-128	4-64	chr	[30]
	VanE	D-Ala-D-Ser	8-32	0.5	chr	[40, 41]
	VanG-1	D-Ala-D-Ser	16	0.5	chr	[42, 43]
	VanG-2	D-Ala-D-Ser	16	0.5	chr	[42]
	VanL	D-Ala-D-Ser	8		chr	[42]
E. faecium	VanA	D-Ala-D-Lac	64-1024	4*-512	Tn*1546*,	[30]
	VanB-1	D-Ala-D-Lac	≥256	0.064-1		[38]
	VanB-2	D-Ala-D-Lac	≤4-≥256	0.094-0,38	Tn*5382*, Tn*1549*	[38]
	VanD	D-Ala-D-Lac	64-128	4-64	chr	[44]
E.gallinarum	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546*,	[6, 30]
	VanB-2	D-Ala-D-Lac	32	0.25		[38]
	VanC-1	D-Ala-D-Ser	2-32	0.5-1	chr	[6, 30]
E.casseliflavus	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546*	[6, 30]
	VanC-2	D-Ala-D-Ser	2-32	0.5-1	chr	[6, 30]
	VanC-4	D-Ala-D-Ser	2-4	<2	chr	[45]
E.flavescens	VanC-3	D-Ala-D-Ser	2-32	0.5-1	chr	[6]
E.avium	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546*,	[30]
E.durans	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546-like*	[30, 39]
E.mundtii	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546-like*	[39]
E.rafinosus	VanA	D-Ala-D-Lac	64-1024	16-512	Tn*1546*	[30]

*modifications in central region Tn*1546* (e.g. *vanS*:IS*1261Y*) can cause chanches of MIC for teicoplanin > 256 to <4.

Tolerance to Vancomycin

Tolerance to vancomycin occurs in 100% of enterococci producing biofilms. For *E. faecalis*, MIC_{90}/MBC_{90} = 4/>128 mg L^{-1}, the MBIC values for *E. faecium* and *E. faecalis* exceed 4096 mg L^{-1} [3]. A biofilm was produced by 57-100% *E. faecalis* and 16-48% *E. faecium* [26]. Tolerance to glycopeptides occurs also in *S. aureus*, VTSA (vancomycin tolerant *S. aureus*) characterize MBC of vancomycin ≥32 MIC. The tolerance of *S. aureus* to vancomycin may be caused by the ability to produce a biofilm or by changed autolysins activity [1, 6, 50, 51].

ABBREVIATIONS

CLSI = Clinical Laboratory Standards Institute

EARSS = European Antimicrobial Resistance Surveillance System

EUCAST = European Committee on Antimicrobial Testing

GI = Genomic islands

h-VISA = Hetero-vancomycin intermediate *Staphylococcus aureus*

MIC = Minimal inhibitory concentration

MIC_{50} = Minimal inhibitory concentration for 50% of strain

MIC_{90} = Minimal inhibitory concentration for 90% of strain

MBC = Minimal bactericidal concentration

MBC_{50} = Minimal bactericidal concentration for 50% of strain

MBC_{90} = Minimal bactericidal concentration for 90% of strains

MBIC = Minimum biofilm inhibitory concentration

MLS-B = Macrolide, lincosamide and streptogramin B

MRSA = Methicillin resistant *Staphylococcus aureus*

MR-CNS = Methicillin resistant - coagulase negative staphylococci

PBP = Penicillin binding protein

PRSP = Penicillin resistant *Streptococcus pneumoniae*

SCC*mec* = Staphylococcal chromosomal cassette mec

VISA = Vancomycin intermediate *Staphylococcus aureus*

VRE = Vancomycin resistant enterococci

VREfl = Vancomycin resistant *Enterococcus faecalis*

VREfm = Vancomycin resistant *Enterococcus faecium*

VRSA = Vancomycin resistant *Staphylococcus aureus*

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