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Macrolide resistance mechanisms and *in vitro* susceptibility patterns of viridans group streptococci isolated from blood cultures

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***Objectives*: Our aim was to study the macrolide resistance mechanisms and antimicrobial susceptibilities of viridans group streptococci (VGS) isolated from blood cultures.**

***Methods*: *In vitro* susceptibilities to nine antimicrobials were studied for 85 VGS isolated from blood cultures by agar dilution. Pheno- and genotyping of erythromycin-resistant isolates were studied by the double disc test and PCR.**

***Results*: Resistance to erythromycin was found in 27% (n = 23) of the isolates. Erythromycin-resistant *Streptococcus oralis* (n = 13) predominated among the other erythromycin-resistant species isolated. The phenotypes among 23 erythromycin-resistant isolates were as follows: 12 constitutive macrolide–lincosamide–streptogramin ($cMLS_B$) resistance phenotype and 11 macrolide (M) resistance phenotype. Of the $cMLS_B$ isolates 11 had *erm*(B) genes and 11 of the M phenotype isolates had *mef*(A) genes. Four of the $cMLS_B$ isolates had both *erm*(B) and *mef*(A) genes. None of the isolates had *erm*(TR) genes. Combined resistance to erythromycin with penicillin, clindamycin, chloramphenicol, tetracycline and quinupristin/dalfopristin was found in 100, 61, 74, 100 and 100% of the isolates, respectively. No resistance was found for vancomycin, linezolid and levofloxacin.**

***Conclusions*: The macrolide resistance mechanisms of our VGS isolates revealed that the $cMLS_B$ phenotype associated with *erm*(B) and the M phenotype associated with *mef*(A) genes are found with similar frequencies.**

Keywords: erythromycin resistance, MLS_B, M phenotype, *erm*(B), *mef*(A), *S. oralis*

Introduction

Although they are part of the commensal flora of the human upper respiratory tract, viridans group streptococci (VGS) have been recognized in the aetiology of various diseases such as endocarditis and bacteraemia, and have been associated with shock and respiratory distress syndrome in febrile neutropenic patients.[1]

It has been shown that VGS can play a significant role as a reservoir of antimicrobial resistance genes transferring different resistance traits to more pathogenic organisms like *Streptococcus pneumoniae* and *Streptococcus pyogenes*.[2] Penicillin has been the first choice treatment in VGS infections but resistance to β-lactams and other antimicrobial agents is increasing.[1] There are three different resistance mechanisms causing macrolide resistance in streptococcal isolates; first is the target site modification mediated by the erythromycin resistance methylases encoded by the *erm*(A) or *erm*(B) genes conferring resistance to macrolide, lincosamide and streptogramin B antibiotics (MLS_B phenotype). Expression of MLS_B resistance can be constitutive ($cMLS_B$) or inducible ($iMLS_B$).[2] The second is the active drug efflux mediated by the membrane-bound efflux protein encoded by the *mef*(A) gene conferring resistance to 14- and 15-membered macrolides only (M phenotype). There are two subclasses of the *mef*(A) gene [the *mef*(A) gene in *S. pyogenes* and the *mef*(E) gene in *S. pneumoniae* strains]. They have been considered a single class of *mef*(A) gene and MefA protein due to their high homology but a recent study found that *mef*(A) and *mef*(E) elements had genetic differences and proposed to refer them as *mef*(A) subclass *mef*(A) or subclass *mef*(E). The element that contains the *mef*(E) gene is a macrolide efflux genetic assembly (MEGA) and the *mef*(A)-carrying element is a defective transposon (Tn*1207.1*). The elements both contain are an open reading frame adjacent to *mef* and *mel* in MEGA, which

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shares 35% identity with the *msr*(A) gene of *Staphylococcus aureus*.[3] The third mechanism is mutation in the streptococcal 23S rRNA or ribosomal protein genes leading to resistance to macrolide and streptogramin B antibiotics (MS phenotype).[2]

The objectives of the present study were to determine the macrolide resistance mechanisms and antimicrobial susceptibilities of viridans streptococci isolated from blood cultures.

Materials and methods

Bacterial strains and identification

Eighty-five VGS isolates recovered from blood cultures at Hacettepe University Hospital, Ankara during January 1996 to December 2004 were included in the study. The isolates were identified by colony morphology, Gram stain, catalase and optochin tests. Species identification was determined by standard methods and BD Phoenix Streptococci, SMIC/ID Panel [Becton and Dickinson Diagnostic Systems (BD), Pont de Claix, France].[4]

Antimicrobial susceptibility and macrolide pheno- and genotyping

Penicillin G, erythromycin, clindamycin, chloramphenicol, tetracycline, levofloxacin, linezolid, quinupristin/dalfopristin and vancomycin susceptibility were determined by the agar dilution method recommended by the CLSI (formerly the NCCLS), using Mueller–Hinton agar supplemented with 5% (v/v) sheep blood.[5] Plates were incubated in a 5% CO_2 atmosphere for 20–24 h at 35°C. *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213 were used as quality controls for all antimicrobials tested. The phenotypes of erythromycin-resistant isolates were determined by the double disc test with erythromycin (15 μg) and clindamycin (2 μg) discs as described previously.[2] Genomic DNA was extracted as described previously.[6] The DNA from erythromycin-resistant isolates was amplified with primers specific for the *erm*(B), *erm*(TR) and *mef*(A) genes.[6,7]

Results

The clinical isolates included *Streptococcus oralis* (n = 22), *Streptococcus mitis* (n = 21), *Streptococcus sanguinis* (n = 13), *Streptococcus anginosus* (n = 7), *Streptococcus parasanguinis* (n = 6), *Streptococcus gordonii* (n = 5), *Streptococcus cristatus* and *Streptococcus sobrinus* (n = 3, each), *Streptococcus intermedius* (n = 2), *Streptococcus constellatus*, *Streptococcus salivarius* and *Streptococcus vestibularis* (n = 1, each). To determine the association between erythromycin resistance and the resistance to the other antibiotics, the isolates were divided into two groups according to their erythromycin susceptibility (susceptible ≤0.25 mg/L; intermediate resistant = 0.5 mg/L; resistant ≥1 mg/L) (Table 1). Twenty-three strains (27%) were resistant to erythromycin. Erythromycin-resistant strains were also resistant to penicillin, tetracycline and quinupristin/dalfopristin. Sixty-one and 74% of the clindamycin- and chloramphenicol-resistant strains were also resistant to erythromycin, respectively. No resistance to vancomycin, linezolid or levofloxacin was detected.

The phenotypes among the 23 erythromycin-resistant isolates were as follows: 52% $cMLS_B$ (n = 12) and 48% M phenotype (n = 11). The strains with the $cMLS_B$ phenotype had higher MICs (all >256 mg/L) of erythromycin than the M phenotype (range = 2–8 mg/L) strains. Of the $cMLS_B$ isolates, 11 of the 12 carried *erm*(B) either alone or together with *mef*(A) but one isolate did not carry any of the macrolide-resistance genes studied here. All M phenotype isolates were explained by the presence of the *mef*(A) gene and none had *erm*(B) in combination. None of the isolates amplified the *erm*(TR) gene. The distribution of the erythromycin-resistant isolates in accordance to species type is shown in Table 2.

Discussion

Of the 85 viridans streptococcal blood culture isolates of this study 27% were resistant to erythromycin. The erythromycin-resistance level was comparable to previous reports from different countries in which Rodriguez-Avial *et al.*[8] from Spain reported 45.6% and Uh *et al.*[9] from Korea reported 33.9% erythromycin resistance among their blood culture isolates.

The phenotype and genotype frequencies from different countries have usually shown that $cMLS_B$ and *erm*(B) gene (~60%) are the most prevalent resistance mechanism among the VGS isolates from blood cultures.[8–10] We have detected methylase type

Table 1. Activities of eight antimicrobial agents against 85 VGS isolated from blood according to their susceptibility to erythromycin

Antimicrobials	Susceptibility breakpoints (mg/L)	Erythromycin susceptible (n = 62) range (≤0.016–0.25 mg/L)			Erythromycin resistant (n = 23) range (0.5 to >256 mg/L)		
		Range	$MIC_{50/90}$	%S[a]	range	$MIC_{50/90}$	%S
Penicillin	≤0.125	≤0.016 to 0.5	0.125/0.25	63	0.5 to 2	1/2	0[b]
Clindamycin	≤0.25	≤0.016 to 0.06	0.03/0.06	100	0.06 to >2	>2/>2	39
Chloramphenicol	≤4	<0.06 to 4	2/4	100	4 to 16	8/8	26
Tetracycline	≤2	<0.03 to >8	0.25/>8	82	>8	>8/>8	0
Quinupristin/dalfopristin	≤1	≤0.016 to 1	–	100	2 to 4	2/2	0[c]
Vancomycin	≤1	≤0.016 to 0.5	–	100	0.5 to 1	–	100
Linezolid	≤2	≤0.016 to 2	–	100	2	–	100
Levofloxacin	≤2	0.03 to 2	–	100	2	–	100

[a]Percentage susceptibility.
[b]All were intermediate penicillin-resistant isolates.
[c]Twenty-two were intermediate quinupristin/dalfopristin-resistant isolates and one was a fully quinupristin/dalfopristin-resistant isolate.

Table 2. Distribution of the erythromycin-resistant isolates in accordance to species type

Erythromycin resistant (no.)	MIC range (mg/L)	Phenotype	No.	Genotype		
				erm (B)	*mef* (A)	*erm*(B) + *mef*(A)
S. oralis (13)	0.5–4	M	8	0	8	0
	>256	$cMLS_B$	5	3	0	2
S. mitis (4)	8	M	1	0	1	0
	>256	$cMLS_B$[a]	3	2	0	0
S. cristatus (3)	2	M	1	0	1	0
	>256	$cMLS_B$	2	1	0	1
Other[b] (3)	0.5	M	1	0	1	0
	>256	$cMLS_B$	2	1	0	1
Total (23)						

[a]One strain did not express *erm*(B), *erm*(TR) or *mef*(A).
[b]*S. intermedius*, *S. parasanguinis* and *S. salivarius*.

resistance responsible for the $cMLS_B$ phenotype in 52% of the erythromycin-resistant isolates and all of the isolates have harboured the *erm*(B) gene, except one. Strains carrying *erm*(B) genes displayed higher MICs of erythromycin (>256 mg/L) than those with a *mef*(A) gene (MICs from 2–8 mg/L) but in 4 of 12 isolates with the $cMLS_B$ phenotype, the *erm*(B) gene was combined with a *mef*(A) gene. Combination of both genes has already been detected in erythromycin-resistant *S. pneumoniae* and *Streptococcus agalactiae* and this accumulation of genes in strains of VGS has been suspected of being a possible reservoir and source of genetic exchange of the resistance genes to pathogenic streptococci.[10] Efflux type resistance (M phenotype) was found in 48% of our erythromycin-resistant isolates and in all these isolates *mef*(A) was detected while none carried *erm*(B). The molecular characterization of our VGS isolates revealed that the *erm*(B) and *mef*(A) genes are found with similar frequencies.

A common point among studies of VGS blood isolates is that the erythromycin-resistant isolates either did not show or very rarely harboured the $iMLS_B$ type of resistance.[8–10] We also did not find this phenotype. In order not to misidentify the $iMLS_B$ phenotype, we also performed the double disc test with 4 mm rather than the 15–20 mm distance between the erythromycin and clindamycin discs as suggested by Seppala *et al.*,[2] and could not find any difference from the conventional method. The PCR results have also revealed that our isolates do have *erm*(B) and *mef*(A) genes alone.

The most commonly isolated erythromycin-resistant species in our study in rank order were *S. oralis*, *S. mitis*, *S. cristatus*, *S. intermedius*, *S. parasanguinis* and *S. salivarius*. In accordance with a previous report from the UK (51%), we also noted a high rate (57%) of *S. oralis* ($n = 13$) isolates resistant to erythromycin.[1] The M phenotype and *mef*(A) gene predominated among *S. oralis* isolates which was different from other reports.[3,10]

Erythromycin-resistant VGS isolates were also resistant to tetracycline, penicillin and quinupristin/dalfopristin (Table 1). The resistance to tetracycline might be due to a transposon encoding the genes of *tet*(M) and *erm*(B).[3] Thus the erythromycin-resistant isolates were also resistant to tetracycline but the presence of intermediate penicillin- and quinupristin/dalfopristin-resistant isolates was noteworthy.

As the VGS have an ability to spread antibiotic resistance genes to other bacteria, especially to *S. pneumoniae*, and can exhibit cross-resistance to many antimicrobial agents, continuous surveillance of the isolates should be performed in routine laboratories.

Transparency declarations

No declarations were made by the authors of this paper.

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