Prevalence of phenotypic resistance of *Staphylococcus aureus* isolates to macrolide, lincosamide, streptogramin B, ketolid and linezolid antibiotics in Turkey

ABSTRACT

The incidence of drug-resistant pathogens differs greatly between countries according to differences in the usage of antibiotics. The purpose of this study was to investigate the phenotypic resistance of 321 methicillin resistance *Staphylococcus aureus* (MRSA) and 195 methicillin susceptible *S. aureus* (MSSA) in a total of 516 *S. aureus* strains to macrolide, lincosamide, streptogramin B (MLS_B), ketolid, and linezolid. Disk diffusion method was applied to determine MLS_B phenotype and susceptibility to different antibiotic agents. It was found that 54.6% of the isolates were resistant to erythromycin (ERSA), 48% to clindamycin, 55% to azithromycin, 58.7% to spiramycin, 34.7% to telithromycin, and 0.4% to quinupristin-dalfopristin, respectively. No strain resistant to linezolid was found. The prevalence of constitutive ($cMLS_B$), inducible ($IMLS_B$), and macrolides and type B streptogramins (M/MS_B) among ERSA isolates (237 MRSA, 45 MSSA) was 69.6 %, 18.2%, and 12.2 % in MRSA and 28.9%, 40%, and 31.1% in MSSA, respectively. In conclusions, the prevalence of $cMLS_B$ was predominant in MRSA; while in MSSA strains, $iMLS_B$ and M/MS_B phenotype were more higher than $cMLS_B$ phenotype resistance. The resistance to quinupristin-dalfopristin was very low, and linezolid was considered as the most effective antibiotic against all *S.aureus* strains.

Keywords: *Staphylococcus aureus*, macrolide, lincosamide, streptogramin B, ketolid, linezolid, MLS_B.

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INTRODUCTION

Macrolides (e.g., erythromycin, azithromycin, spiramycin), lincosamides (e.g., clindamycin, lincomycin), and streptogramin B (e.g., quinupristin) are groups of antibiotic collectively named MLS_B.[1] They are chemically distinct, but have similar inhibitory effects on bacterial protein synthesis. MLS_B, commonly used in treatment of staphylococcal infections,[1] and clindamycin is a frequent choice for some staphylococcal infections, particularly skin and soft-tissue infections, and it is an alternative in the penicillin-allergic patients.[2] The macrolide antibiotic resistance in *S. aureus* is usually caused either by ribosomal modification mediated by 23S rRNA methylases encoded by *erm* genes, or by active efflux of the antimicrobial agent by an ATP-dependent pump encoded by *msrA* gene. Methylases confer inducible ($iMLS_B$) or constitutive ($cMLS_B$) resistance, while the efflux mechanism affects only macrolides and type B streptogramins (M/MS_B). Other more rare macrolide resistance mechanisms include ribosomal mutations and antibiotic inactivation by specific hydrolases or phosphotransferases.[3] Ketolides belong to the MLS_B family, and telithromycin is the first commercially available ketolide.[4] Oxazolidinones and specifically linezolid are new class of compounds that binds to the 23S portion of the 50S ribosomal subunit, preventing initiation complex formation with activity against methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE).[5] Quinupristin-dalfopristin (Synercid, 30:70 ratio) is the first parenteral streptogramin that has recently been licensed for clinical use in the United States and Europe for the treatment of infections caused by multidrug resistant and Gram-positive pathogens.[6]

Quinupristin and dalfopristin enter bacterial cells by diffusion and bind to different sites on the 50S ribosomal subunit, resulting in an irreversible inhibition of bacterial protein synthesis. The combination synergistic effect appears to result from the fact that these compounds target early and late steps in protein synthesis.[7]

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In vitro tests show that strains with constitutive resistance are resistant to all macrolides, which comprise 14-(e.g.erythromycin), 15-(e.g.azithromycin), and 16-membered rings (e.g. spiramycin), lincosamides, and streptogramin B, while inducibly-resistant strains are resistant only to 14- and 15- membered-ring macrolides.[3] The objective of the present study was to investigate prevalence of MLS_B, ketolid, and linezolid phenotypic resistance in clinical *S. aureus* strains.

MATERIAL AND METHODS

Bacterial strains

Between January 2006 and April 2007, 321 MRSA and 195 MSSA, a total of 516 *S. aureus* isolates were obtained from different clinical specimens at Haydarpasa Numune Education and Research Hospital in Istanbul, Turkey. The isolates were identified according to Gram stain, catalase, and coagulase production (Slidex Staph Plus, Biomérieux, France). Duplicate isolates was not included. *S. aureus* ATCC 25923 was used as quality control in susceptibility testing.

Antimicrobial disks

Antimicrobial disks were purchased from Oxoid (Hemakim, Istanbul, Turkey).

Determination of antimicrobial susceptibility test and MLS_B phenotype resistance patterns

MLS_B phenotype resistance pattern was determined according to the method advised by Clinical and Laboratory Standards Institute (CLSI).[8] Briefly, an overnight culture of each isolate was adjusted to 0.5 McFarland (10^8 cf/mL) and spread on unsupplemented Mueller-Hinton agar (HIMEDIA, Himedia Laboratories, Mumbai , India). The following antibiotic disks were applied on an inoculated media: azithromycin (Az-15 µg), spiramycin (Sp 100 µg), telithromycin (Te-15 µg), quinupristin-dalfopristin (Q-D-15 µg), and linezolid (Li-30 µg), erythromycin (E-15 µg), and clindamycin (Cl-2 µg) disks were placed by hand to provide distances of 15-26 mm from edge to edge. Following incubation for 16 to 18 hours at 35° C, zone diameters were measured in the usual manner; any flattening or blunting of clindamycin zone shape (D-shape), indicating $iMLS_B$, while resistance to both erythromycin and clindamycin indicated $cMLS_B$. Lack of a D-shaped zone in erythromycin resistant and clindamycin-susceptible isolates were interpreted as M/MS_B. Due to lack of CLSI zone diameters criteria for spiramycin, we used the Comite´ de l'Antibiogramme de la Socie´te´ Française de Microbiologie recommendation of zone diameters ≥ 24 mm as susceptible, and < 19 mm as resistance.[9]

RESULTS

Of the 516 isolates, 237 MRSA and 45 MSSA, a total of 282 (54.6%) *S. aureus* isolates were found to be resistant to erythromycin (ERSA) and the rest was susceptible to erythromycin (ESSA), 248 (48%) isolates were resistant to clindamycin, 284 (55%) to azithromycin, 303 (58.7%) to spiramycin, 179 (34.7%) to telithromycin, and two (0.4%) strains to quinupristin-dalfopristin. No strain resistant to linezolid was found. As for phenotypic resistance of ERSA isolates, the rate of $cMLS_B$, $iMLS_B$, and M/MS in 282 ERSA strains was 178 (63%) $cMLS_B$, 61 (22%) $iMLS_B$, and 43 (15%) M/MS_B, respectivley. The distribution of $cMLS_B$, $iMLS_B$, and M/MS_B in ERSA-MRSA isolates was 69.6 %, 18.2%, and 12.2 %; and in ERSA-MSSA isolates it was 28.9%, 40%, and 31.1%, respectively, which showed a predominance of $cMLS_B$ in MRSA, while $iMLS_B$ and M/MS_B phenotypic resistance patterns were higher in MSSA isolates (Table 1).

DISCUSSION

This study was conducted at the largest educational hospital in Istanbul-Turkey to investigate the prevalence of MLS_B, ketolid, and linezolid in 516 *S. aureus* isolates. The prevalence of ERSA in Turkish isolates was found to be higher (54.6%) than those obtained (39%) in a study perfomed by the neighbours of Turkey with the participation of 20 European university hospitals.[10] This differance is more likely attributed to the high proportion of MRSA (62.2%) in our S .aureus isolates compaed to that of the European study (22%). However, they also reported[10] higher rate of $cMLS_B$ in MRSA (93%) and MSSA (44%) than we obtained in MRSA (69.6%) or in MSSA isolates (28.9%). Aktas *et al.*[11] conducted a study at the University hospital in Turkey on only 22 MRSA and found that 63%, 18%, and 18% of the isolates exhibited $cMLS_B$, $iMLS_B$, and M/MS_B, respectively. Spiliopoulou *et al.*[12] have mentioned in a study on ERSA strains that only 5.3% of MRSA isolates expressed $iMLS_B$ and the rest displayed $cMLS_B$, while in MSSA, 78.3% were $iMLS_B$ and 21.7% were M/MS_B, similar with our finding in which the percentage of M/MS_B (31.1%) in MSSA was twofold higher (12.2%) than in MRSA. A study of Janapatla *et al.*[13] from Taiwan reported that $iMLS_B$ was predominant in MSSA (8%) than in MRSA (4%). Otsuka *et al.*[14] have also reported that 61.3% of the Japanese MRSA isolates expressed $cMLS_B$ and 94% of the MSSA isolates displayed $iMLS_B$. A retrospective study conducted by Modak *et al.*[15] on 13,946 *S. aureus* strains collected between 1994-2005 revealed a stable incidence of $cMLS_B$ strains, but also a significant increase in the incidence of isolates that were susceptible to clindamycin and resistant to erythromycin, and in $iMLS_B$. They attributed this high incidence to the increased use of macrolides and clindamycin during the same period. Merino-Díaz *et al.*[16] reported that the rate of $iMLS_B$ resistance was significantly higher in *S. aureus* (5.2%) than the rate of $cMLS_B$ (1.7%) in cutaneous strains from Spain. On the other hand, 41.5% of the ESSA isolates (44.7% of MSSA and 35.7% of MRSA) were highly resistant to spiramycin, in contrast to the low resistance rate to clindamycin (4%) and azithromycin (1%). The ribosomal mutations and antibiotic inac-

Table 1. Resistance of S. aureus Strains with different MLSB phenotypic patterns to various antibiotics

Antibiotic	Erythromycin-resistant *S.aureus* (ERSA: n= 282)												Erythromycin susceptible *S. aureus* (ESSA: n=234)			Total *S. aureus strains* (n= 516)		
	cMLSB (n=178)			iMLSB (n= 61)			M/MSB (n=43)											
	MRSA (n=165)	MSSA (n=13)	Total n	(%)	MRSA (n=43)	MSSA (n=18)	Total n	(%)	MRSA (n=29)	MSSA (n=14)	Total n	(%)	MRSA (n=84)	MSSA (n=150)	Total n	(%)	MRSA (n=321)	MSSA (n=195)	Total n	(%)
Erythromycin	165	13	178	(100)	43	18	61	(100)	29	14	43	(100)	0	0	0	(0)	237	45	282	(54.6)
Clindamycin	165	13	178	(100)	43	18	61	(100)	0	0	0	(0)	0	9	9	(4)	208	40	248	(48)
Azithromycin	165	13	178	(100)	43	18	61	(100)	29	14	43	(100)	2	0	2	(1)	239	45	284	(55)
Spiramycin	165	13	178	(100)	19	0	19	(31)	7	2	9	(21)	30	67	97(41.5)		221	82	303	(58.7)
Telithromycin	165	13	178	(100)	1	0	1	(2)	0	0	0	(0)	0	0	0	(0)	166	13	179	(34.7)
Quinupristin-dalfopristin	2	0	2	(1)	0	0	0	(0)	0	0	0	(0)	0	0	0	(0)	2	0	2	(0.4)
Linezolid	0	0	0	(0)	0	0	0	(0)	0	0	0	(0)	0	0	0	(0)	0	0	0	(0)

tivation are the mechanisms that might play a role in the last resistance of ESSA and differ from MLS_B.[3] Only 0.4% of the *S. aureus* isolates were resistant to quinupristin-dalfopristin and this agent was effective in all of the examined isolates. There was concern regarding the use of streptogramin antibiotic (virginiamycin) as a feed additive in the animal husbandry and development of cross-resistance against this antibiotic.[17] Although the first resistance to linezolid was reported in 2001 due to mutations in the 23S rRNA,[18] we did not detect any resistance or even decreased susceptibility to this antibiotic, and most reports have shown that the resistance rate to this agent is still low.[18-20] Our study and review of the studies related to macrolides resistance in *S. aureus* demonstrated that methicillin resistance leads physicians to use different macrolides, mainly eryhromycin, azithromycin, and spiramycin or lincosamides, such as clindamycin and lincomycin which facilitate development of different MLS_B phenotypic patterns, and which mostly end with resistance to macrolides, lincosamide, streptogramin B, and ketolid ($cMLS_B$). We believe that this is the reason behind the increased prevalence of $cMLS_B$ in geographical area with high prevalence of MRSA, and vice versa.

Our study showed that the prevalence of MLS_B in Turkish *S. aureus* isolates was high and that the predominant phenotype was $cMLS_B$ in MRSA and $iMLS_B$ and M/MS_B in MSSA isolates, which is in agreement with reports of most countries. Linezolid and quinupristin-dalfopristin were very effective and promising. The accurate use of these new agents might avoid treatment failure especially in macrolid-resistant *S. aureus* infections.

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