The Antibiotic Susceptibilities of Methicilline-Resistant Staphylococcus aureus Strains Isolated From Various Clinical Samples

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Abstract

Background and Objective::

In this study, it was aimed to determine the in vitro susceptibilities of Methicillin-Resistant Staphylococcus aureus (MRSA) strains to fluoroquinolone, linezolid, tigecycline, and quinupristin /dalfopristin as well as the macrolide-lincosamide-streptogramin B (MLSB) resistance phenotype.

Materials and Methods

A total of 94 MRSA strains isolated from various clinical samples in our hospital laboratory between January 2020 and September 2020 were included. The in-vitro susceptibilities of MRSA strains against fluoroquinolone, linezolid, tigecycline, and quinupristin/dalfopristin were determined by Kirby-Bauer disc diffusion assay according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST). The E test assay was used for evaluation of tigecycline susceptibility. The D-zone test was performed with erythromycin (15 µg) and clindamycin (2 µg) discs to determine the MLSB resistance. Besides, bacterial identification, antibiotic susceptibility tests including methicillin resistance and MLSB phenotype determination were performed by using VİTEK 2 Gram-positive diagnostic kits(Bio-Mérieux/France).

Results

Results: Among 94 MRSA strains included, resistance rates to ciprofloxacin, moxifloxacin, tigecycline, and quinupristin/dalfopristin were found as 71% (67 isolates) 64%(60 isolates), 17%(16 isolates), and 2% (2 isolates), respectively.. Resistance was not detected for linezolid. A total of 36(49%) isolates showed cMLSB resistance phenotype while 18(19%) had iMLSB resistance. The MS phenotype – strains resistant to erythromycin and susceptible to clindamycin- was not detected.

Conclusion

Very little or no resistance was found to linezolid, quinupristin/dalfopristin and tigecycline. Therefore, these antibiotics may be beneficial for the proper treatment of infections caused by MLSB-resistant isolates.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most important causes of infections caused by multiple resistant microorganisms, which makes treatment difficult and reduces treatment options[1,2]. Resistance to beta-lactam group and fluoroquinolones leads to the use of last-option drugs such as vancomycin and teicoplanin, thus increases the resistance rates of these drugs. Therefore, the

need for new antimicrobial drugs has come to the fore and various antibiotics have been developed for the treatment of infections caused by this bacterial group[3, 4].

Tigecycline (GAR-936), is a semi-synthetic analogue of classical tetracyclines which has activity against both Gram-positive and Gram-negative bacteria [5]. Tigecycline prevents the aminoacyl tRNA from entering its target by binding to the 30S ribosomal subunit. This prevents the bacteria's protein synthesis and stops its growth[6,7,8]. Linezolid from the oxazolidinone group is another antimicrobial agent used in the treatment of MRSA infections. Linezolid prevents the formation of the initial complex in protein synthesis by binding to the 50S subunit in ribosomes. The absence of intrinsic resistance gene against linezolid is an advantage for Gram-positive bacteria [9,10]. Quinupristin / dalfopristin is a combination of semisynthetic streptogramins containing 30:70 ratio of quinupristin and dalfopristin. This macrolide-lincosamide-streptogramin B (MLSB) group antibiotic is effective against Gram-positive bacteria. The drug acts by binding to the 50S ribosomal subunit and inhibits protein synthesis[11,12]. Frequent use of MLSB group antibiotics in MRSA infections is important in terms of leading to the increase of the number of resistant strains. Methylase enzymes encoded by methylase genes (erm), which is associated with the development of resistance to erythromycin, play a role in the development of resistance[13]. MLSB resistance phenotypes are of two types, structural (cMLSB) and inducible (iMLSB). Strains with inducible MLSB resistance are crucial as erythromycin treatment causes enzyme induction in the bacterium, leading to resistance to macrolides and lincosamides[14].

This study aims to investigate in-vitro susceptibilities of MRSA strains isolated from various clinical samples to fluoroquinolone, linezolid, tigecycline, and quinupristin /dalfopristin and to determine the MLSB resistance phenotype.

Materials And Methods

This study is a retrospective study and it was conducted in accordance with ethical principles for medical research with the Declaration of Helsinki. A total of 94 MRSA strains isolated from various clinical samples in the laboratory of our hospital between January 2020 and September 2020 were included.

Identification

S. aureus strains were identified by conventional methods -colony morphology, hemolysis type, Gram stain, catalase, and coagulase tests- and VITEK 2 automated system (Bio-Mérieux / France).

Detection of antibiotic susceptibilities

Methicillin resistance and antibiotics susceptibility testing (AST) were investigated by the Kirby-Bauer disk diffusion method according to the recommendations of The European Committee on Antimicrobial Susceptibility Testing (EUCAST)[15]. Cefoxitin (30 µg) (Oxoid, England) disc was tested for methicillin resistance. Isolates with a cefoxitin inhibition zone diameter of less than 21 mm were defined as methicillin resistant. The MRSA isolates were subjected to the antibiotic susceptibility test with

ciprofloxacin (5µg), moxifloxacin (5µg), linezolid (30µg), quinupristin/dalfopristin (15µg) discs (Oxoid, UK) and tigecycline E test strips (Bio-Mérieux / France). A suspension of 0.5 McFarland fresh bacterial culture in sterile physiological saline was prepared and spread on two separate Mueller Hinton agar (Oxoid, England) plates. Ciprofloxacin (5µg), moxifloxacin (5µg), linezolid (30µg), quinupristin / dalfopristin (15µg) discs (Oxoid, UK) were placed on one plate, and tigecycline on the other one. After incubation at 35 ± 2oC for 18–24 hours, the minimal inhibitor concentration (MIC) of tigecycline and the inhibition zone diameters of other antibiotics were measured and the results were evaluated according to EUCAST criteria. In addition, D-test was performed with erythromycin (15 µg) and clindamycin (2 µg) discs adjacent to each other in order to detect MLSB resistance. The flattening of the clindamycin inhibition zone - defined as the (D) zone- facing the erythromycin disc was evaluated as inducible clindamycin resistance (iMLSB). Strains without an inhibition zone around the clindamycin and erythromycin discs were defined as constitutive clindamycin resistant (cMLSB). AST was also performed by VITEK 2 Gram-positive diagnostic kits (Bio-Mérieux / France) automatically.

Quality control

S. aureus ATCC 25923 and *S. aureus* ATCC 29213 and 43300 were used as quality control strains in the study.

Statistical methods

The results were evaluated in terms of frequency and percentage, in line with the purpose of the study.

Results

Out of 94 MRSA strains included in the study, 67 (71%) were resistant to ciprofloxacin, 60 (64%) to moxifloxacin, 16 (17%) to tigecycline, 2 (2%) to quinopristin / dalfopristin. There was no resistance to linezolid. The sensitivity of MRSA strains to antibiotics is shown in Table-1.

Of all the MRSA strains examined, 46 (49%) had cMLSB resistance, 18 (19%) had iMLSB resistance, and 30 (32%) had no resistance. In the strains included in the study, inducible resistance was found in all strains resistant to erythromycin and susceptible to clindamycin (Table-2).

VITEK 2 (Bio-Mérieux / France) results were concordant with classical microbiological identification tests and antibiotic susceptibility test results.

Discussion

In recent years, infections caused by multi-drug resistant MRSA have increased all over the world. MRSA strains resistance to various antimicrobials such as fluoroquinolones have led to use of glycopeptide antibiotics as the first and sometimes the only option[2]. With the reporting of glycopeptide resistance in

MRSA infections, it has brought the use of antimicrobials such as linezolid, tigecycline and quinopristin / dalfopristin in treatment[16,17].

In this study, a very high rate of fluoroquinolone resistance was found. 71% of 94 MRSA strains were resistant to ciprofloxacin and 64% to moxifloxacin. The fluoroquinolone resistance rate reported for MRSA strains in our country is between 33% and 85.9%; in other countries it ranges from 9.2–85%. Similar to this study, in a study in which Dündar et al.; investigated the antimicrobial susceptibility of S. aureus strains in a 3-year period (2005–2007) and reported ciprofloxacin resistance rates as 87%, 90% and 92%, respectively[18].

Linezolid and tigecycline are reported to be highly effective in MRSA strains. Linezolid resistance has been reported to be less than 0.1% in various surveillance programs since linezolid resistance, which was first published in 2001[19–22]. In this study, no resistance to linezolid was found among the MRSA strains. Similar results have been obtained in various studies, too. In a study conducted by Dizbay et al. in 2005 on 120 MRSA strains isolated from various clinical samples, all strains were found to be susceptible to linezolid[23]. In another study conducted with 1707 MRSA strains between 1997–1999, again, linezolid sensitivity was found to be 100%[23]. A study conducted in Korea retrospectively examined antibiotic susceptibility tests of a total of 22,067 MRSA isolates over 4 years, and only 110 (0.5%) were found to be resistant to linezolid[24].

In various studies, MRSA strains were found to be highly susceptible to tigecycline and resistance was not reported. For example; Arslan et al. investigated tigecycline in 100 MRSA strains isolated from various clinical specimens and linezolid in 80 of them and found all strains susceptible to linezolid and tigecycline[4]. Similarly, Goff et al. found all strains susceptible to tigecycline and linezolid in a study they conducted between January 2004 and September 2005 on 879 MRSA strains[26]. Behera et al. found 21 MRSA strains isolated from a hospital in India to be 100% susceptible to tigecycline[27]. In a study conducted in Malesia, five isolates (5.6%) were found, tigecycline-resistant but they were not linezolid resistance in 90 MRSA[28]. In this study, 16 (17%) of the MRSA strains were found to be resistant to tigecycline. Similar to this study, in a study by Kaya et al. investigating the in-vitro activity of tigecycline and linezolid in 60 MRSA strains; while they found all strains susceptible to linezolid, they found resistance against tigecycline in 1 strain[29]. Hoban et al. reported tigecycline sensitivity as 98.9% in a study they conducted with 5348 MRSA strains in 2004[30]. The lower rate of tigecycline resistance in various studies conducted in the past years may be attributed to the resistance of MRSA strains to this antibiotic over time.

In a review article published in 2020, quinupristin/dalfopristin resistance was found as 0.7% (0.3-1%) in MRSA strains[31]. Additionally, in some studies investigating the susceptibility of MRSA to quinupristin/dalfopristin abroad, the rate of resistance was reported to be between 0–31%[13,32,33]. Kim et al. did not find resistance to quinopristin/dalfopristin in any of 439 MRSA strains in Korea[13]. Baddour et al. found that all 512 MRSA strains in Saudi Arabia were susceptible to quinopristin/dalfopristine[34]. Luh

et al. determined this rate as 31% in Taiwan[32]. In our country, Baysallar et al. and Yavuz et al. found the quinopristin/dalfopristin resistance to be 1% for MRSA strains and it was found as 2.3% by Tünger et al.[35,36,37]. Kılıç et al. found no resistance in MRSA strains in the study they conducted in 2001 and 2002 while they reported that they found 2% resistance in 2003[2]. In this study, similar to various studies conducted in our country, quinopristin / dalfopristin resistance was found to be 2%.

Although macrolides and lincosamides are used effectively in MRSA infections, they cause problems in treatment due to MLSB resistance detected recently. Among 94 MRSA isolates included in this study, MLSB resistance was determined as 49% and iMLSB as 19%; no resistance was found in 30 MRSA strains (32%). These rates are similar to various studies conducted in our country. For example; In the study conducted by Doğruman et al. on 63 MRSA strains isolated from various clinical samples in Ankara between 2005 and 2006; 32 (50.8%) had cMLSB resistance, 13 (20.6%) had iMLSB resistance and 18 (28.6%) had no resistance[14]. In the strains included in the study, no strains resistant to erythromycin, susceptible to clindamycin but not inducible resistance (MS phenotype) were detected. In the study conducted by Azap et al. in Ankara, similar to the results of this study, cMLSB resistance was found as 45% and iMLSB resistance was found as 37%[38].

Different resistance rates were found in the studies abroad examining MLSB resistance in MRSA infections. Otsuka et al. In Japan, reported that they found iMLSB resistance is 38.7%, cMLSB resistance is 61.3%; Fiebelkorn et al. reported that they found iMLSB resistance as 29.8% and cMLSB resistance as 34.2% in the USA[39,40].

Conclusion

Determining the resistance against MLSB group antibiotics will be useful in providing appropriate and effective treatment in MRSA infections. Thus, selection of appropriate and effective drugs before treatment will both prevent the increase in resistance and increase the chance of treatment. Lack of resistance or low rate of resistance to antimicrobial agents such as quinupristin / dalfopristin, linezolid and tigecycline in MLSB-resistant MRSA infections will positively affect the success of the treatment.

Significance Statement

Aims of antimicrobial susceptibility tests are contributing to prescribed appropriate antibiotics and monitoring resistant pathogens. Researches of Antibiotic susceptibility, conducted with genotypic or phenotypic methods, contribute to provide epidemiological data, as well as regulation of correct antibacterial treatment regimens. In addition, by collecting data on regional antibiotic susceptibility test results, the types of resistance detected can guide empirical treatment selection.

Results of this study is shown that there is widespread resistance to other antibiotics besides methicillin resistance in *S. aureus* strains and it emphasized that to importance of antibiotic susceptibility tests.

Declarations

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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Tables

Table 1. Antibiotic Sensitivity of MRSA Strains

	CIP		MXF		TGC		QD		LZD	
	n	%	n	%	n	%	n	%	n	%
Sensitive (S)	27	29	34	36	78	83	92	98	94	100
Resistant (R)	67	71	60	64	16	17	2	2	-	-

CIP: Ciprofloxacin**, MXF: Moxifloxacin, TGC: Tigecycline, LZD: Linezolid, QD: Quinupristin / Dalfopristin**

Table 2. Distribution of MLSB Resistance in MRSA Strains

MLSB Resistance	n	%
Number of strains with cMLSB resistance	46	49
Number of strains with iMLSB resistance	18	19

$cMLS_B$ **:** Constitutive macrolide-lincosamide-streptogramin B

$iMLS_B$ **:** Inducible macrolide-lincosamide-streptogramin B