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Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Isolates Against Newly Marketed Antibiotics: A Report From Imam Reza Hospital of Mashhad, Iran

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Abstract

Background: Infections due to *Staphylococcus aureus* have long been considered as a big challenge to clinicians. The innate ability of this microorganism to develop resistance to different antibiotics, has led to the appearance of MRSA (methicillin-resistant *Staphylococcus aureus*) and lately VRSA (vancomycin-resistant *Staphylococcus aureus*) strains, which are considered as major problems for both patients and clinicians.

Objectives: In this study, we tried to evaluate susceptibility pattern of *S. aureus* isolates against some prevalent antibiotics as well as some infrequent ones.

Materials and Methods: This inquiry was performed on 238 clinical samples, collected from different wards of Imam Reza Hospital of Mashhad between 2011 and 2012, which were previously defined as *S. aureus* and stocked in -70°C. Kirby-Bauer's disc diffusion was performed for the following antibiotics: quinupristin-dalfopristin, linezolid, cefoxitin and mupirocin according to EUCAST 2014 (v. 4), co-trimoxazole, doxycycline, tigecycline, oxacillin based on CLSI 2012 (M100-S22) and vancomycin according to CLSI 2007 guidelines.

Results: Out of 238 samples, 5.88% were resistant to quinupristin-dalfopristin; 5.46% to linezolid; 60.92% to Co-trimoxazole; 31.93% to doxycycline; 18.90% to tigecycline; 5.04% to vancomycin; 9.24% to mupirocin; 43% to oxacillin and 46.21% of our isolates were resistant to cefoxitin.

Conclusions: Coming across isolates with reduced susceptibility to quinupristin-dalfopristin and resistant to linezolid in this study are worrisome although these antibiotics are not used in our hospital. This might be a new challenge in the treatment of MRSA.

Keywords: Methicillin, Vancomycin, Quinupristin-Dalfopristin, Linezolid, Methicillin-Resistant *Staphylococcus aureus*, *Staphylococcus aureus*

1. Background

Antibiotic resistance in pathogens is a worrisome phenomenon which today is seen with many available antibiotics. Among antibiotic resistant pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) is of special interest (1), mainly due to its ability to cause both healthcare-associated and community-acquired infections, which may be fatal (2). There are several antibiotics in different antibacterial classes available in the market with in vitro and in vivo activity against MRSA (3). Among these, vancomycin was considered as the drug of choice for treatment of MRSA infections until recently (2, 4). In the past decade, vancomycin-resistant strains of *S. aureus* have been reported from different parts of the world (5, 6). This fact has raised concerns about the efficacy of vancomycin for treatment of infections caused by *S. aureus* in near future (7, 8).

In the past decades, many anti-MRSA agents have been introduced to the market, including ceftobiprole, linezolid, tigecycline, daptomycin, dalbavancin and quinupristin-dalfopristin. Linezolid is one of these antibiotics with good anti-gram positive activity and is considered as a good alternative to vancomycin in the treatment of MRSA infections (2). Quinupristin-dalfopristin is another antibiotic which is used in treatment of MRSA infections as an alternative to vancomycin; however, shortly after the introduction of this agent, reports confirmed the resistance development among *S. aureus* isolates (9).

2. Objectives

As mentioned above, there are limited number of antibiotics available which can be used to fight infections due to MRSA and other resistant pathogens. Thus, it is of great importance to evaluate the resistance against these antibiotics. To achieve this aim, we evaluated the antibiotic susceptibility pattern of *S. aureus* isolates collected from different wards of Imam Reza hospital of Mashhad, Iran.

3. Materials and Methods

3.1. Sample Isolation and Identification

This cross-sectional study was performed on 238 clinical samples collected from different wards of Imam Reza hospital of Mashhad between 2011 and 2012. All clinical samples which were sent to microbiology laboratory and identified as *S. aureus* by different standard biochemical tests including gram staining, catalase test, oxidase test, DNase and coagulase tests were included in this study. All samples which were collected from nostrils and other sites indicating colonization were excluded.

3.2. Evaluating the Antimicrobial Susceptibility

All the specimens were sub-cultured on blood agar and incubated for 24 hours. The isolates were then cultured on Mueller-Hinton agar and their antimicrobial susceptibility was evaluated by Kirby-Bauer disc diffusion method using the following antibiotic discs: quinupristin-dalfopristin (MAST, 15 μg), linezolid (MAST, 10 μg), mupirocin (MAST,200 μg), tigecycline (MAST, 15 μg), trimethoprim/sulfamethoxazole (MAST, 1.25/23.75 μg), doxycycline (MAST, 30 μg), vancomycin (MAST, 30 μg), cefoxitin (MAST, 30 μg) and oxacillin (MAST, 1 μg).

3.3. Adopting the Susceptibility Breakpoints

Since none of the available guidelines had susceptibility breakpoints for all antibiotics tested in this study, we used three different guidelines. We used the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2014 (v. 4) guidelines to define the resistance pattern to quinopristin-dalfopristin, linezolid and mupirocin and the Clinical and Laboratory Standard Institute (CLSI) 2012 guidelines (M100-S22) to delineate the susceptibility pattern to co-trimoxazole, doxycycline, tigecycline and oxacilin. To evaluate the resistance pattern of our isolates to vancomycin, we used CLSI 2007 guidelines.

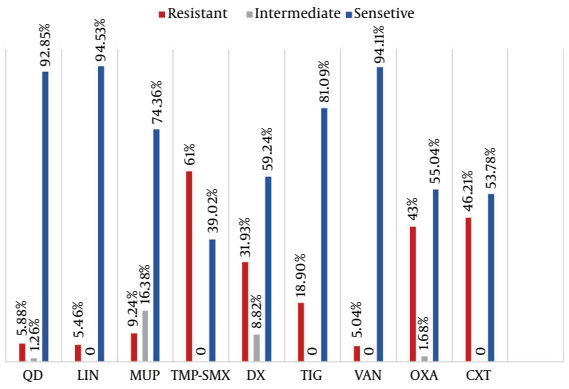
4. Results

Our study showed that out of 238 *S. aureus* isolates, 221 were susceptible, 14 were resistant and 3 were intermediate to quinupristin-dalfopristin. 225 isolates were susceptible to linezolid; whereas, 13 were resistant. 22 out of 238 isolates were resistant to mupirocin and 39 were intermediately resistant.

145 isolates showed resistance to co-trimoxazole and 93 were susceptible. 76 isolates were resistant and 141 were susceptible to doxycycline and 21 isolates showed intermediate resistance. 193 samples were susceptible and 45 were resistant to tigecycline. 131 isolates were susceptible to oxacilin, while 103 were completely resistant and 4 were intermediate. Among our isolates, 110 isolates were resistant to cefoxitin, while 128 isolates were susceptible and 224 isolates were susceptible to vancomycin, while 14 showed resistance to it (Figure 1).

In this study, all isolates which showed resistance to linezolid (14 isolates), were also resistant to Co-trimoxazole, doxycycline, tigecycline, vancomycin, oxacillin and cefoxitin. Cross-resistance to tested antibiotics is shown in Table 1.

Figure 1. Susceptibility Pattern of *S. aureus* Isolates to Selected Antibiotics



Abbreviations: CXT, cefoxitin; DX, doxycycline; LIN, linezolid; MUP, mupirocine; QD, quinupristin-dalfopristin; TIG, Tigecycline; TMP-SMX, Co-trimoxazole; VAN, vancomycin, OXA, oxacillin.

Table 1. Cross-Resistance of *S. aureus* Isolates to the Selected Antibiotics[a]

Antibiotics	n	Number of Isolates Resistant to the Selected Antibiotics								
		QD	LIN	MUP	TMP-SXT	DX	TIG	VAN	OXA	CXT
QD	14	14 (100)	12 (85.71)	13 (92.85)	14 (100)	13 (92.85)	12 (85.71)	12 (85.71)	14 (100)	14 (100)
LIN	13	12 (92.30)	13 (100)	12 (92.30)	13 (100)	13 (100)	13 (100)	12 (92.30)	13 (100)	13 (100)
MUP	22	13 (59.09)	12 (54.54)	22 (100)	20 (90.90)	15 (68.18)	15 (68.18)	12 (54.54)	19 (86.36)	17 (77.27)
TMP-SXT	145	14 (9.65)	13 (8.96)	20 (13.79)	145 (100)	67 (46.30)	36 (24.82)	13 (8.96)	95 (65.51)	96 (66.20)
DX	76	13 (17.10)	13 (17.10)	15 (19.73)	67 (88.15)	76 (100)	34 (44.73)	13 (17.10)	62 (81.57)	64 (84.21)
TIG	45	12 (26.66)	13 (28.88)	15 (33.33)	36 (80)	34 (75.55)	45 (100)	13 (28.88)	32 (71.11)	34 (75.55)
VAN	14	12 (85.71)	12 (85.71)	12 (85.71)	13 (92.85)	13 (92.85)	13 (92.85)	14 (100)	13 (92.85)	13 (92.85)
OXA	103	14 (13.59)	13 (12.62)	19 (18.44)	95 (92.23)	62 (60.19)	32 (31.06)	13 (12.62)	103 (100)	96 (93.20)
CXT	110	14 (12.72)	13 (11.81)	17 (15.45)	96 (87.27)	64 (58.18)	34 (30.90)	13 (11.81)	96 (87.27)	110 (100)

Abbreviations: CXT, cefoxitin; DX, doxycycline; QD, quinupristin-dalfopristin; LIN, linezolid; MUP, mupirocine; OXA, oxacillin; TIG, Tigecycline; TMP-SXT, Co-trimoxazole; VAN, vancomycin.

[a]Data are presented as number (%) of isolates resistant to a single drug.

5. Discussion

Today there is an imperative need for new antibiotics because pathogens are developing resistance against many available antibiotics in an exponential manner. MRSA is one of these pathogens which due to its high prevalence has become one of the main interests in developing new antibiotics (3). A systematic review showed that almost 53% of *S. aureus* isolates reported from Iran until early 2012 were *mecA* positive MRSA (10). In this study we found that 46% of our isolates were resistant to cefoxitin. This rate is almost similar to the prevalence of MRSA in other parts of Iran, although our isolates need to be confirmed as MRSA by detection of the *mecA* gene.

In the past decades many antibiotics with anti-MRSA activity were introduced to the market, some of which are available in Iran. Linezolid is among these agents which is not widely available and it is not in use in many Iranian hospitals including ours. Despite the mentioned fact, 5.6% of isolates in our study were resistant to linezolid which is worrisome. Garcia et al. have reported the first clinical outbreak of linezolid resistant *S. aureus* (LRSA) from a tertiary teaching university hospital in Madrid, Spain (11). In their article they reported 12 patients with LRSA from which 6 patients died (with one death ascribed to LRSA infection). They also stated that this outbreak was associated with nosocomial transmission and extensive use of this antibiotic. Gu et al. in their systematic review have reported that until early 2012, more than 98% of staphylococci were susceptible to linezolid worldwide; they identified resistance in 0.05% *Staphylococcus aureus* isolates and in 1.4% of coagulase-negative staphylococci (12).

In a Study performed by Ruzbahani et al. 70% of their isolates were MRSA which were confirmed by PCR (13). They have reported that all of the MRSA isolates were susceptible to vancomycin; however, 100% of them were resistant to oxacilin and 34% to co-trimoxazole. In our study 60% of our isolates were resistant to co-trimoxazole which is high compared to the aforementioned study. This is noteworthy considering the fact that in our study 56% of the isolates were methicillin-susceptible, which may suggest that a great amount of *S. aureus* isolates develop resistance to co-timoxazole, even though they are susceptible to methicillin.

Shahsavan et al. in their inquiry which was performed in 2012, have reported that 58.8% of their *S. aureus* isolates were resistant to methicillin and 40% were resistant to mupirocin (14). The rate of mupirocin resistance in our study was lower (i.e. 9.20%). Contrary to our findings, in inquiries performed by Dibah et al. (15) and Rahimi et al. (16) all isolates were susceptible to linezolid, mupirocin and quinupristin-dalfopristin. Our results showed that 93.6% of our isolates were susceptible to quinupristin-dalfopristin. In a report from Taiwan published in 2000, the authors found that 31% of their MRSA isolates were resistant to quinupristin-dalfopristin, despite the fact that at the time of their study, this antibiotic was not available in Taiwan (17). Although 6.4% of our isolates were resistant to quinupristin-dalfopristin by disc diffusion method, the resistance should be confirmed by evaluating minimum inhibitory concentration (MIC) as suggested by Kali et al. (18).

In a recent study performed in Tehran, 28.3% of MRSA isolates were resistant to doxycycline and trimethoprim (19). Similarly in our study we found that 31.6% of our isolates were resistant to doxycycline and also 9.6% showed intermediate resistance to this antibiotic.

Tigecycline is another novel antibiotic which many authors believe to be an effective alternative to vancomycin for infections due to MRSA (20, 21). Tigecycline resistance among staphylococci has been rarely reported in the literature (22). Despite the aforementioned fact, in our study 18.40% of isolates showed resistance to tigecycline which should be confirmed with other methods.

According to what discussed before, developing resistance among staphylococci to different types of antibiotics is a worldwide issue. Coming across isolates with reduced susceptibility to quinupristin-dalfopristin and resistant to linezolid and tigecycline in this study raise concern although these antibiotics are not used in our hospital. This mandates more detailed studies evaluating the resistance pattern of *S. aureus* isolates especially to the newly marketed antibiotics and indicates the importance of designing concrete infection control policies.

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Footnotes

Authors' Contribution: Study concept and design: Mahboubeh Naderinasab, Emran Askari; acquisition of data: Arash Arianpoor, Emran Askari, Fatemeh Estaji; analysis and interpretation of data: Arash Arianpoor, Fatemeh Estaji; drafting of the manuscript: Arash Arianpoor, Fatemeh Estaji, Emran Askari; critical revision of the manuscript for important intellectual content: Mahboubeh Naderinasab, Emran Askari; statistical analysis: Arash Arianpoor; administrative, technical, and material support: Mahboubeh Naderinasab, Arash Arianpoor; study supervision: Mahboubeh Naderinasab.

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References

1. Cookson B, Bonten MJ, Mackenzie FM, Skov RL, Verbrugh HA, Tacconelli E, et al. Meticillin-resistant Staphylococcus aureus (MRSA): screening and decolonisation. *Int J Antimicrob Agents*. 2011;**37**(3):195–201. doi: 10.1016/j.ijantimicag.2010.10.023. [PubMed: 21163631]
2. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. *Clin Infect Dis*. 2011;**52**(3):e18–55. doi: 10.1093/cid/ciq146. [PubMed: 21208910]

3. Shlaes DM, Spellberg B. Overcoming the challenges to developing new antibiotics. *Curr Opin Pharmacol.* 2012;**12**(5):522–6. doi: 10.1016/j.coph.2012.06.010. [PubMed: 22832234]
4. van Hal SJ, Fowler VG. Is it time to replace vancomycin in the treatment of methicillin-resistant Staphylococcus aureus infections? *Clin Infect Dis.* 2013;**56**(12):1779–88. doi: 10.1093/cid/cit178. [PubMed: 23511300]
5. Askari E, Tabatabai SM, Arianpoor A, Nasab MN. VanA-positive vancomycin–resistant Staphylococcus aureus: systematic search and review of reported cases. *Infect Dis Clin Prac.* 2013;**21**(2):91–3.
6. Rossi F, Diaz L, Wollam A, Panesso D, Zhou Y, Rincon S, et al. Transferable vancomycin resistance in a community-associated MRSA lineage. *N Engl J Med.* 2014;**370**(16):1524–31. doi: 10.1056/NEJMoa1303359. [PubMed: 24738669]
7. Hiramatsu K. Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance. *Lancet Infect Dis.* 2001;**1**(3):147–55. doi: 10.1016/S1473-3099(01)00091-3. [PubMed: 11871491]
8. Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Crispell EK, Riahi F, et al. Activities of vancomycin, ceftaroline, and mupirocin against Staphylococcus aureus isolates collected in a 2011 national surveillance study in the United States. *Antimicrob Agents Chemother.* 2014;**58**(2):740–5. doi: 10.1128/AAC.01915-13. [PubMed: 24247138]
9. Yu F, Lu C, Liu Y, Sun H, Shang Y, Ding Y, et al. Emergence of quinupristin/dalfopristin resistance among livestock-associated Staphylococcus aureus ST9 clinical isolates. *Int J Antimicrob Agents.* 2014;**44**(5):416–9. doi: 10.1016/j.ijantimicag.2014.06.020. [PubMed: 25218154]
10. Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Naderinasab M. Epidemiology of mecA-Methicillin Resistant Staphylococcus aureus (MRSA) in Iran: A Systematic Review and Meta-analysis. *Iran J Basic Med Sci.* 2012;**15**(5):1010–9. [PubMed: 23493646]
11. Sanchez Garcia M, De la Torre MA, Morales G, Pelaez B, Tolon MJ, Domingo S, et al. Clinical outbreak of linezolid-resistant Staphylococcus aureus in an intensive care unit. *JAMA.* 2010;**303**(22):2260–4. doi: 10.1001/jama.2010.757. [PubMed: 20530779]
12. Gu B, Kelesidis T, Tsiodras S, Hindler J, Humphries RM. The emerging problem of linezolid-resistant Staphylococcus. *J Antimicrob Chemother.* 2013;**68**(1):4–11. doi: 10.1093/jac/dks354. [PubMed: 22949625]
13. Ruzbahani M, Rahdar HA, Babaei Z, Rezaeyan MH, Jafari M, Rezai M. Identification of Antibiotic Resistance Patterns of Methicillin-Resistant Staphylococcus Aureus Isolates from Patients in Selected Hospitals in Isfahan. *Glob J Med Res Stud.* 2014;**1**(2):37–40.
14. Shahsavan S, Emaneini M, Noorazar Khoshgnab B, Khoramian B, Asadollahi P, Aligholi M, et al. A high prevalence of mupirocin and macrolide resistance determinant among Staphylococcus aureus strains isolated from burnt patients. *Burns.* 2012;**38**(3):378–82. doi: 10.1016/j.burns.2011.09.004. [PubMed: 22040930]
15. Dibah S, Arzanlou M, Jannati E, Shapouri R. Prevalence and antimicrobial resistance pattern of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from clinical specimens in Ardabil, Iran. *Iran J Microbiol.* 2014;**6**(3):163–8. [PubMed: 25870749]
16. Rahimi F, Bouzari M, Katouli M, Pourshafie MR. Antibiotic resistance pattern of methicillin resistant and methicillin sensitive Staphylococcus aureus isolates in Tehran, Iran. *Jundishapur J Microbiol.* 2013;**6**(2):144–9.
17. Luh KT, Hsueh PR, Teng LJ, Pan HJ, Chen YC, Lu JJ, et al. Quinupristin-dalfopristin resistance among gram-positive bacteria in Taiwan. *Antimicrob Agents Chemother.* 2000;**44**(12):3374–80. [PubMed: 11083643]
18. Kali A, Stephen S, Umadevi S, Kumar S. Detection of quinupristin-dalfopristin resistance in methicillin-resistant Staphylococcus aureus in South India. *Indian J Pathol Microbiol.* 2013;**56**(1):73–4. doi: 10.4103/0377-4929.116164. [PubMed: 23924572]
19. Hassanzadeh S, Pourmand MR, Hadadi A, Nourijeylani K, Yousefi M, Mashhadi R, et al. Frequency and Antimicrobial Resistance Patterns of Methicillin-Resistant Staphylococcus aureus in Tehran. *J Med Bacteriol.* 2013;**2**(3, 4):41–6.
20. Yin LY, Lazzarini L, Li F, Stevens CM, Calhoun JH. Comparative evaluation of tigecycline and vancomycin, with and without rifampicin, in the treatment of methicillin-resistant Staphylococcus aureus experimental osteomyelitis in a rabbit model. *J Antimicrob Chemother.* 2005;**55**(6):995–1002. doi: 10.1093/jac/dki109. [PubMed: 15857944]
21. Florescu I, Beuran M, Dimov R, Razbadauskas A, Bochan M, Fichev G, et al. Efficacy and safety of tigecycline compared with vancomycin or linezolid for treatment of serious infections with methicillin-resistant Staphylococcus aureus or vancomycin-resistant enterococci: a Phase 3, multicentre, double-blind, randomized study. *J Antimicrob Chemother.* 2008;**62 Suppl 1**:i17–28. doi: 10.1093/jac/dkn250. [PubMed: 18684703]
22. Verkade EJ, Verhulst CJ, Huijsdens XW, Kluytmans JA. In vitro activity of tigecycline against methicillin-resistant Staphylococcus aureus, including livestock-associated strains. *Eur J Clin Microbiol Infect Dis.* 2010;**29**(5):503–7. doi: 10.1007/s10096-010-0886-2. [PubMed: 20186450]