



Frequency of Multidrug-Resistant, Extensively Drug-Resistant, and Pandrug-Resistant Phenotypes among Clinical Isolates of *Staphylococcus aureus*

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ABSTRACT

Background: In recent years, the widespread prevalence of Multidrug-Resistant (MDR) *Staphylococcus aureus* strains and the increase in the number of Extensively Drug-Resistant (XDR) and Pandrug-Resistant (PDR) phenotypes amongst *S. aureus* strains have become one of the greatest challenges. This study aimed to determine the incidence of MDR, XDR, and PDR phenotypes in *S. aureus* strains in a teaching hospital in Gorgan, Golestan province, Iran.

Materials & Methods: Clinical samples of blood, urine, wound, and sputum were collected from all hospitalized patients during April to June 2019. *S. aureus* strains were identified using conventional biochemical methods, and antibiotic susceptibility assessment was performed by Kirby-Bauer disc diffusion method.

Findings: A total of 73 isolates were identified as *S. aureus*. The majority of *S. aureus* isolates were collected from wound specimens (31 out of 73). Most of the isolates were recovered from internal ward (35 out of 73), followed by intensive care unit (ICU) (16 out of 73). The highest susceptibility was observed to glycopeptides category (100%), and the lowest susceptibility was observed to erythromycin (54.7%), followed by cefoxitin (49.3%). Out of the 73 isolates, 32 (43.8%) were found to be methicillin-resistant *S. aureus* (MRSA) isolates. Among MRSA isolates, 96.8 and 12.5% were MDR and XDR, respectively. All of the MRSA isolates, were susceptible to vancomycin. No PDR phenotype was observed among the isolates as all of them were sensitive to vancomycin (100%).

Conclusion: Based on the obtained results, the highest and lowest antibiotic resistance was observed against erythromycin and vancomycin, respectively, which is consistent with similar studies conducted in the country. Therefore, these antibiotics should not be used in the empirical therapy of *S. aureus* infections.

Keywords: Drug -resistance, Phenotype, Prevalence, *Staphylococcus aureus*.

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Introduction

Staphylococcus aureus is found everywhere in the environment and inhabits on the skin of approximately 20% of the world population as normal flora. Also, 60% of the population are sometimes asymptomatic carriers of it during their life time. However, it is an opportunistic pathogen when entering the bloodstream and tissues [1]. *S. aureus* is responsible for both community-acquired and hospital-acquired infections. It causes a wide variety of diseases, including skin infections, pneumonia, osteomyelitis, meningitis, toxic shock syndrome, endocarditis, and septicemia [2].

At present, *S. aureus* is the most important and the second leading cause of infection among hospitalized patients and outpatients, respectively. In recent years, it has become the most important cause of nosocomial pneumonia and a major cause of bloodstream infections worldwide [3]. This bacterium is able to adapt to different antibiotics and environmental conditions [1, 3]; thus, it could rapidly develop antibiotic resistance and spread in the hospital environment [4]. As a result, multidrug resistant (MDR) *S. aureus* strains including methicillin-resistant *S. aureus* (MRSA) have emerged [3]. The emergence and spread of MDR strains is a serious global threat to public health and treatment of staphylococcal infections [1]. Infections caused by these organisms are a serious threat, especially for patients, as they increase morbidity, mortality, length of hospital stay, and costs [4]. Currently, MRSA annually causes nearly 95,000 cases of invasive infections and 19,000 deaths in the USA, which is higher than the mortality rates of other threatening diseases such as AIDS, hepatitis, and combined tuberculosis and influenza [5]. Therefore, knowledge of regional antimicrobial resistance profiles and prevalence of MRSA could be useful for physicians in policy-making related to

empirical therapies, healthcare settings, and infection control within the country [6].

Recently, there have been some reports about the widespread prevalence of MDR *S. aureus* strains and also the increase in the number of extensively drug resistant (XDR) and pan drug resistant (PDR) phenotypes amongst *S. aureus* strains all over the world [7].

Objectives: The present study aimed to determine the incidence of MDR, XDR, and PDR phenotypes in *S. aureus* strains in a teaching hospital in Gorgan, Golestan province, Iran. As described by Magiorakos et al. (2012), MDR is defined as non-susceptibility to ≥ 1 antimicrobial agent in ≥ 3 antimicrobial categories, XDR is defined as non-susceptibility to ≥ 1 antimicrobial agent in all the antimicrobial categories, except in ≤ 2 , and PDR is defined as non-susceptibility to all antimicrobial agents in all antimicrobial categories. In addition, according to Magiorakos et al. (2012), a MRSA is always considered as MDR [8].

Materials and Methods

Sample collection and Identification:

In this cross-sectional descriptive study, *S. aureus* strains were collected from patients admitted to an educational hospital in Gorgan, Northeast of Iran, from April to June 2019. They were collected from patients' clinical specimens, including blood, urine, wound, and sputum samples. The isolates were transferred to the laboratory of Islamic Azad University, Gorgan Branch. Information about patients, including sex, age, sample type, and admission ward type was recorded anonymously. To confirm the isolates, they were cultured on Manitol salt agar (Himedia Company, India) aseptically, and the plates were incubated at 37 °C for 24 hrs under aerobic conditions. Then the isolates were identified based of Gram staining, colonial morphology, catalase, OF, DNase, and Coagulase tests [9].

Antibiotic Susceptibility Assessment: Antimicrobial susceptibility tests were performed for the isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using the Kirby-Bauer disk diffusion method ^[10]. In this study, *S. aureus* ATCC 29213 strain (provided by Tehran University, Faculty of veterinary medicine) was used as a quality control. After incubation at 37°C for 18 hrs, the results were interpreted by measuring the inhibition zone diameter against each of the isolates. The susceptibility of the test isolates to each antibiotic was interpreted according to CLSI guidelines. Methicillin resistance was evaluated using 30 µg cefoxitin disk (≤21 mm indicated MRSA) and 1 µg oxacillin disk (≤10 mm indicated MRSA) ^[11]. The isolated bacteria were classified as MDR, XDR, and PDR as described by Magiorakos et al. (2012) ^[8]. The antimicrobial agents used to define MDR, XDR and PDR in *S. aureus* strains are

shown in Table 2. All discs were obtained from MAST Company (MAST Chemical Co, UK). Data were analyzed by SPSS software Version 16 using Chi-square test. A *p*-value less than .05 (*p*<.05) was considered as statistically significant.

Findings

A total of 73 isolates were identified as *S. aureus*. Of which 26 (35.5%) and 47 (64.5%) isolates were from males and females, respectively. The highest incidence rate of *S. aureus* (35.5%) was in the age group of 20 to 40 years. The majority of *S. aureus* isolates were collected from wound (31 out of 73) and sputum (19 out of 73) samples. Most of the isolates were recovered from internal ward (35 out of 73), followed by intensive care unit (ICU) (16 out of 73). The characteristics of 73 *S. aureus* strains isolated are showed in Table 1. There was no significant difference between different age groups and hospital

Table 1) Characteristics of 73 *S. aureus* strains isolated from different samples and wards

Age Groups		Total	0-20	20-40	40-60	>60	P-Value
Variables		No (%)	No (%)	No (%)	No (%)	No (%)	
Ward	Internal	35 (47.9)	0(0)	20 (57.1)	10 (28.5)	5 (14.2)	0.076
	ICU	16 (21.9)	0(0)	2 (12.5)	4 (25)	10 (62.5)	
	Neonatal	7 (9.5)	7 (100)	0(0)	0(0)	0(0)	
	Burn	4 (5.4)	0(0)	4(100)	0(0)	0(0)	
	Infectious	2 (2.73)	0(0)	0(0)	2 (100)	0(0)	
	Orthopedic	3 (4.1)	3 (100)	0(0)	0(0)	0(0)	
	Surgery	6 (8.2)	0(0)	0(0)	3 (50)	3(50)	
Specimens	Wound	31 (42.4)	0(0)	8 (25.8)	6 (19.3)	17 (54.8)	*0.037
	Urine	16 (21.9)	0(0)	3 (18.7)	5 (31.2)	8 (50)	
	Blood	7 (9.5)	2(28.5)	1(30)	2(28.5)	2(28.5)	
	Sputum	19 (26)	3 (15.7)	3 (15.7)	6 (31.5)	7 (36.8)	

*Significant difference between the study groups based on the Chi-Square test

Table 2) Antimicrobial susceptibility of 73 *S. aureus* isolates against 10 antimicrobial agents

Antimicrobial Categories	Antimicrobial Agents	Number of Isolates (%)		
		R	I	S
Aminoglycosides	Gentamicin (10 µg)	4 (5.4)	2 (2.7)	67 (91.7)
Ansamycins	Rifampin (10 µg)	5 (6.8)	0 (0)	68 (93.1)
Anti-staphylococcal b-lactams	Oxacillin (1 µg)	32 (43.8)	0 (0)	41 (56.1)
	Cefoxitin (30 µg)	36 (49.3)	0 (0)	37 (50.6)
Fluoroquinolones	Ciprofloxacin (5 µg)	8 (10.9)	3 (4.1)	62 (84.9)
Folate pathway inhibitors	Trimethoprim-sulphamethoxazole (1.25 µg)	11 (15)	1 (1.3)	61 (83.5)
Glycopeptides	Vancomycin (30 µg)	0 (0)	0 (0)	73 (100)
Macrolides	Erythromycin (30 µg)	40 (54.7)	9 (12.3)	24 (32.8)
Tetracyclines	Doxycycline (30 µg)	31 (42.4)	4 (5.4)	38 (52)
Lincosamides	Clindamycin (2 µg)	14 (19.1)	2 (2.7)	57 (78)

wards under study in terms of the isolation rate ($p=.076$).

Antimicrobial susceptibility of 73 *S. aureus* isolates against 10 antimicrobial agents from 9 categories is shown in Table 2. The highest susceptibility was shown to glycopeptides category (100%), and the lowest susceptibility was shown to erythromycin (54.7%), followed by cefoxitin (49.3%). Out of the 73 isolates, 32 (43.8%) were found to be MRSA. Among MRSA isolates, 96.8 and 12.5% were MDR and XDR, respectively. All MRSA isolates exhibited susceptibility to vancomycin. No PDR phenotype was observed among the isolates, as all the isolates were sensitive to vancomycin (100%).

Discussion

In recent years, the widespread prevalence of MDR *S. aureus* strains, the increase in the number of XDR and PDR phenotypes amongst *S. aureus* strains, and the limited therapeutic options for invasive infections caused by

drug-resistant strains have become one of the greatest challenges [7, 12]. Therefore, Knowledge of regional antimicrobial resistance patterns and prevalence of various phenotypes of drug resistance among clinical isolates of *S. aureus* could be useful for physicians in policy-making related to empirical therapies, hospital settings, and infection control within the country [6]. The present study aimed to determine the incidence of MDR, XDR, and PDR phenotypes in *S. aureus* strains in a teaching hospital in Gorgan, Golestan province, Iran. In this study, the highest antibiotic resistance was observed against erythromycin (54.7%), which is consistent with similar studies in Shahrekord, Zabol, and Tehran [13-15]. In agreement with some studies results, more than 90% of the isolates were susceptible to vancomycin [1, 3, 15-17]. Out of the 73 isolates, 32 (43.8%) were MRSA, this finding is in line with the results of the studies by Dibah et al. (2014)

[16] and Mir et al. (2019) [14] in Ardabil and Zabol, respectively; however, dissimilar prevalence rates have been reported by other researchers in Iran and other countries [1,3, 5, 7, 9, 13, 17, 18-19].

Among MRSA isolates, 96.8% were found to be MDR, which is similar to the finding of Kot et al. (2020) in Poland [20]. Based on the obtained results, 12.5% of MRSA isolates were XDR, while in the studies of Sabir et al. (2020) and Eshetu et al. (2018), 20 and 0.5% of the isolates were characterized as XDR [7, 21]. In the current study, none of the isolates were PDR, but in a study from Pakistan, 13.3% of the isolates were PDR [6].

Considering the results of all the mentioned studies, including the present study, the susceptibility rate of *S. aureus* isolates to antimicrobial agents and the incidence of drug-resistant isolates vary in different geographical regions. The prevalence rate of MDR, XDR, and PDR strains could be varied from 43 to 92.9, 0.5 to 20, and 0 to 13.3%, respectively [3, 6, 20-21]. Differences in antibiotics used, geographical distribution of resistant strains, and history of antibiotic use may be the reasons for these differences [4].

Knowledge of regional antimicrobial resistance profiles and prevalence of MRSA could be useful for adopting appropriate strategies to control the spread of resistant strains. Thus, detection, infection control policies, and tracking antibiotic resistance are highly suggested. Finally, sensible use of antibiotics, antibiogram testing to select a suitable antibiotic for infections treatment, and prevention of self-medication seem to be inevitable measures.

The limitation of the present study is that this study was a single-center study performed for only a three-month period in a teaching hospital in Gorgan. To reveal the growing trend of infections caused by drug-resistant bacteria with different phenotypes,

performing a multicenter study involving all types of medical systems in the region for at least one year is recommended.

Conclusion

Today, the emergence of MRSA and drug-resistant strains with different phenotypes has become a major concern worldwide [22]. In this study, the highest and lowest antibiotic resistance was observed to erythromycin and vancomycin, respectively, which is consistent with similar studies conducted in the country. In addition, the incidence of MRSA among the isolates was 43.8%, which emphasizes the importance of local and continuous monitoring of MRSA and MDR strains to reduce the threat of antimicrobial resistance as a current global challenge.

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