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***Correspondence**
Wadhah Hassan Edrees
Email:
edress2020@gmail.com

Prevalence of Methicillin- Resistant *Staphylococcus aureus* and Antibacterial Susceptibility among Patients with Skin and Soft Tissue Infection at Ibb City, Yemen

Rashad Saleh Al-Khawlany[1], Wadhah Hassan Edrees[2,3*], Ahamed Yehia AL-Jaufy[4], Mohammed AM Nasher[4], Wadee Abdullah Al-Shehari[1], Alariqi Reem[4], Maged Mohammed Almezgagi[4]

[1]Medical Microbiology Department, Faculty of Medical Sciences, Ibb University, Yemen.
[2]Medical Laboratory Department, Faculty of Medical Sciences, Al-Razi University, Yemen.
[3]Medical Microbiology Department, Faculty of Applied Sciences, Hajjah University, Yemen.
[4]Medical Laboratory Department, Faculty of Medical Sciences, Sana'a University, Yemen.

Abstract:
The increase of methicillin-resistant *Staphylococcus aureus* among patients is receiving more attention to health care. The present study aimed to evaluate the prevalence of Methicillin-resistant *S.aureus* (MRSA) between patients with infected skin and soft tissue at Ibb city, Yemen, and determine their antibacterial susceptibility profiles. A total of 189 patients were enrolled in this study between October 2007 and September 2008 at Al-Thawrah Hospital. Two specimens of each patient (1 of the nose and 1 of the infected site) were collected by sterile swabs and the MRSA was isolated and identified by standard bacteriological methods. The antibacterial susceptibility profiles were performed for the isolated MRSA. The overall prevalence rate of MRSA infection was 9.3% isolated from the nose and infected sites. The prevalence of isolated MRSA from the nose among males (45.2%) was higher than in females (4.8%). While a similar prevalence of MRSA among males and females with infected sites was found. The high prevalence of MRSA was among the age group of 18 to 29 years. Most MRSA isolates were isolated from the abscesses, ulcers, wound infection, and post-operation wounds. All isolates of MRSA were sensitive to vancomycin and completely resistant to oxacillin. The low rate prevalence of MRSA isolates in this work is considered the precursor for serious health problems. So, avoiding the empirical use of antibiotics and introducing effective guidelines for using antibiotics is required to control MRSA transmission in the hospital. The results of antimicrobial susceptibility testing should be combined with clinical information and experience when selecting the most appropriate antibiotic.

INTRODUCTION

Staphylococcus aureus often exists asymptomatically on most of the parts of the human body as a commensal microorganism. It colonizes the skin surface, oral, skin glands, and respiratory and gastrointestinal tracts of healthy persons (Olowe *et al*., 2007). Around 20% of individuals are persistent nasal carriers of *S. aureus* and about 30% are recurrent carriers, while about 50% are non-carriers (Wertheim *et al*., 2005).

S. aureus is considered as one of the most common hospital-acquired bacterial infections and continuously resistant increasing to antibiotics since 1942 when penicillin approved for diseases treatment (Lobanovska and Pilla, 2017). The action of penicillin representing inhibiting penicillin-binding protein (PBP), which plays a vital role in the bacterial cell-wall synthesis that eventually leads to dying cell due to leakage of the cell components. In brief, *S. aureus* strains started showing resistance to methicillin treatment and became recognized as MRSA (Rajaduraipandi *et al*., 2006; Hena and Sudha, 2011).

The MRSA term is used to explain *S. aureus* strains that developed resistance to *ß*-lactam antibiotics, like methicillin, cephalosporins, and oxacillin (Al-Anazi, 2009; Hena and Sudha, 2011). Also, the MRSA strains are accountable for numerous cases of nosocomial and community-acquired pathogenic bacteria and deaths. The mortality rates resulting from MRSA infection are higher than infections due to methicillin-susceptible *S. aureus* (MSSA) (Hena and Sudha, 2011; Peters *et al*., 2013).

In a hospital, the MRSA infection is generally associated with complicated soft tissue and skin infections among patients who stay for a long-time resulting in increased costs of healthcare (Nathwani *et al*., 2014; Edelsberg *et al*., 2015). The patient acquisitions the MRSA infections regularly throughout hospitalization particularly after an operation and throughout stays in long-term care facilities. Also, MRSA infections are most prevalent among patients who have indwelling vascular catheters for treatment purposes (Lakhundi and Zhang, 2018).

The prevalence of MRSA infections was reported by many researchers. Globally, the prevalence of MRSA infections was ranged between 13%-74% (Köck *et al*., 2010), while in 2014, it ranged from 0.9% to 56% in Europe (Hassoun *et al*., 2017). Also, the Center for Disease Control and Prevention (CDC) documented that 50% of isolated *S. aureus* from intensive care units in the US were resistant to methicillin (Cassandra *et al*., 2003).

A study conducted on seven (7) hospitals in Riyadh, Saudi Arabia, revealed that the prevalence of MRSA infections was ranged from 12-49% (Ahmad *et al*., 2009). In Bahrain, the MRSA infections were 4.3% reported among patients with complicated skin and soft tissue infections (Lopez *et al.,* 2017). In Yemen, an investigation by Alyahawi et al. (2018) found that the frequency of MRSA infection was 17.6% among patients in Sana'a. Various antibiotics, plant parts, or complex compounds could be an important source of new therapeutic agents against pathological damage due to free radicals and microbial infections (Iqbal and Ashraf, 2019; 2020; Mouffouk *et al.,* 2019).

The lack of data about the MRSA infections prevalence among patients with skin and soft tissue infection stays for a long-time in hospital at Ibb City, Yemen. So, this work aimed to evaluate the occurrence of Methicillin-resistant *S. aureus* colonization and infection among patients with skin and soft tissue infections admitted to Al-Thawrah hospital in Ibb city, Yemen, and determine their antibacterial susceptibility profiles.

MATERIALS AND METHODS

Study design and area

This is a cross-sectional descriptive study carried out at Al-Thawrah Hospital in Ibb city, Yemen, between October 2007 and September 2008.

Ethical approval

The protocol of this work was approved by the Medical Ethics Committee of the Sana'a University, Medical College, and health officials in Ibb city. Before starting data and specimens' collection, the aims and methods of study were clarified to participated patients and take them an agreement.

Selection of participants

One hundred and eighty-nine (189) patients (aged ≥18 years) have prospectively participated in this study with main complaints of skin and/or soft tissue infection(s) and with a positive culture of *Staphylococcus aureus*. The patients were admitted into the inpatient department suffering from one of the following types of skin or soft tissue infections: necrotizing soft tissue infection, cellulitis, wound infection, boil, ulcer, or abscess.

Data collection

A structured questionnaire was subjected for each patient and filled with the patient's personal and clinical information by interview face to face. The study questionnaire was approved by the Sana'a University, Yemen, and authorization to start data collection.

Samples collection and processing

Two specimens from each patient (1 nose plus 1 infection site isolate) were obtained from the anterior nose and the infection sites for S. aureus by sterile swabs. Specimens were transported to the laboratory and directly cultured on Mannitol salt agar individually and incubated aerobically at 35°C for 24-48 h (Bannerman, 2003).

Identification of *S. aureus* isolates

All isolated *S. aureus* were identified according to standard microbiological procedures. The morphological characteristics such as colony properties and Gram stain as well as the biochemical tests were performed to confirm and purification of the *S. aureus* (Bannerman, 2003; Iqbal *et al.,* 2015; Saleem *et al.,* 2018; Shahzad *et al.,* 2017; Yunus *et al.,* 2016).

However, all isolates of *S. aureus* were subjected to Methicillin resistance by using the Kirby-Bauer disc diffusion technique with Methicillin and oxacillin discs (Oxoid) on Mueller-Hinton agar and incubated at 35°C for 24 h. The inhibition zone of bacterial growth equal to or less than 9 and 10 mm around the Methicillin and oxacillin discs, respectively, were considered resistant (CLSI, 2016).

Antibacterial sensitivity testing

Susceptibility of all Methicillin-resistant *S. aureus* isolates to tested antibiotic agents was determined by the Kirby-Bauer disc diffusion procedure on Mueller-Hinton agar according to CLSI protocol (CLSI, 2016; Iqbal *et al.,* 2016). The antibacterial drug discs that were used in this study were oxacillin, clindamycin, erythromycin, ofloxacin, tetracycline, gentamicin, and vancomycin (HiMedia, India).

Statistical analysis

The obtained data were analyzed by the Epi Info 2000-CDC. The Pearson Chi-square ($\chi 2 \geq 3.84$) test was used to analyze the significance of the difference in proportion and the P-value (≤ 0.05) was considered statistically significant.

RESULTS

The distribution of collected samples according to age and gender was listed in Table (1). The age groups of patients were ranged from 18 to >59 years, with a mean age of 38.6 years. The majority of the study group, 75 (39.7%) patients were at the age of 18-29 years old.

The present results regarding types of infection showed the abscess was the most common type of infection among males and females (78%). As regards the type of infection between males and females, the ulcer was statistically significant, (*P* = 0.01) (Table 2).

The current results showed the MRSA and MSSA in addition to other species that were isolated from the nose and other infected sites. It was reported that the MSSA isolated from the

nose was higher than those isolated from infected sites (Table 3).

Table 1. Distribution of collected samples according to age and gender

Age group (in years)	Male		Female		Total	
	No.	%	No.	%	No.	%
18 – 29	61	41.5	14	33.3	75	39.7
30 – 39	23	15.6	7	16.7	30	15.9
40 – 49	15	10.2	9	21.4	24	12.7
50 – 59	17	11.6	4	9.5	21	11.1
> 59	31	21.1	8	19.1	39	20.6
Total	147	100	42	100	189	100

Table 2. The types of infection among male and female patients

Type of infection	Male	Female	Total	RR	χ^2	P
	No.(%)	No.(%)	No.(%)			
Cellulitis	5 (3.4)	0(0)	5(2.6)	1.3	1.47	0.22
Abscess	114 (77.6)	34(81)	148(78.3)	0.96	0.22	0.63
Ulcer	1 (0.7)	3(7.1)	4(2.1)	0.3	6.59	0.01
Boil	9 (6.1)	4(9.5)	13(7)	0.88	0.59	0.44
Wound infection	8(5.4)	0(0)	8(4.2)	1.3	2.4	0.12
Accident	1(0.7)	0(0)	1(0.5)	1.3	0.29	0.6
Surgical wound	4(2.7)	0(0)	4(2.1)	1.22	0.9	0.34
Post operation	5(3.4)	1(2.4)	6(3.2)	1.07	0.11	0.73
Total	147 (100)	42 (100)	189 (100)			

$P < 0.05$: significant, $\chi^2 \geq 3.84$: significant.

Table 3. The MRSA, MSSA, and other species isolated from the nose and infected sites.

Culture results	Nose		Infected sites		Total	
	No.	%	No.	%	No.	%
MRSA	14	7.4	21	11.1	35	9.3
MSSA	64	33.9	60	31.7	124	32.8
Other than coagulase (+) ve *Staphylococcus*	75	39.7	65	34.4	140	37
No growth	36	19	43	22.8	79	20.9
Total	**189**	**100**	**189**	**100**	**378**	**100**

The current work revealed that the number of isolated MRSA from the nose of males was higher than those isolated from females. However, the difference between the numbers of MRSA isolated from the nose of both genders was not statistically significant, (P= 0.077). The frequency rate of isolated MRSA from the infected sites of females was significantly (P= 0.0001) higher than that in the males (Table 4).

Table 4. The isolated MRSA and MSSA from the nose and infected sites according to gender.

Gender	From the nose					From infected sites				
	MRSA No. (%)	**MSSA No. (%)**	***RR***	**χ^2**	***P***	**MRSA No. (%)**	**MSSA No. (%)**	***RR***	**χ^2**	***P***
Male (n= 147)	12 (8.2)	45 (30.6)	0.7	3.12	0.077	16 (10.9)	48 (32.7)	1.14	0.25	0.61
Female (n = 42)	2 (4.8)	19 (45.2)	1.5	3.12	0.077	5 (11.9)	12 (28.6)	0.3	25.7	<0.0001
Total (n= 189)	**14 (7.4)**	**64 (33.9)**				**21 (11.1)**	**60 (31.7)**			

P < 0.05: significant χ^2 ≥ 3.84: significant.

Table 5 revealed the frequency rate of MRSA of patients according to age groups. It was found that the high number of MRSA isolates from the nose and infected sites were isolated among the age group of 18 to 29 years old.

Table 5. The isolated MRSA and MSSA from the nose and infected sites according to age group

Age groups (Years)	From the nose					From infected sites				
	MRSA No. (%)	**MSSA No. (%)**	***RR***	**χ^2**	***P***	**MRSA No. (%)**	**MSSA No. (%)**	***RR***	**χ^2**	***P***
18 – 29 (n= 75)	8 (10.6)	22 (29.3)	0.55	2.5	0.11	6 (8)	25 (32)	0.38	5.77	0.016
30 – 39 (n = 30)	1 (3.3)	8 (26.6)	0.66	0.16	0.68	2 (6.7)	7 (20)	1.7	0.52	0.47
40 – 49 (n = 24)	2 (8.3)	8 (33.3)	2.6	2.24	0.13	4 (16.7)	10 (41.7)	2.75	3.44	0.06
50 – 59 (n = 21)	0 (0)	8 (38)	0.0	1.04	0.306	3 (14.3)	8 (38.1)	2.86	2.8	0.09
> 59 (n = 39)	3 (7.7)	18 (46.2)	0.64	0.58	0.44	6 (15.4)	10 (25.6)	2.3	3.04	0.08
Total (n= 189)	**14 (7.4)**	**64 (33.9)**				**21 (11.1)**	**60 (31.7)**			

P < 0.05: significant. χ^2 ≥ 3.84: significant.

Regarding the soft tissue infections, it was found that the high numbers of MRSA isolates from the abscesses, ulcers, wound infection, and post-operation wounds were significantly higher than those isolated from other types of tissue infections (Table 6).

The antibacterial sensitivities of MRSA isolates against the different clinically used antibacterial drugs are shown in Table (7). Vancomycin was the most effective antibacterial against all the MRSA isolates, whereas all the MRSA isolates were resistant to oxacillin.

Table 6. The isolated MRSA and MSSA from the different types of soft tissue infections

Type of infection	MRSA	MSSA	RR	χ2	P
	No. (%)	No. (%)			
Abscess (n= 148)	14 (9.5)	50(33.8)	0.13	86.22	0.0001
Ulcer (n= 4)	2(50)	0(0)	ND	93	0.0001
Boil (n= 13)	1(7.7)	6(46.2)	2.3	0.62	0.43
Wound infection (n= 8)	3(37.5)	0(0)	ND	68	0.0001
Surgical wound (n = 4)	0(0)	2(50)	0.0	0.04	0.83
Post operation (n = 6)	1(16.7)	2(3.3)	15.3	9.02	0.002
Total (n= 189)	21(11.1)	60(31.7)			

P < 0.05: significant. χ2 ≥ 3.84: significant ND: Non defined

Table 7. The antibacterial sensitivity patterns of MRSA isolates

Antibacterial drug	MRSA of nose (n = 14)		MRSA of infected sites (n= 21)		Overall MRSA (n = 35)	
	No.	%	No.	%	No.	%
Vancomycin	14	100	21	100	35	100
Clindamycin	9	64.3	16	76.2	25	71.4
Gentamicin	10	71.4	14	66.6	24	68.6
Tetracycline	9	64.3	14	66.6	23	65.7
Ofloxacin	8	57.1	12	57.1	20	57.1
Erythromycin	7	50	12	57.1	19	54.3

DISCUSSION

MRSA infections have recently become a prime hospital-acquired pathogen for patients attending hospitals during the past ten years. Besides, the continuous increase of MRSA infection among patients and the community is receiving more attention from health professionals (Cooke and Brown, 2010; Al-Haik *et al*., 2017; Alhlale *et al*., 2019).

In the current study, it was found that the overall rate of MRSA infection was 9.3% of the nose and infected sites, 7.4% and 11.1% of the nose and infected sites, respectively. This result is higher than finding in Denmark 0.8% (Stefani and Varaldo, 2003) and Bahrain 4.3% (Lopez *et al*., 2017).

However, this finding is lower than reported in Japan 60% (Hashimoto *et al.*, 1994), USA 35% (CDC, 2002), 54% in Portugal, 34% in Greek hospitals, 28% in the UK, and 43-58% in Italy (Stefani and Varaldo, 2003), Germany 17.5% (Stefani and Varaldo, 2003), Saudi Arabia 12-49% (Ahmad *et al.,* 2009), and Sana'a 17.6% (Alyahawi *et al*., 2018).

The variation in results obtained from different countries may be affected by differences in patient populations, activities of infection control, practices of hospital care, study time, and the biological characteristics of the *S. aureus* strains.

Moreover, the high rate of MRSA infection among patients with infected sites in this study may be resulting from using antibiotics without prescription. Also, the use of antibiotics with broad-spectrum such as imipenem for infection treatment maybe increase the MRSA rate and other resistant pathogens (Alyahawi *et al*., 2018; Ashraf and Iqbal, 2020; Edrees and Anbar, 2020).

The *S. aureus* nasal colonization rates among selected patients in this study were within the

normal range, the nasal colonization of *S. aureus* was 41.2% and the colonization rate may range from 20 to 55% in the normal adult population (Marjolcin *et al.,* 1999; Orrett*,* 1999). This value is within the range that might be predictable among cases with active skin infections (Grundmann *et al.,* 2002).

In the present work, it was found that 7.4% of nasal of isolated *S. aureus* were MRSA. These results are first-time in a group of non-hospitalized, largely non–nursing facility patients and are higher than might be expected among healthy individuals as reported 3% (Ellis *et al.,* 2004). Moreover, the present finding is higher than the rate of MRSA among 833 homeless living in San Francisco in 1999 and 2000 which was 12% of nasal *S. aureus* and 2.8% of all nasal isolates (Charlebois *et al.,* 2002), but lower than that reported in Chicago area hospital in which the MRSA represents 60% of all *S. aureus* (April *et al.*, 2008).

In the present study, the rate of MRSA colonization among males was (8.2%) higher than among females (4.8%). In contrast, the rate of MRSA infection among females (11.9%) was slightly higher than in males (10.9%). Also, the highest number of MRSA isolates was reported among the age group of 18 to 29 years old. In similar work by Valderrama-Beltrán et al. (2019) found that the higher rate of MRSA isolates was (54.2%) reported among the age group of 18-44 years old as well as the males had 53.7% higher MRSA when compared to the females by 46.3%.

Similar findings have been reported in Saudi Arabia that showed the MRSA was more common among males (59%) (Atif and Aiman, 2006). A study by Alyahawi et al. (2018) observed that the male had a higher rate (83.29%) of MRSA than females (17.6%). Also, they found a higher prevalence of MRSA among the age of 46-60 years old.

The most important result was the strong relationship between the abscess with the occurrence of MRSA, both in the nose and infection site (P >0.0001). This result is in agreement with preceding reports document that the MRSA outbreaks responsible for the skin and soft tissue infections in communities (Dufour *et al.,* 2002; Baggett *et al.,* 2004). As noted by another study, the abscess is the most commonly documented presentation of community-acquired MRSA (Cohen and Kurzrock, 2004).

However, a highly significant association was found between MRSA and ulcers, wound infection, and post-operation infection, where MRSA formed 50% of ulcers (P <0.0001), 37.5% of wound infection (P <0.0001), and 16.7% of post-operation infection (P=0.002).

All isolated MRSA in the present work showed uniform resistance to oxacillin (a surrogate for Methicillin). However, most MRSA isolates were susceptible to several antibacterial other non–β-lactam, which includes 100% susceptible to vancomycin, and was similar to that reported in Saudi Arabia (Atif and Aiman, 2006; Alhlale *et al.,* 2020) and the USA (Bradley *et al.,* 2005). Other studies found that the increase of MRSA in hospitals or communities lead to the increasing demand for vancomycin for treating MRSA (Freidlin *et al.*, 2007; Edrees and Al-Awar, 2020).

In this study, it was found that MRSA which was isolated from infected sites were more susceptible than MRSA isolated from the nose to other antibacterial drugs. The susceptibility to other antibacterial drugs in this study was less than reported in the USA (Bradley *et al.*, 2005) and Hong Kong (Leung *et al.*, 2008), but it is higher than what is reported in Saudi Arabia (Atif and Aiman, 2006) and Yemen (Al-Rubasi and Al-Muaid, 2007).

In the present work, the rate of fully MRSA susceptible to clindamycin was 71.4%. This antibacterial drug has been used successfully to treat MRSA infections in community settings and it also can suppress MRSA virulence factors which include Panton-Valentine Leukocidin (Stevens *et al.*, 2007).

The limitation in the present work, this work was conducted in one hospital in Ibb city and was restricted to patients with skin and soft tissue infections only to study MRSA infection and colonization. MRSA nasal colonization was only

tested because the nasal is considered the most frequent reservoir for *S. aureus*.

CONCLUSION

The prevalence of MRSA isolates even at low rates in this study considers the precursor for the health problems in a feature. Therefore, awareness of and demanding attention to strict adherence to infection control procedures can reduce MRSA prevalence among patients in hospitals. The regular cleaning and disinfecting of hospital environmental surfaces, use of personal protective equipment, hand washing, disposable instrument, and devices will reduce the MRSA in the hospital. Also, it must perform the susceptibility testing for isolated *S. aureus* and avoid use the initial empirical treatment for *S. aureus* particularly the antibiotics that lead to an increase in MRSA in hospitals and communities to prevent the prevalence of MRSA among communities.

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CONFLICT OF INTEREST

The authors declare that this article's content has no conflict of interest.

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