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Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* from the Isolated Wound Culture in the Northwest Region, Kingdom of Saudi Arabia

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Abstract

The present study aimed to investigate the antibiotic susceptibility pattern of Methicillin-resistant *Staphylococcus aureus* (MRSA) in isolated wound cultures of the patients admitted in King Khalid Hospital, Tabuk, Kingdom of Saudi Arabia. A retrospective cohort study of 54 patients admitted with wound infections in the surgical department. Ethics committee approval was granted by the University of Tabuk and King Khalid Hospital, Kingdom of Saudi Arabia. Fifty-four adult patients (>18 years old) diagnosed with moderate to severe skin and soft tissue infections were included in the study. 26 patients with isolated cultures of MRSA were compared with 28 patients with Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolated cultures using Graph pad prism 4.0 version statistical databases. Overall, there was no significant difference in sensitivity (P=0.2445) and resistance (P=0.4215) between MRSA and MSSA cultures. However, it is interesting findings that Oxacillin and Fusidic acid had higher resistance in MRSA isolated cultures compared MSSA culture, on the other hand, Linezolid, Tigecycline and Nitrofurantoin shows 100% sensitivity in both MRSA and MSSA isolates. No significant difference between male and female regarding the sensitivity (P=0.0638) and resistance (P=0.3638). The current study emphasizes that Tigecycline, Nitrofurantoin and Fusidic acid were the best drugs in both MRSA and MSSA isolates. While, oxacillin showed 100% resistance to MRSA; but retain its efficacy on MSSA isolates.

Keywords: Antibiotic, Cultures, Sensitive

1. Introduction

Wound infections have been a problem in the field of medicine for a long time. The presence of foreign materials increases the risk of serious infection even with relatively small bacterial inoculums[1]. Advances in control of infections have not completely eradicated this problem because of the development of drug resistance[2]. The widespread misuses of antibiotics over a long time have led to emergences of resistant organisms contributing to morbidity and mortality[3–5]. Antimicrobial resistance can increase complications and costs associated with procedures and treatment[6].

The most common isolated aerobic microorganisms were *Staphylococcus aureus*, Coagulase-negative *staphylococci* (CoNS), *Enterococci*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* species, *Proteus mirabilis*, *Candida albicans* and *Acinetobacter*[7,8].

Wound infections can be caused by different groups

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of microorganisms like bacteria, fungi, and protozoa. However, different microorganisms can exist in polymicrobial communities especially in the margins of wounds and in chronic wounds[9]. The infecting microorganism may belong to aerobic as most commonly isolated aerobic microorganism include *Staphylococcus aureus*, Coagulase-negative *staphylococci* (CoNS), *Enterococci*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* species, *Proteus mirabilis*, *Candida albicans* and *Acinetobacter*[7,8].

The genus *Staphylococcus* includes pathogenic organisms in which *Staphylococcus aureus* is most important. It overcomes most of the therapeutic agents that have been developed in the recent years; hence, the antimicrobial chemotherapy for this species has always been empirical[10]. After the emergence of MRSA as a nosocomial pathogen in the early 1960s[11], an increasing number of outbreaks due to MRSA infections in hospitals have been reported from many countries, ranging from abscesses to life-threatening sepsis, endocarditis, and osteomyelitis[12].

Early diagnosis of MRSA and treatment by following standard antibiotic guidelines will reduce morbidity and mortality rate in tertiary care hospitals. Understanding the antibiotic susceptibility pattern of MRSA in a specific geographic region is vital in the selection of the appropriate empirical antibiotic therapy. The present study was carried out to determine the antibiotic susceptibility pattern of MRSA from the isolated wound cultures, and update the clinicians about the optimal antibiotics to treat wound infections.

2. Methods

A retrospective study conducted at King Khalid Hospital in Tabuk City during the period from June to December 2014, following the ethical guidelines for patient data privacy fifty-four wound cultures of adult patients (>18 years old) and sensitivity forms referred from the surgeons from the surgical department were reviewed. The research was cleared by the ethical committee of the University of Tabuk and King Khalid Hospital, Tabuk, Kingdom of Saudi Arabia. Graph pad Instat Prism 4.0 version was used for data analysis. The t-test was performed to compare the sensitivity and resistance pattern between MRSA and Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates.

Information collected includes: sex, type of specimen included is wounds. Media used for bacterial isolation was carried using the serial dilution technique on the wound swabs to isolate *Staphylococcus aureus*.

2.1 Specimen Collection

Samples were collected from the patients with complaints of wound sepsis. The wound samples were collected by using a sterile cotton swab, the inner surface of the infected area was swabbed gently and then the swabs were transported to the laboratory.

2.2 Bacteriology and Antibiotic Susceptibility Testing

Bacterial isolation according to morphology followed according to Benson *et al.*, 1994[13]. Antibiotic sensitivity test was performed by using the Kirby-Bauer disk diffusion method recommended by the National Committee for Clinical Laboratory Standard (NCCLS, 2000)[14] for the following antibiotics: cefoxitin, penicillin, oxacillin, gentamicin, tobramycin, levofloxacin, moxifloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tetracycline, tigecyclin, fosfomycin, nitrofurantoin, fusidic acid, mupirocin, rifampicin, trimethoprim-sulphamethoxazole etc. The Vitex 12, Phoenix, and Micro scans were used. MRSA test was performed using cefoxitin 30 μg disc on Mueller-Hinton agar with 24 hours incubation at 35°C. The antibiotic discs used for the susceptibility tests were from Hi-Media Laboratories Pvt. Limited, India. A zone of inhibition less than 10 mm or any discernible growth within a zone of inhibition was indicative of methicillin resistance. *Staphylococcus aureus* ATCC 25923 (Manassas, VA, USA) was used as a standard control strain.

Table 1. Minimum inhibitory concentration (MIC) of the antibiotics

S.No.	Antibiotic	MIC*
1	Cefoxitin	POS**
2	Penicillin	≥0.5
3	Oxacillin	≥4
4	Gentamicin	≥16
5	Tobramycin	8
6	Levofloxacin	4
7	Moxifloxacin	1
8	Erythromycin	≥8
9	Clindamycin	≤0.25
10	Linezolid	2
11	Teicoplanin	2
12	Vancomycin	≤0.5
13	Tetracycline	2
14	Tigecyclin	≤0.12
15	Fosfomycin	32
16	Nitrofurantoin	≤16
17	Fusidic acid	≥32
18	Mupirocin	≤2
19	Rifampicin	≤0.5
20	Trimethoprim+Sulphamethoxazole	≥320

*MIC: Minimum Inhibitory Concentration
**POS: Positive

3. Results

Out of 199 wound sepsis samples at the King Khalid Hospital, Tabuk, Kingdom of Saudi Arabia, July to December 2014, predominant *Staphylococcus aureus* (27%; n=54) followed by *Pseudomonas aeruginosa* (20%; n=39), *E. coli* (15%; n=30) etc. (Figure 1). Among the *Staphylococcus aureus*, MRSA resistance (48%) and 28 (52%) with MSSA resistance (Figure 2) observed in the *Staphylococcus aureus* isolates. Gender distribution reveals predominant male population in MRSA (n=25; 96%) and MSSA resistance samples (n=16; 57%) (Figure 3).

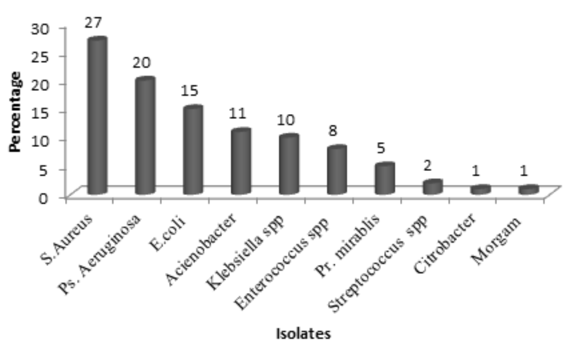

Figure 1. Percentage of isolates from wound sepsis (n=199).

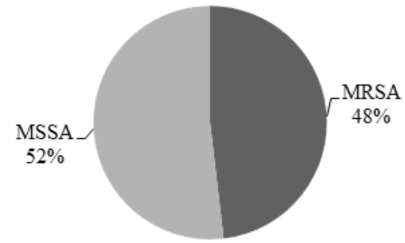

Figure 2. Percentage of MRSA and MSSA among the culture sample (n=54).

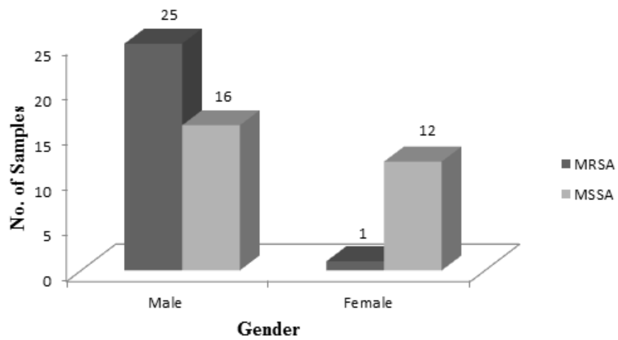

Figure 3. Gender distribution among the culture sample (n=54).

3.1 Association Antibiotic Sensitivity and MRSA

Linezolid, Teicoplanin, Vancomycin, Tigecycline, Nitrofurantoin and Mupirocin were found to have 100% sensitivity against MRSA isolates; however, Penicillin and Oxacillin were found to be 100% resistant. On the other hand, Gentamycin, Tobramycin, Linezolid, Tetracycline, Fosfomycin and Nitrofurantoin were found to have 100% sensitivity against MSSA isolates. Both sensitivity (P=0.2445) and resistance (P=0.4215) were found statistically not significant. However, a significant difference was observed between isolates of MRSA sensitivity and resistance (P<0.0001). Similarly, MSSA isolates also shows a significant difference (P<0.0001). Interestingly, the present study observed some findings as follows 1. Oxacillin shows 96% sensitivity in MSSA isolates and 0% sensitivity in MRSA isolates, 2. Fusidic acid was 93% sensitivity in MSSA Vs. 46% in MRSA isolates. 3. Linezolid, Tigecycline, and Nitrofurantoin shows 100% sensitivity in both MRSA and MSSA isolates (Table 2).

Table 3. Antibiotic sensitivity in Methicillin sensitive staphylococcus aureus among the gender (n=54)

Antibiotic	Male (16)				Female (12)			
	S	%	R	%	S	%	R	%
Cefoxitin	0	0	16	100	0	0	12	100
Penicillin	0	0	16	100	0	0	12	100
Oxacillin	15	93	1	7	12	100	0	0
Gentamicin	16	100	0	0	12	100	0	0
Tobramycin	16	100	0	0	12	100	0	0
Levofloxacin	13	81	3	19	11	92	1	8
Moxifloxacin	12	75	4	25	11	92	1	8
Erythromycin	14	88	2	12	11	92	1	8
Clindamycin	15	93	1	7	11	92	1	8
Linezolid	16	100	0		12	100	0	0
Teicoplanin	15	93	1	7	12	100	0	0
Vancomycin	15	93	1	7	11	92	1	8
Tetracycline	16	100	0	0	12	100	0	0
Tigecyclin	16	100	0	0	12	100	0	0
Fosfomycin	16	100	0	0	12	100	0	0
Nitrofurantoin	16	100	0	0	12	100	0	0
Fusidic acid	15	93	1	7	10	62	2	38
Mupirocin	15	93	1	7	12	100	0	0
Rifampicin	4	25	12	75	9	56	3	44
Trimethoprim+Sulphamethoxazole	15	93	1	7	12	100	0	0

Male Vs. Female culture sensitivity - P=0.0638; Not significant
Male Vs. Female culture resistance - P=0.3638; Not significant
Male sensitivity Vs. Resistance - P<0.0001; Significant
Female sensitivity Vs. Resistance - P<0.0001; Significant

3.2 Antibiotic Sensitivity in MSSA among the Gender Distribution

In the present study, we thought to investigate the sensitivity of MSSA among the gender. While cefoxitin and penicillin were found to have 100% resistance in both genders, Tetracycline, Tigecycline, Nitrofurantoin were found to have 100% sensitive in both genders. On the contrary, rifampicin was found to have 25% and 56% sensitivity for male and female respectively. However, no statistical significance between male and female in both culture sensitivity and resistance. Although, a significant difference was noted between sensitivity and resistance in both male and female (P<0.0001) Table 3.

4. Discussion

The aim of the present study is to understand the association of wound culture and MRSA, since the wound is most commonly caused by MRSA, which is well documented already[15]. MRSA is a challenging issue in healthcare during the selection of antibiotic across the world. In Saudi Arabia, its prevalence keeps uprising with significant morbidity and mortality. The present study shows 48% MRSA among the fifty-four isolates, by a study conducted the by Ghazal *et al*., 2010 in Saudi Arabia[16].

Draghi *et al*., 2006[17] and Neela *et al*., 2008[18] already reported the sensitivity of Vancomycin, Linezolid, and Teicoplanin. The present study substantiates the same result and in addition, to that the present study exploring the sensitivity of Nitrofurantoin and Mupirocin. Although Madani *et al*., 2001[19] reported vancomycin resistance is emerging problem, our study shows 100% sensitivity (Table 2).

Nagwa *et al*., 2012[20] indicates variable resistance towards tetracycline, rifampicin, trimethoprim/sulphamethoxazole, levofloxacin, erythromycin, and clindamycin, etc. and the present study substantiates the same (Table 2). However, the present study indicates levofloxacin found to have 12% resistance in contrast to the result of Nagwa *et al*., 2012[20] showing 66% resistance. This discrepancy mandates further study with larger samples.

Oxacillin was found to have 100% resistance in MRSA isolates and 4% resistance in MSSA isolates. Yasoka Hosaka *et al*., 2006 already addressed this issue in their

Table 3. Association antibiotic sensitivity and methicillin resistant *Staphylococcus aureus* (n=54)

Antibiotic	MRSA (26)				MSSA (28)			
	S	%	R	%	S	%	R	%
Cefoxitin	21	81	5	19	0	0	28	100
Penicillin	0	0	26	100	0	0	28	100
Oxacillin	0	0	26	100	27	96	1	4
Gentamicin	20	77	6	23	28	100	0	0
Tobramycin	18	69	8	31	28	100	0	0
Levofloxacin	23	88	3	12	25	92	3	8
Moxifloxacin	23	88	3	12	24	85	4	15
Erythromycin	20	77	6	23	25	92	3	8
Clindamycin	20	77	6	23	26	93	2	7
Linezolid	26	100	0	0	28	100	0	0
Teicoplanin	26	100	0	0	27	96	1	4
Vancomycin	26	100	0	0	26	93	2	7
Tetracycline	19	73	7	27	28	100	0	0
Tigecyclin	26	100	0	0	28	100	0	0
Fosfomycin	25	96	1	4	28	100	0	0
Nitrofurantoin	26	100	0	0	28	100	0	0
Fusidic acid	12	46	14	54	27	93	2	7
Mupirocin	26	100	0	0	27	96	1	4
Rifampicin	6	23	20	77	7	26	21	74
Trimethoprim+Sulphamethoxazole	20	77	6	23	28	100	0	0

MRSA Vs. MSSA culture sensitivity - P=0.2445; Not significant
MRSA Vs. MSSA culture resistance - P=0.4215; Not significant
MRSA sensitivity Vs. Resistance - P<0.0001; Significant
Non- MRSA sensitivity Vs. Resistance - P<0.0001; Significant

earlier report that when treating OS-MRSA infections, we should take precautions because treatment with β-lactam antibiotics may cause the emergence of high-resistant MRSA (HA-MRSA type), which is attributable to the presence of the *mecA* gene. Balode *et al*., 2013[21] and Yao *et al*., 2009[22] reported the effectiveness of Tigecycline and Linezolid respectively on MRSA and the present study consistent with their statement.

The present study made an attempt to understand the impact of the gender on antibiotic susceptibility of MSSA and the result shows no significant difference between male and female which is in agreement with Kimberly *et al*., 2013[23]. However, due to the small size of females among (3.8%; n=1) MRSA isolates, it was impossible to compare with males. Our study claims more studies with larger sample size to address this issue.

5. Conclusion

The current study recommends Linezolid, Tigecycline and Nitrofurantoin can be used as drugs of choice in both MRSA and MSSA isolates. However, Oxacillin should not be used in MRSA isolates, additionally cefoxitin not to be used in MSSA. The study is useful to understand antibiotic susceptibility in Tabuk, which is located in the north-west region of Saudi Arabia and also to guide the clinicians choosing empirical antibiotic treatment.

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