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Prevalence and Antimicrobial Susceptibility of Methicillin-Resistant *Staphylococcus aureus* in an Egyptian University Hospital

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Abstract

The relative high burden of morbidity and mortality caused by *Staphylococcus aureus* (SA) in healthcare and community settings is a major concern worldwide. It can cause invasive infections, sepsis and deaths. Despite progress in methicillin-resistant *S. aureus* (MRSA) prevention in healthcare settings, there is a critical need for assessment of the problem in both healthcare and community settings. This study was conducted for examining the prevalence, risk factors and antimicrobial susceptibility of MRSA in Mansoura University Hospitals (MUHs), Egypt. Samples were collected from patients in MUHs with clinically suspected nosocomial infections. MRSA isolates were identified by the standard bacteriological methods, biochemical reactions and disc diffusion method as recommended by the Clinical & Laboratory Standards Institute (CLSI), then confirmed by *MecA* gene PCR. A total of 2006 isolates was obtained. SA (32%) was the most frequently isolated pathogen. MRSA (130 isolates) represented 20% of SA and 6.48 % of all isolates. The *mecA* PCR identified SA as MRSA in 99.2% of cases. MRSA was isolated with another organism (mostly Gram-negative bacilli) from 40.8% of cases while 59.2% of MRSA was isolated alone. The most important reported risk factors for MRSA infections were prolonged hospital stays, recent antibiotic therapy, ICU admission, indwelling devices and presence of surgical sutures. MRSA was resistant to many antibiotics but sensitive to vancomycin in 99.2% of cases. Minimizing exposure to the risk factors with rapid diagnosis of MRSA infections are essential for early initiation of appropriate antibiotic treatment and limitation of the non-optimal use of glycopeptides and deaths.

Keywords: Antibiotic resistance, β-lactamase, β-lactams, MRSA, *Staphylococcus aureus*.

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Abbreviations: BSI: Blood-stream infection; CLSI: Clinical & Laboratory Standards Institute; EB: Ethidium bromide; LRTI: Lower respiratory tract infection; MDICU: Microbiology diagnostics and infection control unit; MH: Mueller– Hinton; MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*; MUHs: Mansoura University Hospitals; PCR: Polymerase chain reaction; SA: *Staphylococcus aureus*; SSI: Surgical site infection; URTI : Upper respiratory tract infection; UTI: Urinary tract infection; VA: Vancomycin; VISA: Vancomycin intermediate-resistant *Staphylococcus aureus*; VRSA: Vancomycin resistant *Staphylococcus aureus*.

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INTRODUCTION

SA is a leading cause of human bacterial infections in healthcare facilities and in the community all over the world. The severity of these infections varies widely from minor skin infections to sepsis, fatal necrotizing pneumonia and deaths. In the United States, an estimated 19,832 deaths from 119,247 SA bloodstream infections occurred in 2017[1].

About 20–30% of the global population is persistently colonized with SA in the anterior nares, with 60–100% of persons suspected to be transiently colonized at some time during their lives. SA infection frequently follows its nasal carriage[2].

Antibiotic resistance in SA is becoming increasingly significant[3]. The β-lactamase-resistant penicillins (oxacillin, methicillin, flucloxacillin and cloxacillin) were developed as a treatment for penicillin-resistant SA. In 1959, methicillin, a semi-synthetic penicillin, was introduced to treat SA infections, but only 2 years later, the 1st case of MRSA was reported in England**.** Despite this, MRSA generally remained an uncommon finding even in hospitals till the 1990s when the occurrence of MRSA has rapidly increased as nosocomial and community-acquired infections resulting in a serious problem worldwide. Children, old ages, pregnant females and immunocompromised patients, for example those suffering from cancer, blood dialysis and transplantation, frequently become infected by SA, mostly the multi-antibiotic-resistant MRSA strains leading to longer hospitalization duration, more health care costs, treatment failures and deaths[4].

The prevalence of MRSA infections shows considerable worldwide geographical variation, that has been related to efforts to decrease colonization and spread of these highly adaptive organisms. Multidrug resistant epidemic can occur from the endemic organisms. MRSA prevalence was reported to be 62.5% in Pakistan and ranged from 12% to 49.4% in six different hospitals of Saudi Arabia. In European countries, MRSA rates varied from 45% in Ireland, Italy, Belgium, Greece and United Kingdom down to 0.6% in Sweden and most nearby countries. The low MRSA prevalence in Sweden, Denmark, Norway, Finland, Iceland and the Netherlands has been suspected to be due to proper infection control measures in these countries. In the Mediterranean area, the highest overall proportions of MRSA were reported in Egypt, Jordan and Cyprus, where more than 50% of the SA blood culture isolates were resistant to methicillin[5].

The commonest effective therapeutic option against the multi-antibiotic-resistant MRSA is VA or linezolid[6-7]. VA is a glycopeptide antibiotic that is active against Gram-positive bacteria including *Staphylococci* and *Enterococci*, however, Gram-negative bacteria are naturally resistant to it, mainly because of its outer membrane that acts as a penetration barrier[8].

The non-optimal use of VA in treatment of methicillin-resistant *Staphylococcal* infections (both coagulase-positive &-negative), preceded the development of VA-resistant *Staphylococci*. Clinically, the first reported *Staphylococcal* resistance to VA was in a strain of *Staphylococcus haemolyticus***.** In 1997, the 1st reported VA intermediate-resistant SA (VISA) was in Japan, and then subsequent cases were reported in other countries. The VISA isolates were all MRSA[9]. The 1st reported VRSA cases were in the USA, Jordan and Brazil in the 2002[10].

A critical assessment of the prevalence and the recent trends of MRSA is central to formulating a framework of approaches and informing public health policy to further prevent SA infections.

Objective

Screening for the prevalence, risk factors and antimicrobial susceptibility of MRSA in Mansoura University Hospitals (MUHs), Egypt.

MATERIALS AND METHODS

Bacterial strains

The study was conducted over a period of 12 months starting from January till December, 2015. During the 1st 6 months, 130 MRSA isolates were isolated from patients with clinically suspected nosocomial infections. The isolates were from different medical and surgical departments of the MUHs dealing with the Microbiology diagnostics and infection control unit (MDICU) in the Microbiology and Immunology department, Faculty of Medicine, Mansoura University, Egypt.

Materials used

1. Equipments for samples collection and processing: Sterile cotton swabs, wide-necked

leak-proof containers, syringes, Eppendorf tubes and pipettes.

2. Blood culture bottles (Egyptian Diagnostic Media) (EDM): The Composition: Trypticase (1.5 gm%), Dextrose (0.5 gm%), Yeast extract (0.25 gm%),Agar (0.075 gm%), L-cystine (0.05 gm%) Sodium thioglycolate (0.05 gm%), Resazurin (0.0001 gm%)

3. Media: Stuart's transport medium (STM) (Oxoid), Nutrient agar (Oxoid), Blood agar (Oxoid), Chocolate agar (Oxoid), MSA (Oxoid), Cysteine Lysine Electrolyte Deficient (CLED) medium (Becton-Dickinson), Mueller– Hinton (MH) agar (Oxoid), DNase agar (Oxoid), Nutrient broth (Oxoid).

4. Chemicals: Hydrogen peroxide (3% H2O2), Hydrochloric acid (3.6% HCl).

5. EDTA-anticoagulated human plasma.

6. Antibiotics: Antibiotic disks (Oxoid).

7. McFarland 0.5 standards that were used as references to adjust the bacterial suspensions' turbidity as recommended by the CLSI 2014 [11].

Culture conditions and Antibiotics' sensitivity testing

The isolates were obtained as pure growth from the clinical samples and identified by the standard bacteriological methods and biochemical reactions[12-13]. Antibiotics' sensitivity testing, including the identification of methicillin resistance by the cefoxitin-based method, was conducted by the disc diffusion method as recommended by the CLSI 2014[11].

Confirmation of MRSA isolates by *MecA* gene amplification PCR

Chromosomal DNA extraction was done according to Aushbel *et al.,* 1990[14] to get the DNA templates. *MecA* gene amplification by PCR was done using a pair of primers (Sigma) selected according to *Bignardi et al,1996*[15] and the sequence of the primer used was:

F: 5'-CTCAGGTACTGCTATCCACC-3'

R: 5'-CACTTGGTATATCTTCACC-3'

The thermal cycler program was adjusted and proceeded as the following; initial denaturation for 5 minutes at 95°C, thirty cycles of 30 seconds denaturation then 30 seconds annealing then 30 seconds extension at 94°C, 42°C, 72°C respectively, followed by final extension for 10 minutes at 72°C. Agarose gel (1.5%) electrophoresis of the amplified *MecA* gene was done according to Davis *et al,* 1986[16] using the DNA molecular marker (100 bp DNA Ladder; Lonza Rockland. Inc, USA) to detect the expected (448 bp) bands visualized by staining with ethidium bromide (EB).

Determination of the MIC of VA on MRSA

This was done according to Sarker *et al,* 2007 using a microtitre plate incorporating resazurin as an indicator of bacterial growth[17].

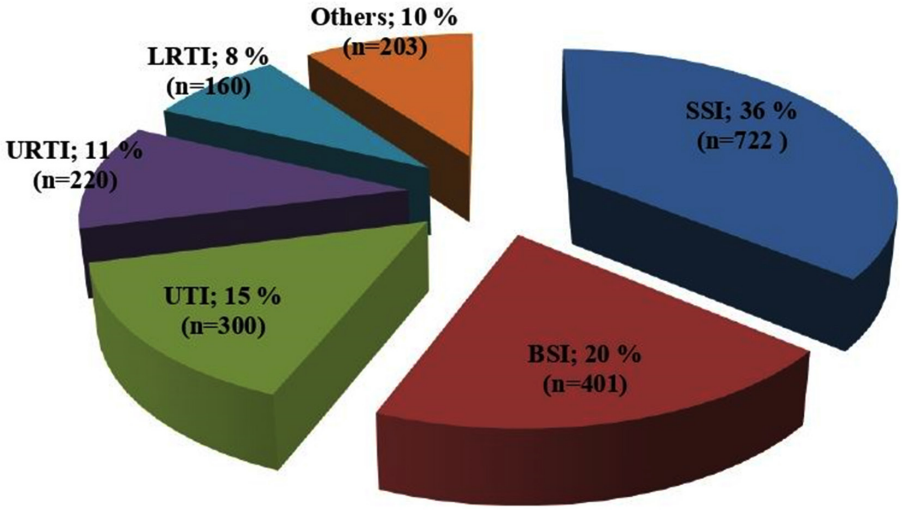

Fig. 1. Different types of nosocomial infections in MUHs: A total of 2006 nosocomial isolates was obtained. Surgical site infection (SSI) represented the commonest nosocomial infection (36%) followed by blood stream infection (BSI), urinary tract infection (UTI), upper respiratory tract infection (URTI) then lower respiratory tract infection (LRTI).

Statistical analysis

The χ^2 was done using a computer programme embedded in Microsoft Excel. Tests were considered non-significant if the probability of error is equal or more than 5% ($p \geq 0.05$), significant if ($p < 0.05$), highly significant if ($p < 0.001$)[18].

RESULTS

Different samples were received from 2856 patients with clinically suspected nosocomial infections, from different medical and surgical departments of the MUHs dealing with the MDICU in the Microbiology and Immunology department, Faculty of Medicine, Mansoura University, Egypt. A total of 3047 clinical samples were collected. Samples were examined in MDICU laboratory after being processed and cultured on appropriate media under appropriate incubation conditions. Infections were detected in 1881 samples. Single pathogen was detected in 1756 samples whereas 125 samples yielded 2 pathogens. Consequently, the total number of the isolated nosocomial pathogens was 2006.

Surgical site infection (SSI) [722 isolates] represented the commonest nosocomial infection during the study period (36%) (Fig. 1). SA [642 isolates] was the most frequently isolated pathogen representing 32% of all isolates while *Klebsiella* species was the most common Gram-negative organism accounting for 22.1%.

One hundred & thirty MRSA isolates were detected by phenotypic methods representing 20% of SA. The pattern of resistance to methicillin was homogenous in 66 cases (50.8%) wile heterogenous in 64 cases (49.2%). MRSA represented 6.48% of all nosocomial isolates. One hundred and twenty-nine isolates (99.2% of cases) were positive for the *mecA* gene by PCR.

MRSA was isolated alone from 59.2% of cases (77 cultures) while 40.8% of MRSA isolates (53 cultures) were isolated with another organism. Gram negative bacilli (44 isolates;) were common co-pathogens as the following; *Klebsiella* (15 isolates), *E. coli* (11 isolates), *Pseudomonas* (10 isolates) and *Proteus* (8 isolates) (Fig. 2). All the associated *Enterococci* (5 isolates) were VA sensitive.

MRSA causing SSI (61 isolates) was the most predominant type for MRSA nosocomial infections representing (8.45%) "Fig. 3". MRSA was isolated from surgical patients in 58.46% of cases (76 cases) and was from medical patients in 41.54% of cases (54 cases).

The most important reported risk factors for the development of MRSA infections were recent antibiotic therapy (especially β-lactams), prolonged hospital stays (especially if more than

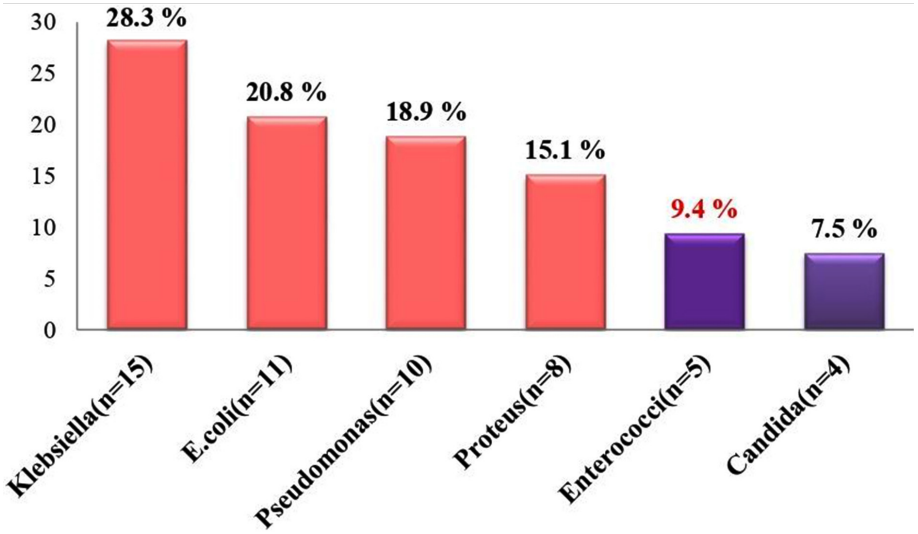

Fig. 2. Associated co-pathogens with nosocomial MRSA isolates: Gram negative bacilli were common co-pathogens. All the associated *Enterococci* were VA sensitive.

7 days), indwelling devices (especially if more than 2 devices simultaneously), ICU admission and presence of surgical sutures (Table 1).

Regarding β-lactams as a risk factors for methicillin resistance, they were prescribed alone in 73.1% of cases and in combination with other antibiotics in 7.7% of cases (Table 2).

Table 1. The risk factors for methicillin resistance among *Staphylococcus aureus* isolates

Risk factor		130 MRSA	512 MSSA (control)	Odds ratio (95%CI)	*P* value
Antibiotic therapy		117(90.0%)	235(45.9%)	10.61(5.66-20.27)	<0.001**
Hospitalization > 1 week		110(84.6%)	214(41.8%)	7.66(4.50-13.16)	<0.001**
Indwelling devices	*IV catheter*	97(74.6 %)	125(24.4 %)	9.10(5.72-14.54)	<0.001**
	Urinary catheter	64(49.2%)	201(39.3%)	1.50(1.0-2.25)	0.049*
	Wound drains	48(36.9 %)	111(21.7 %)	2.11(1.37-3.26)	0.005*
	Orthopedic prosthesis	10(7.7%)	33(6.4%)	1.21(0.54-2.64)	0.75
	Central venous line	7(5.4%)	20(3.9%)	1.40(0.52-3.59)	0.61
	ETT (Ventilator)	7(5.4%)	17(3.3%)	1.66(0.61-4.36)	0.39
Old age (≥ 50)		32(24.6 %)	110(21.5 %)	1.19(0.74-1.92)	0.51
ICU patients		33(25.4 %)	87(17.0 %)	1.66(1.02-2.69)	0.039*
Previous hospital admission		26(20.0%)	76(14.8%)	1.43(0.85-2.41)	0.19
Diabetes mellitus		32(24.6%)	166(32.4%)	0.76(0.47-1.21)	0.27
Surgical sutures		76(58.5%)	247(48.2%)	1.51(1.00-2.27)	0.047*
Pressure ulcers		12(9.2%)	44(8.6%)	1.08(0.52-2.20)	0.96
Burn		13(10.0%)	39(7.6%)	1.35(0.66-2.71)	0.48
Malignancy		19(14.6%)	53(10.4%)	1.48(0.81-2.69)	0.22

The table illustrates the reported risk factors for infection with MRSA. Recent antibiotic therapy, prolonged hospital stays, indwelling devices (such as IV lines, urinary catheters and wound drains), ICU admission & presence of surgical sutures were the most important reported risk factors for the development of MRSA infections. * Significant ($p < 0.05$); **Highly significant ($p < 0.001$).

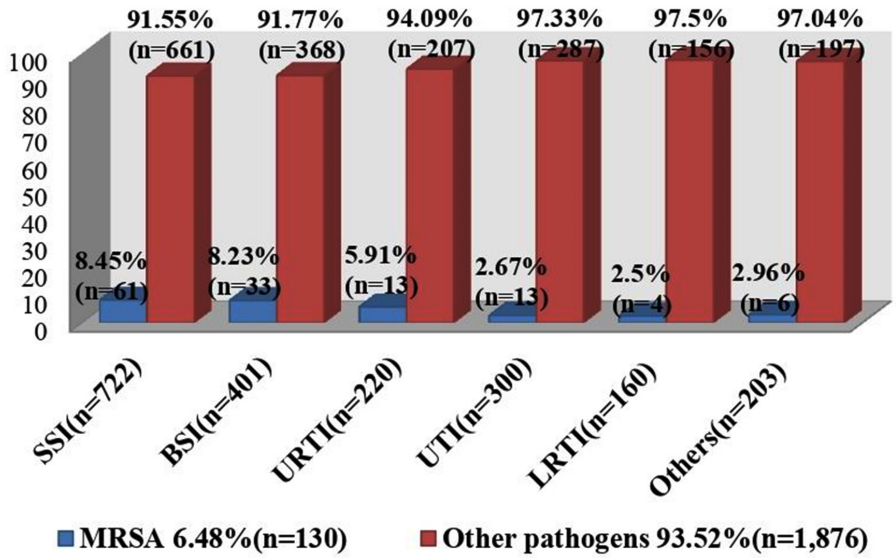

Fig. 3. Distribution of MRSA isolates according to the type of nosocomial infection in MUHs: Surgical site infection (SSI) was the most prevalent (8.45 %) followed by blood stream infection (BSI), upper respiratory tract infection (URTI) urinary tract infection (UTI), then lower respiratory tract infection (LRTI).

Table 2. Antibiotic therapy of the 130 MRSA infected patients during their hospital stay

Antibiotic therapy		Patients infected	
		No.	%
No antibiotic therapy		13	10.0
Single antibiotic type	β-*lactam antibiotics*	95	73.1
	Others	12	9.2
More than one antibiotic type (including β-lactams)		10	7.7
Total		130	100

Table shows the antibiotics prescribed for the 130 MRSA infected patients during their hospital stay. The β-lactam antibiotics were the commonest.

Table 3. Age and gender distribution of the 130 MRSA infections

Age group (years)	Test group 130 MRSA	
	Males	Females
<10	5(62.5%)	3(37.5%)
10-	3(30.0%)	7(70.0%)
20-	4(57.1%)	3(42.9%)
30-	10(37.0%)	17(63.0%)
40-	19(41.0%)	27(59.0%)
50-	13(54.2%)	11(45.8%)
60-	3(37.5%)	5(62.5%)
Total	57(43.8%)	73(56.2%)

Table shows the age and gender distribution of MRSA patients. The highest overall age incidence was in the age group between 40-50 years.

The highest overall age incidence was in the age group 40-50 years and nearly the prevalence of MRSA infections among males is equal to the prevalence of MRSA infections among females (Table 3).

All isolates were resistant to penicillin, ampicillin and cephradine antibiotics. MRSA isolates were resistance to rifampicin (10%), fusidic acid (64%), ciprofloxacin (65%), trimethoprim-sulfamethoxazole (71%), gentamicin (80%), erythromycin (88%), clindamycin (89%) and tetracycline (92%). Fortunately, one hundred and twenty-nine MRSA isolates (99.2%) in the current study, were sensitive to VA (Table 4).

DISCUSSION

As strains of SA with reduced susceptibility to many antibiotics continue to emerge, with increasing the rates of morbidity and death in the hospitals and community, there is a clinical need to fully characterize them and conduct a well-designed research and epidemiological studies. The current MRSA strains in hospitals and community are alarming condition to the clinicians[19].

Development of resistance to antibiotics in developing countries, like ours, seems to be mainly related to the unreasonable usage of antibiotics due to their easy availability without prescription at the drug stores, non-optimal use in hospitals, animal husbandry, fisheries and agriculture[20].

The accurate and rapid diagnosis of MRSA infections is of major importance**.** Susceptibility testing of MRSA may be problematic owing to the phenotypic heterogeneous resistance (heteroresistance) displayed by many isolates to anti-*Staphylococcal* β-lactams**.** Consequently, many laboratory methods have been developed to increase the resistance expression, including prolonging the incubation period to 24 hours and the supplementation of media with NaCl[21].

This study aimed to estimate the role of MRSA as a causative agent of nosocomial infections in MUHs. During the study period, a total of 642 SA isolates were isolated. One hundred and thirty MRSA isolates were isolated representing 20% of all SA nosocomial infections. This result is nearly like the result of Viswanathan *et al.*[22] who have reported that MRSA accounts for 20-40 % of all SA infections, and of Saunders and Holmes[23] who reported that this percentage reached up to 30%.

Also, it was stated by Dhanalakshmi, *et al.*[24] that methicillin resistance represented 31.3% of SA isolates. Moreover, Abd El-Baky *et al.*[20] found that methicillin resistance represented 25.4% of SA isolates in the study conducted for documentation of VRSA in the Minia University, Egypt.

Higher rates were reported in U.S.A. hospitals by Diekema *et al.*[25] who mentioned that MRSA accounts for 30-50% of all nosocomial SA isolates and Wisplinghoff *et al.*[26]*,* who found that

Table 4. Antibiotic sensitivity of the MRSA isolates by the disc diffusion method

Antibiotic	Result	MRSA (No. = 130)	
		No.	%
		No.	%
Cefoxitin (FOX)	Sensitive	0	0
	Resistant	130	100
Methicillin (MET)	Sensitive	0	0
	Resistant	130	100
Oxacillin (OX)	Sensitive	0	0
	Resistant	130	100
Penicillin G (P)	Sensitive	0	0
	Resistant	130	100
Ampicillin (AMP)	Sensitive	0	0
	Resistant	130	100
Amoxycillin/K+	Sensitive	1	0.8
clavulanate (AMC)	Resistant	129	99.2
Cephradine (CE)	Sensitive	0	0
	Resistant	130	100
Cefuroxime (CXM)	Sensitive	4	3
	Resistant	126	97
Clindamycin (DA)	Sensitive	14	11
	Resistant	116	89
Erythromycin (E)	Sensitive	16	12
	Resistant	114	88
Gentamicin (CN)	Sensitive	26	20
	Resistant	104	80
Tetracycline (TE)	Sensitive	10	8
	Resistant	120	92
Sulphamethoxazole/	Sensitive	38	29
Trimethoprim (SXT)	Resistant	92	71
Ciprofloxacin (CIP)	Sensitive	45	35
	Resistant	85	65
Fusidic acid (FD)	Sensitive	47	36
	Resistant	83	64
Imipenem (IPM)	Sensitive	72	55
	Resistant	58	45
Rifampicin (RD)	Sensitive	117	90
	Resistant	13	10
Vancomycin (VA)	Sensitive	129	99.2
	Resistant	1	0.8

the proportion of MRSA increased from 22% in 1995 to 57% in 2001.

Higher rates were reported in the Kingdom of Saudi Arabia by Baddour *et al.*[27] who reported that MRSA accounted for 77.5% of all SA nosocomial infections in a study conducted in several hospitals in Riyadh, and by Alzolibani *et al.*[28] who reported that 90% of SA strains were resistant to methicillin in a study conducted in the Qassim region for documentation of VRSA among children with atopic dermatitis. These higher rates could be attributed to the vulnerable study group.

In this study, MRSA constituted 6.48% of the total nosocomial infections. This result is not coming with the higher rates that were reported by Hsueh *et al.*[29] who stated that a rapid emergence of nosocomial MRSA infection (from 26.3% in 1986 to 77% in 2001) was found in a university hospital in Taiwan.

The prevalence of MRSA infection shows marked variation. The difference in the MRSA rates is likely related to differences in the populations of the studies and variations in the infection control measures applied. Some investigators used active surveillance cultures, and others used only culture of clinical specimens. In addition, infection control measures for patients with MRSA colonization or infection in some hospitals are stricter than in other hospitals.

Out of the 130 MRSA isolates, MRSA was isolated alone from 77 cultures (59.2%) while MRSA was isolated with another organism from 53 cultures (40.8%). Gram negative bacilli were common co-pathogens that are resistant to VA required for the MRSA treatment, so they may need to be treated by β-lactam antibiotics which may induce VA resistance among MRSA isolates[30,31].

SSIs were by far the commonest sites for MRSA infections in this study, followed by blood stream infections. Similar results have been obtained by Carla *et al.*[32] who found that the most common sites infected by MRSA were surgical wounds (21%), intra venous sites (18%), and bacteremia (13%).

These results do not agree with the results coming from United Kingdom, Ireland and Greece that reported one of the highest rates of MRSA from bloodstream isolates (44%) in 2004[33].

In the present study, MRSA was noticed to be more prevalent in surgical patients (58.46%) than in medical patients (41.54%). In agreement with our results, Gordon and Lowy[34] reported that MRSA was isolated from surgical patients, ICU patients and from medical patients represented (40%, 27%, 33% respectively).

Regarding the risk factors, it was revealed that recent antibiotic therapy (especially β-lactam antibiotics) and prolonged hospital stay for > 7 days are important risk factors for the expansion of MRSA infections (90.0% & 84.6% of cases, respectively). This was supported by many reports[35-37]. This may be owing, in part, to the more likelihood over time of becoming colonized with MRSA from either horizontal nosocomial transmission or endogenous emergence of resistance.

Similarly, Raygada and Levine[38] have demonstrated a close association between recent antibiotic usage and the development of subsequent antibiotic resistance in both Gram positive and negative bacteria.

In the current study, β-lactams alone were the antibiotics prescribed for 73.1% of patients. Other drugs prescribed ranged among fluoroquinolones, monobactams, macrolides and aminoglycosides. These findings are consistent with Borg *et al.*[39] who stated that *mecA* expression is either constitutive or inducible by some β-lactam antibiotics. Also, extensive antibiotics usage within a hospital may partly explain differences among hospitals in transmission rates of resistant organisms[40].

During the study, it was noticed that invasive indwelling devices (such as IV lines, urinary catheters and wound drains), ICU admission and presence of surgical sutures were also important risk factors for the emergence of MRSA infections. This agreed with data mentioned by Ricarda *et al.*[41]

Moreover, the greatest incidence of acquisition of MRSA infections occurred when ≥ 2 different devices were used for the same patient. Likewise, Sadoyama and Gontijo-Filho[42] concluded that most nosocomial MRSA infections occur in persons with multiple risk factors for infection.

Concerning the antibiotics sensitivity patterns of the MRSA isolates, it was noticed that, all isolates were resistant to penicillin, ampicillin and cephradine antibiotics. These findings agreed with Noto *et al.*[43] who stated that more than 95% of patients with SA infections worldwide do not respond to first-line antibiotics such as penicillin or ampicillin.

During the study, MRSA was found to be resistance to ciprofloxacin (65%), trimethoprim-sulfamethoxazole (71%), clindamycin (89%) and erythromycin (88%). Similarly, Adwan *et al.*[44] reported that up to 82.1% of nosocomial MRSA isolates were resistant to erythromycin and therefore, the macrolides cannot be considered first line therapy for serious *Staphylococcal* infections.

However, in a study done by Al-Tawfiq[45], nosocomial MRSA isolates showed lower rates of resistance to trimethoprim-sulfamethoxazole (68%), clindamycin (76.6%) and erythromycin (68%) but showed higher rates of ciprofloxacin resistance (76.6%).

In studying sensitivity to gentamicin, MRSA isolates were resistant in 80% of cases. This does not correlate with results obtained in Cyprus by Gourni *et al.*[46], who found that MRSA were gentamicin resistant in 18.75 only.

In this study, thirteen MRSA isolates were found to be resistant to rifampicin (10%). This agreed with the Turkish study that reported the emergence of rifampicin-resistant MRSA in 3 wards of a university hospital in Turkey[47].

All MRSA isolates detected by Colakoglu *et al.*[48] were found to be sensitive to fusidic acid, rifampicin and tetracycline, but, some of the isolates that were detected in our study were resistant to these antibiotics.; for fusidic acid (64%), rifampicin (10%) and for tetracycline (92%) were resistant.

The variations in the antibiotics resistance patterns among different studies can be explained by selection pressure of certain drugs used according to the local hospital policy.

Glycopeptides antibiotics are the treatment of choice of MRSA infections. Appearance of different degrees of VA resistance occurred due to its widespread use to treat MRSA and other Gram-positive infections. The first strain of SA with reduced susceptibility to VA and teicoplanin (glycopeptide-intermediate SA [GISA]) was reported in the 1997 from Japan, whereas VRSA isolates were first reported in the 2002 from the USA, Jordan and Brazil[10].

The reasons for decreased VA susceptibility may be owing to non-optimal utilization of VA and other antibiotics or due to use of antimicrobial agents in food-producing animals as documented in Saudi Arabia in Qassim area[28]. These strains represent a crucial challenge for antimicrobial therapy, testing antimicrobial susceptibility and infection control in hospitals[20].

Fortunately, one hundred and twenty-nine of MRSA (99.2%) in the current study, were VA sensitive, which agreed with Sievert *et al.*[49] and Antonanzas *et al.*[50] who included VA in antibiotic susceptibility testing of MRSA.

The present study encountered one VRSA strain (0.8%) with VA MIC 32µg/ml. Many studies encountered VRSA strains at higher frequencies than that reported in our study; Saderi *et al.*[51] reported that 3.5% of SA isolates were VRSA and Hakim *et al.*[52] reported VISA (13%). On the other hand, Dhanalakshmi, *et al.*[24] reported that no VISA or VRSA were found among MRSA strains.

The VRSA case reported in our study[31] showed multi-drug resistance to penicillin, amoxicillin/clavulanic, ampicillin/sulbactam, cefazolin, cefuroxime, gentamicin and ciprofloxacin but showed susceptibility to linezolid. Multi-drug resistance of VRSA was also reported by many studies[3,28,53].

CONCLUSION

SA infections account for substantial morbidity and mortality in MUHs. The large scale of spread of methicillin resistance has been considered as a fearsome threat to the already challenging treatment of *staphylococci*[54].

Minimizing the risk factors exposure with rapid MRSA diagnosis are essential for early initiation of appropriate therapy and limitation of the non-optimal usage of glycopeptides and the number of deaths. Antibiotic prescription practices should be based on the in vitro antibiotic susceptibility testing and should be reviewed by the hospital administers with implementation of policies aiming at reduction of their non-optimal use. Attention to MRSA prevention and control must remain a constant team effort, concerning all health care professionals, because the new therapeutic agents alone will not provide the long-term solution[55]. Adherence to the CDC recommendations[56] for preventing device- and procedure-associated infections is needed to further prevent SA infections

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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None

AUTHORS' CONTRIBUTION

AET designed and performed the research work. MFB, FEE and EH supervised and performed the laboratory techniques. AET wrote the manuscript. MFB, FEE and EH revised the manuscript.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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