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Research Paper

Methicillin-Resistant Staphylococcus Aureus Prevalence, Response and Resistance to Antimicrobial Agents at King Khaled Hospital in Najran (KSA)

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Abstract: *Methicillin Resistant Staphylococcus Aureus (MRSA) has been recognized as important nosocomial pathogens worldwide. Across-sectional hospital-based study was conducted from March 2015 to Jan. 2016 at King Khaled hospital in Najran. The aim of this study was to assess the prevalence of MRSA colonization beside determining antimicrobial susceptibility. A total of 158 patients and 54 ICU professionals were recruited to participate in this study. A self-administered questionnaire and laboratory investigations were used to collect data. The mean age for patients and ICU professionals was 39.4 years ± SD 23.1 and 36.5 years ± SD 9.3 respectively. Among the patients, male were 91(57.41%) while the majority of professionals 31(57.41%) were females. The mean ICU stay for patients was 7.6 ± 8.5 days. The prevalence of bacterial pathogens among the samples was found to be 81 (38.21%). The main organisms detected were Staphylococcus 26(32.1%), Escherichia coli 18(22.22%), Enterococcus spp. 13 (16.05%), Klebsiella spp. 9(11.11%), Pseudomonas aeruginosa 9(11.11%), and Proteus mirabilis 6(7.41%). The prevalence of MRSA colonization among patients and ICU professionals was 10.13% and 5.56% respectively. All MRSA isolates were highly resistant to Penicillin, Oxacillin, Gentamycin and Erythromycin. On the other hand, all MRSA isolates 19(100%) were highly sensitive to vancomycin. Although the rate of MRSA colonization in this study was less than what reported about other areas in Saudi Arabia, but still there a need for local surveillance to monitor MRSA. Preventive measures like hand hygiene, use of sterile aprons, masks and avoiding touching one's nose during work may reduce transmission rate.*

Keywords: *Staphylococcus aureus, MRSA, Nosocomial pathogens*

I. INTRODUCTION

Microscopically, Staphylococcus aureus (S. aureus) is a Gram positive organism characterized by individual cocci measuring 0.5 – 0.7 µm in diameter. The organisms can occur singly, in pairs, or in short chains with a strong tendency to form clusters. The three main species considered clinically important include S. aureus, S. epidermidis and S. saprophyticus.[1] Staphylococcus aureus colonizes the skin and nasal carriage, occurs in about 25 – 30% of healthy people. It is among the most common human pathogens, capable of causing infections of any body parts that ranging from mild to fatal forms both in community and hospital settings.[2]
Methicillin- resistant Staphylococcus aureus (MRSA) is a subgroup within a group of Staphylococcus aureus organisms. This strain is resistant to treatment with commonly used antibiotics, this implies resistance to all Penicillinase-resistant Penicillins and essentially, all other β-lactam antibiotics. Moreover, MRSA has also been found to be resistant to other unrelated antibiotics, such as streptomycin, Tetracyclines, Chloramphenicol, erythromycin, Lincomycin, Clindamycin and kanamycin.[3] It was first reported in 1960, within a year of methicillin introduction. In contrast to the remainder of the Staphylococcus aureus groups which are referred to as Methicillin-sensitive Staphylococcus aureus (MSSA).[4]

Since the introduction of Methicillin for clinical use, the proportion of MRSA strains isolated worldwide has risen sharply, and it was considered an endemic by the year of 1990. Although Methicillin (Forerunner of Flucloxacillin) is no longer used to treat patients, but it is still used to test the susceptibility to Flucloxacillin i.e. Methicillin resistance means the same as Flucloxacillin resistance. MRSA infections that acquired in hospitals are becoming a serious concern globally due to their severe complications, they may cause

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a variety of serious healthcare-associated infections that impose serious economic costs on patients and hospitals.[5,2]

The reason why bacteria can become resistant to antibiotics is their genetic plasticity and their ability to evolve and adapt to environmental changes. This adaptation and evolvement resulted from the pressure of the overuse or misuse of antibiotics.[6]

So far, 16 epidemic strains of MRSA have been discovered, but particular two strains (clones 15 and 16) are thought to be more transmissible than others. The most common strains of MRSA are healthcare-associated that typically seen in hospitalized patients or among those receiving treatment in other healthcare facilities such as day units and nursing homes. The most endemic areas inside the hospitals include, nursery, maternity, pediatric, medical, surgery and intensive care units.[7]

In the present decade, the rate of MRSA prevalence is reaching to alarming levels in a large number of countries such as Italy (45%), United Kingdom (40%), France and Greece (35%) each.[8] In the United States, approximately 60% of Staphylococcus infections in the intensive care units are now caused by MRSA.7,3 In Saudi Arabia, the prevalence of MRSA has increased in the past ten years, that ranged from 7.5% up to 33%, additionally, severe community-acquired infections had been reported. The first epidemiological report about MRSA from Saudi Arabia appeared in the year 1994 in the Western region (Jeddah) in which MRSA comprised about 7.5%. The majority of them were resistant to Tetracycline (93%), followed by Gentamicin (83%), Rifampicin (6%) and Ciprofloxacin (1%).[9]

Patients usually become a victim to nosocomial infections (including MRSA) either through infections with an endogenous microbes (normal flora of the body), or through cross contamination from healthcare workers through skin, hands, or through use of contaminated equipments such as needles, razors, sports equipment, towels, or from an exogenous microbes (organisms from the environment or poor crowded living conditions).[10,11]

Colonization is an important risk factor for subsequent infection. Individuals who are either colonized or infected are the major sources of spread to others.[8] Thus, decolonization has two main purposes which are: 1) prevent subsequent infections in individuals who are already colonized, and 2) prevent transmission from colonized individuals to others by eradicating the S. aureus reservoir.[12]

There are several mechanisms responsible for Methicillin resistance. The most important one is the production of the penicillin-binding protein (PBP2a) which encoded by the *mecA*- gene.[13,7]

I.1. The risk of acquiring MRSA

Hospitalized patients are most at risk of acquiring MRSA. Most of these patients are colonized rather than infected. The commonest sites are the nose, groin, and broken skin. In many cases, colonization does not progress to infection, but in a subset of patients, infection does supervene and this ranges from mild skin and soft-tissues infection to life-threatening invasive infection. The factors which influence this process are not fully understood, but include patient susceptibility and variations in the virulence of individual strain of MRSA. Healthy individuals such as healthcare workers, patients' families and household contacts of those discharged from hospitals with MRSA are at very low risk of developing MRSA infection.[14]

II. SAMPLING AND METHODOLOGY

This is a cross-sectional, prospective, hospital-based study that undertaken in Najran city at King Khaled hospital – Intensive care unit (ICU) during the period from March, 2015 up to Jan. 2016. The target population for this study was the healthcare workers in the ICU, regardless their qualifications, specialties or years of experiences, beside the patients who admitted to ICU during this period of study. By adopting convenience sampling technique, 158 patients along with 54 healthcare professionals (ICU staff) had been recruited to participate in this study.

Two tools were developed for collecting data, which were a semi-structured questionnaire for collecting socio-demographic data of the selected samples that include age, sex, previous used antibiotics during the last 6 months, and previous hospitalization. The another tool was the laboratory investigation (Nasal swab culture and sensitivity) for determining the prevalence of MRSA and their antibiotic susceptibilities. Nasal swabs were collected from the anterior nares of the both nostrils with a sterile swab sticks which were moistened with saline and inserted into a depth of approximately 0.75 cm, and rotated four times. The primary inoculations of the collected swabs were done on blood agar medium, and Mannitol salt agar medium. The plates were incubated aerobically at 37^{o}C for 24 hours and they were examined for growth. The staphylococcus aureus which was isolated from the nasal swab samples was identified by standard methods based on the colony morphology, gram staining, the catalase test, slide and tube coagulase test, and the fermentation of Mannitol.

By using the modified Kirby Bauer disc diffusion method, all the isolated staphylococcus aureus strains were tested against different antimicrobial agents resistance. The antibiotics discs that used in the current study were:

Ampicillin (10mcg), Amikacin (30mcg), Oxacillin (10mcg), Ciprofloxacin (10mcg), Erythromycin (10mcg)_, Penicillin G (10 IU), Vancomycin (30mcg), Augmentin (30mcg), and Gentamycin (30mcg).

For Methicillin resistance, the Kirby – Bauer dis diffusion method was used as described above using as Oxacillin (1mcg) disk (Oxoid) on Mueller – Hinton agar supplemented with 4% NaCl and incubated at 35^{o}C for 24h. A zone of inhibition of 13 mm or more was considered as Oxacillin sensitive. The strains with Oxacillin minimum inhibitory concentration (MIC) value ≥ 4 mcg/ml were described as MRSA. All S. aureus isolates were further confirmed by the presence of nuclease gene. Presence of mec*A*-gene was the final marker for confirming MRSA isolates.

The healthcare workers who were found to be colonized with Staphylococcus aureus, were advised to apply 2% Mupirocin ointment in paraffin base into their inner surface of each nostril three times daily for five days, and they were advised too to be re-tested for the nasal carriage of S. aureus after 3 months of treatment.
A pilot study was carried out on 7 healthcare professionals who are currently working in the ICU.

III. STATISTICAL METHODS

The statistical pachage for the social sciences (SPSS – 18, SPSS Inc., Chicago, IL) was used for all statistical analysis. The chi-square test was used for comparison of proportion, univariate and multivariate analysis were used too for analyzing the risk factors for MRSA colonization. A *P*-value of <0.05 was considered statistically significant.

VI. ETHICAL CONSIDERATION

Authorization to carry out the study was obtained from Najran university- college of medicine beside the administration of King Khaled hospital as well as from the head department (ICU). Moreover, a verbal informed consent and confidentiality was assured for all the participated subjects.

V. RESULTS

A total of 158 patients beside 54 healthcare professionals were recruited to participate in this study. The mean age for patients and ICU professionals was 39.4 years ± SD 23.1, and 36.5 years ± SD 9.3 respectively. 61 participants (28.77%) were above 46 years old, among them five (8.2%) were reported positive MRSA. Furthermore, 133 patients (62.74%) their ages ranged between 16 and 45 years old, while only 18 patients (8.5%) were less than 15 years old, among them only one participant (5.6%) was reported a positive MRSA. In regard to patients, male contributed 57.6% (91), while the majority of ICU health professionals 31(57.41%) were females. It is observed in the current study that the highest MRSA colonization among the studied sample in regard to their demographic characteristics was shown in table (1). The overall prevalence of bacterial pathogens among the 12 clinical sample was found to be 81 (38.21%). The main organisms detected among these 81 pathogens were: Staphylococcus 26 (32.1%), Escherichia coli 18 (22.22%), Enterococcus spp. 13(16.05%), Klebsiella spp. 9(11.11%), Pseudomonas aeruginosa 9 (11.11%), and Proteus mirabilis 6 (7.41%) as displayed in figure 1.

The results obtained from this study revealed that S. aureus accounted for 73.08% (19/26) of the total staphylococcus isolates. As identified by univariate analysis in this study, length of ICU stay, previous used antibiotics as well as previous hospitalization experiences were found to be significantly (*P*-value of <0.05) associated with MRSA colonization independently as presented in table (2). The multivariate analysis of risk factors (socio-demographic characteristics) in regard to MRSA colonization among the selected subjects in the current study shows that the level of education has a significantly association with MRSA colonization among the studied group (*P*-value of <0.05) as shown in table (3). Antibiotic resistance among staphylococcus isolates is summarized in table (4), it shows that all MRSA isolates 19 (100%) were susceptible to vancomycin at a rate of 100%, followed by Ciprofloxacin (68.42%), and Augmentin (52.63%). On the other hand, the majority of MRSA strains (62%) have developed resistance to some antibiotics that including Ampicillin (100%), Penicillin G (89.47%), Cefoxitin (89.47%), Erythromycin (89.47%), Oxacillin (89.47%), Gentamycin (78.95%) and Amikacin (78.95%).

The overall MRSA colonization among the participated was 8.96%. Moreover, S. aureus nasal colonization was more common among males than females (7.08% vs. 5.19%) as shown in table (5).

Table (1): MRSA in regard to the demographic characteristics of the studied sample

Characteristics	**Category**	**No.**(%)	**MRSA**		***P*-value**
			+ve (%)	**-ve**	
Age in years	0 -15	**18** (8.5%)	**1** (5.6%)	**17**	0.72
	16 - 30	**46** (21.7%)	**3** (6.5%)	**43**	0.50
	31 - 45	**87** (41.04%)	**10** (11.5%)	**77**	0.31
	≥ 46	**61** (28.77%)	**5** (8.2%)	**56**	0.04
Sex	Male	**134** (63.2%)	**11** (8.2%)		0.12

	Female	**78** (36.79%)	**8** (10.3%)		0.49
Occupation(patients)	Business	**27** (12.74%)	**3** (11.1%)	**24**	0.01
	Farmer	**32** (15.09%)	**7** (21.9%)	**25**	0.71
	Retired	**51** (24.06%)	**4** (7.8%)	**47**	0.46
	Other	**48** (22.64%)	**5** (10.4%)	**43**	0.19
Health professionals	ICU/ Health worker (Male)	**23** (42.59%)	**2** (8.7%)	**21**	0.05
	ICU/ Health worker (Female)	**31** (57.41%)	**1** (3.2%)	**30**	0.41
Education level	No formal education	**6** (2.83%)	**1** (16.7%)	**5**	0.51
	Primary	**41** (19.43%)	**5** (12.2%)	**36**	0.36
	Intermediate & Secondary	**133** (62.7%)	**13** (9.8%)	**120**	0.18
	University or higher	**32** (15 %)	**1** (3.1%)	**31**	0.01

(n=212)

Table (2): Univariate analysis of risk factors in regard to MRSA colonization

Risk factor	**Individuals with +ve MRSA (n=19)**		**Individuals with -ve MRSA (n=193)**		***P*-value**
	ICU professionals 3 (5.56%)	**Patients 16 (10.13%)**	**ICU professionals 51 (94.44%)**	**Patients 142 (89.87%)**	
Age, mean (SD)	38.4(18.7)	40.5(22.4)	36.2(21.2)	49.1(23.1)	0.510
Length of ICU stay, mean (SD)	0.0	16.09(14.6)	0.0	6.75(5.83)	< 0.001
Previous used antibiotics during the last 6 months, n= 38	4.5(2.1)	14.1(11.07)	6.21(4.03)	26.81(9.49)	0.005
Previous hospitalization anywhere & at any hospital department during the last 6months , mean (SD)	2(1.02)	12.1(8.03)	4(6.01)	69.87(17.46)	0.471

(n=212)

Table (3) Multivariate analysis of risk factors (Socio-demographic) in regard to colonization among the studied subjects

Characteristics	**Category**	**COR**	**95% CI**	**AOR**	**95% CI**
Age in years	0 -15	1.82	0.31 – 8.75		
	16 - 30	0.61	0.14 – 3.11		
	31 - 45	0.50	0.21 – 1.61		
	≥ 46	0.73	0.32 – 2.18		
Sex	Male	5.1	1.31 – 24.25	6.71	1.31 – 32.62
	Female	0.14	0.03 – 0.6		
Occupation	Business	0.64	0.31 – 2.03		
	Farmer	0.82	0.3 – 2.37		
	Retired	2.01	0.51 – 16.39		
	Other	0.74	0.01 – 6.53		
Education level	No formal education	0.52	0.31 – 1.15		
	Primary	0.76	0.22 – 2.71		
	Intermediate & Secondary	2.41	0.51 – 16.11		
	University or higher	1.41	0.29 – 4.01		

(n=212)

Table (4): Antibiotics' resistance to MRSA isolates

Variable	Total tested	No. of Resistant Isolates	% of Resistant Isolates	Proportion	Total MRSA resistance	*P*-value
Ampicillin (10mcg)						
Patients	16	16	100%	0.100	100%	0.005
ICU professionals	3	3	100%	0.100		
Amikacin (30mcg)						
Patients	16	14	87.5	0.875	78.95%	0.341
ICU professionals	3	1	3.33	0.333		
Oxacillin (10mcg)						
Patients	16	15	93.75	0.937	89.47%	0.014
ICU professionals	3	2	66.7	0.667		
cefoxitin (30mcg)						
Patients	16	15	93.75	0.937	89.47%	0.511

ICU professionals	3	2	66.7	0.667		
Ciprofloxacin(10mcg)						
Patients	16	6	37.5	0.375	31.58%	0.562
ICU professionals	3	0	0.0	0.00		
Erythromycin(10mcg)						
Patients	16	14	87.5	0.875	89.47%	0.021
ICU professionals	3	3	100	0.100		
Penicillin G (10 IU)						
Patients	16	15	93.75	0.937	89.47%	0.021
ICU professionals	3	2	66.7	0.667		
Vancomycin (30mcg)						
Patients	16	0	0.0%	0.00	0.0%	
ICU professionals	3	0	0.0%	0.00		
Augmentin (30mcg)						
Patients	16	8	50.00	0.500	47.37%	0.511
ICU professionals	3	1	3.33	0.333		
Gentamycin (30mcg)						
Patients	16	14	87.5	0.875	78.95%	0.341
ICU professionals	3	1	3.33	0.333		

(n=19)

Table (5): MRSA and MSSA nasal colonization among the participated sample by gender

	Male	Female	Total (212)	Odds Ratio	*P*-Value
	(n=114)	(n=98)		(95%CI)	
MRSA	11(5.19)	8(3.77)	19 (8.96)	2.11(1.168-3.719)	0.003
MSSA	4(1.89)	3(1.42)	7(3.3)	1.25(0.714-1.731)	0.021
Total	15(7.08)	11(5.19)	26(12.26)		

(n=212)

VI. DISCUSSION

Since the introduction of Methicillin for clinical use, the proportion of MRSA strains isolated worldwide has risen sharply. In this study, the prevalence rate of MRSA is (8.96%) among patients and healthcare professionals which is lower than what has been reported by researchers in other regions of Saudi Arabia[15], as well as in other studies worldwide.[16,17] Similarly, study done by Zaman and Dibb at King Khalid National Guard hospital in Jeddah over a period of three years (1990 -1992) revealed that about 7.5% per annum of all isolated S. aureus were MRSA.[18] Furthermore, Bukhari and abdelhadi reported almost the same prevalence rate of MRSA infection (8.5%) among their sample.[19] Moreover, this result is in consistence with another study conducted by Bloemendaal et al[20], in which the prevalence of MRSA colonization was 9.6%, additionally, the result obtained from the current study is in agreement with Shakya et al. who reported that the international range of MRSA carriage is approximately 6 – 18% among the healthcare workers in hospital settings.[21] This carriage rate of MRSA is remarkably lower than what had been reported in Nigeria[22], Yemen[23], and Libya.[24] In regional perspective, Saudi Arabia has a higher prevalence of MRSA than Bahrain, Kuwait, and Lebanon countries.[25]

However, the prevalence rate in this study is different from that reported by Wang and his colleagues in Taiwan (14.9%), this difference could be due to differences in study design and population as they conducted a case control study.[26]

Our obtained result in this study is coincided with what reported in abha Maternity hospital (South-West Saudi Arabia) in which MRSA colonization at a rate of 71.6%.[27] Gender description in relation to MRSA had been considered in this study which shows that 114(53.8%) were males, while 46.2% were females. A similar sex description was reported from King Fahad hospital in Jeddah in which Bukhari and Abdelhadi[19] reported that male were 63%, while females were 37% in their study sample. Zaman and Dibb also reported 69% among his study sample were males.[18] Madani et al too had reported in their study that 73% male and 27% females.[28]

The prevalence rate of MRSA in the current study was higher among male than females, which is in consistence with a study from India by Rongpharpi et al.[29] found that the prevalence of MRSA nasal carriage was higher among male healthcare workers (54.28%) compared with that for females healthcare workers (45.71%). The reason of high S. aureus carriage among males compared with females reported from different countries are not fully known. Yet this difference might be attributed to the fact that men's maneuvers and their circle size of social contacts, especially in Arab countries and Middle East, is much higher than those of females.

Antimicrobial susceptibility tests performed in this study show that all MRSA isolates were sensitive to Vancomycin at a rate of 100% which is similar to what had been reported by Awadh and Al-Anazi in their

study.[30] In contrast, almost all the 81 microbial isolates in our study showed a high resistance to most of the antimicrobial tested in the current study in terms of Ampicillin (100%) followed by Oxacillin (89.47%), Penicillin G (89.47%), Cefoxitin (89.47%), Erythromycin (89.47%), Gentamycin (78. 95%) and Amikacin (78.95%). The 10% susceptibility of Erythromycin, Penicillin G, and Oxacillin among MRSA isolates in our study is similar to that found in community- associated MRSA in Texas.[31] These findings also were in agreement with those of Abdelmonem who found that MRSA resistance against Penicillin (97.77%) and against Amoxicillin (100%).[23]

VII. CONCLUSION AND RECOMMENDATION

There is existence of MRSA colonization among ICU patients and healthcare professionals. The nasal carriage of MRSA among the healthcare professionals still represents an issue that must be dealt with and addressed. The outbreaks of MRSA may indicate problems with infection control practice within healthcare settings. Currently, MRSA is not only a prominent healthcare-associated pathogens, but also an important cause of community-associated infections. It is also concluded that some antibiotics, such as Ampicillin, Oxacillin, Penicillin G, Cefoxitin and Erythromycin that were associated with all MRSA strains, reflecting their great abuse and/or irrational use in our country. The healthcare professionals should be informed about the potential consequences of the nosocomial infections, and their cooperation should be sought to diminish the carriage of Staphylococcus aureus. Some recommended steps to prevent all nosocomial infections including MRSA may be as follow: 1) Hands must be cleaned and washed carefully with soap and water, or to be cleaned and disinfected by using alcohol-based sanitizers. 2) Wounds must be covered with sterile dry bandages to avoid cross-infection from draining pus which could contains Staphylococcus. 3) Avoid personal items' sharing, such as towels, razors, clothing, washcloths and uniforms. 4) Dirty sheets, linen, cloths and towels must be cleaned with warm/hot water and detergents, and drying them in hot dryer instead of air dryer to kill all bacteria on the fabrics. 5) Healthcare workers must be screened routinely for MRSA carriage and treated if found positive. 6) Patients with high risk for MRSA should be screened upon admission and to be isolated if found positive carriers. 7) Hospital environmental cleaning and disinfection is recommended along with MRSA-infected patient isolation precautions. 8) Increase the national surveillance and implementation of risk adjusting infection rates between hospitals to increase awareness. 9) Development of less invasive infection resistant devices. 10) Better implementation of existing control measures from both hospitals and healthcare workers.

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