Original Research Article

Prevalence, antimicrobial susceptibility pattern and risk factors of MRSA isolated from clinical specimens among military patients at 48 Medical Compound in Sana'a city-Yemen

Abstract

Methicillin-resistant strains of *S. aureus* evolved in the 1970s and have troubled hospitals worldwide with persistent infections in patients. The objectives of this study were to determine the prevalence, antimicrobial susceptibility pattern and risk factors of MRSA isolated from clinical specimens among military patients at 48 Medical Compound in Sana'a city - Yemen. The study included 233 patients of whom suffering from *Staphylococcus aureus* infections. Specimens and data collected from November 2016 to November 2017. Standard methods of isolation and identifications were used to isolate bacteria in pours culture then *Staphylococcus aureus* were identifying using standard cultural techniques. MRSA was determined by the disc diffusion method to oxacillin and antimicrobial susceptibility testing was performed by the disc diffusion method for selected antibiotics. The prevalence rate of MRSA was 19.3% and there was significant association between MRSA and older age patients, and surgical site infections. There was higher rate of antibiotics resistant for tested antibiotics in MRSA isolates comparing with lower rate of antibiotics resistant in MSSA. 60% of the MRSA isolates were resistant to vancomycin. They were also susceptible to erythromycin and rifampicin (100%), but showed resistance to Cotrimoxazole and Gentamycine. In conclusion, the emergence of *S. aureus* isolates resistant to vancomycin and other wide range of antibiotics have raised MRSA in Yemen into a multi-drug-resistant 'Superbug", making it more and more dangerous than ever in our hospital environments. Regular surveillance of hospital associated infections and monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics used within and outside the hospital environments are recommend.

Keywords: MRSA, MSSA, MDR, Superbug, Sana'a city, Yemen

Introduction

Staphylococcus aureus (*S.aureus*) is a bacterium of significant importance because of its ability to cause a wide range of diseases and capacity to adapt to diverse environmental forms.[1] The organism colonizes skin, skin glands and mucous membrane, causing infections both in human and animals such as rashes, inflammations of bones and the meninges as well as septicaemia.[2] At present, the majority of *S. aureus* strains are resistant to most penicillin derivatives and ordinary antimicrobial agents like drugs from the family of aminoglycosides, macrolides, chloramphenicol, tetracyclines and fluoroquinolones.[3] The global spread of MRSA constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections.[4] MRSA carries a uniquely effective antibiotic resistance mechanism that can protect the microorganisms against all members of β-lactam antibiotics. This makes infections caused by these pathogens very difficult to manage and costly to treat.[5, 6]

The objectives of this study were to determine the prevalence, antimicrobial susceptibility pattern and risk factors of MRSA isolated from clinical specimens among military patients at 48 Medical Compound in Sana'a city – Yemen

Subjects and Methods

Ethical Consideration

Ethical clearance for the study was taken from the Faculty of Medicine and Health Sciences Research Review Committee. A written permission was also taken from the administrative Manager of the 48 Medical Compound, Sana'a city, Yemen. Informed Consent was taken from the patients before the questionnaire was filled.

Survey procedure and Laboratory Analysis

This study was conducted during a period of one year, starting in November 2016 and ending in November 2017 at 48 Medical compounds in Sana'a city-Yemen. All clinical specimens were received and collected by laboratory department of the hospital. 233 isolates of *S. aureus* were isolated from all age groups of out- or inpatients hospitalized in different wards. Clinical specimens were taken from various body sites of infection including blood, wound, sputum, urine and others. Clinical and demographic data were collected for all participants' patients. Clinical specimens were collected using standard collection techniques [7, 8], and inoculated on appropriate bacteriological media, including Blood agar, Chocolate agar, Thioglycollate, Mac-Conkey Agar Media. The plates were incubated aerobically at 37 °C for 18–24 hours. The blood cultures were performed using an automated blood culture system, followed by bacterial growth inspection. The identification of isolates was made according to standard methods [9] for any potential clinically significant growth appear on the culture media on the base of quantity, feature of growth, source and site of specimens. The primary identification was made with basic microbiological methods using colony morphology, Gram staining, catalase and coagulase tests.[7] **Antibiotic Resistance Phenotypes (Methicillin/Oxacillin susceptibility testing)**: All the *Staphylococcus aureus* isolates were tested for the susceptibility to 5 µg Methicillin disc and 1 µg Oxacillin disc provided by Difco using the disk diffusion method as described by NCCLS. The resistance breakpoints were ≥14 mm to ≤ 10 mm for 5 µg Methicillin, and ≥ 12 mm to ≤ 10 mm for 1 µg Oxacillin. The ability of other antibiotic disc to inhibit MRSA or MSSA were estimated according to the guidelines provided by NCCLS using commercially available discs which include: azithromycine (AZM, 15ug), chloramphenicol (C, 30ug), gentamycine (GN, 10ug), trimethoprim-sulfamethoxazole (SXT, 1,25/23,75ug), Oxacillin (OX, 1ug), erythromycin (E, 15ug), cefoxitin (FOX, 30ug), nitrofurantoin (NIT, 300ug), tetracycline (T, 30ug), teicoplanin (TE, 30ug), clindamycin (CC, 2ug), rifampicin (RA, 5ug), ciprofloxacin (5 µg, Ci), vancomycin (V, 30ug) and others listed in table 4.

Data analysis

The analysis of data was done by Epi Info version 6 statistical program (CDC, Atlanta, USA), where the chi-square (χ^2) and probability value (*p*) was calculated for the test of significance. In addition, Odd's ratio (OR), confidence interval (CI) were added to estimate the risk factors of contracting MRSA.

Results

Tables 1, 2, 3 show the prevalence of MSSA and MRSA among isolated coagulase positive (*S. aureus*), the potential associated factors of MRSA in patients with *S.aureus* infection and the association between prevalence of MRSA and type of hospital wards. Also, the susceptibility pattern of *S. aureus* strains is presented in table 4. The prevalence rate of MRSA was 19.3% and there was significant association between MRSA and older age patients, and surgical site infections. There was higher rate of antibiotics resistant for tested antibiotics in MRSA isolates comparing with lower rate of antibiotics resistant in MSSA. Also, 60% of the MRSA isolates were resistant to vancomycin. They were also susceptible to erythromycin and rifampicin (100%), but showed resistance to Cotrimoxazole and Gentamycine.

Discussion

We detected MRSA in 19.3% of all *staphylococcus aureus* isolates (table 1). Our result is lower than that reported from USA in which MRSA was isolated from 59% of patients with skin and soft tissue infections.[10] Also our 19.3% MRSA of all *staphylococcus aureus* isolates is lower than that reported from Yemen in previous reports in which MRSA was isolated from 55% of HCWs in Taiz city, Yemen[11] and extremely low from that reported by Al-Baidani *et al*, [12] among HCWs in Hodeida city, Yemen in which the MRSA rate was 86% . In addition, the prevalence of MRSA among our inpatients (HA-MRSA) was 19.4% roughly similar to 19.2% among

outpatients (CA-MRSA) (table .2). Prolonged hospital stays, indiscriminate use of antibiotics, lack of awareness, and receipt of antibiotics before coming to the hospital are some of the possible predisposing factors of MRSA emergence in the hospital and community. Our result is different from that reported from USA in which high rate of MRSA was occurred in hospital acquired *S. aureus* infections (HA-MRSA) (59%), than in community acquired infection *S.aureus* (17%).[13] This variation can be explained by that the biology of CA-MRSA appears to differ from that of HA-MRSA and CA–Methicillin-susceptible *S. aureus* (MSSA), perhaps allowing CA-MRSA to cause disease other than that expected from MSSA.[14,15] As HA-MRSA emerged, it likely did not merely replace HA-MSSA but led to an overall increase in *S. aureus* infections in healthcare settings.[16,17] In addition nearly all the researchers say the same thing that in-patients and out-patients have higher *S. aureus*/MRSA infections than *S. aureus* / MSSA because of wide spread of MRSA in community and hospitals environment.[17, 18,19]

When we considered age as risk factor of MRSA in our study, the highest incidence of MRSA in our cases were in age groups of > median (24 years). The risk age group of in-patients and out-patients in our study was different from that reported previously in several studies in which roughly equal rates of MRSA in the different age groups.[20]. In our study when we considered the association between prevalence of MRSA and sites of infections (type of clinical specimens), the highest prevalence MRSA was 33.3% in surgical site infection (SSI) with associated odds ratio equal to 2.2 (P>= 0.05) (table 2). The general pattern of MRSA infections in this study is not different from that worldwide in which in MRSA in surgical site infection (SSI) was one of the major diagnosis infections by MRSA.[20]

We carried this study because of the knowledge of MRSA prevalence and the current antimicrobial profile is necessary in selection of appropriate empirical treatment of these infections and control of MRSA in hospitals is essential. In our study vancomycin resistant was 40% in isolated MRSA. This result is different from that reported in Asian countries in which the rate of vancomycin resistant was not more than 10%.[21] Also the incidence of VRSA in the Asian countries has been documented by Kaleem *et al*, [22] in Pakistan to be 3.3% , 6% in India, by Sonavane and Mathur [23], 7.5% in Iran by Mehdinejad *et al*,[24], and 9% in Jordan by Al-Zoubi *et al*,.[3]

The results of our study shows a higher rate of resistant of MRSA comparing with a study carried out at India in 2009 which showed variable susceptibility pattern with high resistance rates to tetracycline (82%), clindamycin (79%), and cotrimoxazole (59%), while in our study all MRSA were sensitive to rifampicin different to 50% of rifampicin resistant in India.[25] On other hand resistance to chloramphenicol (50%) and naldixic acid (50%) was lower than Indian studies in which resistance to chloramphenicol was10% and naldixic acid was 9%.[25] Studies carried out at by Kishore *et al*, [26] in India, by Kaleem *et al*, [22] in Pakistani and by Al-Zoubi *et al*, [3] in Jordan showed that less than 10% of MRSA isolates were sensitive to Macrolides, whereas 38% of isolates were found to be sensitive in our study. In our study 100% of the MRSA isolates were sensitive to ciprofloxacin which is harmonized with the results of India , Pakistani, Iranian and Jordan studies in which ciprofloxacin also found to be 100% sensitive to all MRSA isolates.[3,22,26, 27] Effective antimicrobial activity as well as cost effectiveness should be considered in drugs prescribed for MRSA infections. Oral dosing options for antibiotics can allow earlier discharge of hospitalized patients and minimize the chances of VRSA emergence. Good hospital infection control measures prove to be the main stay against these infections because antibiotics can never be an effective alternative to good medical practice. Also, medium sensitivity percentages were found to gentamycine, trimethoprim-sulfamethoxazole and tetracycline (Table 4). Different sensitivity percentages have been reported to these three antibiotics worldwide. [28, 29, 30] These differences might be due to prolonged antibiotic treatment, age, type of infection and geographical variation.

Conclusions

In conclusion, the results of this study showed the importance of regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics used within and outside the hospital environments. Moreover, *in-vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories may be helpful for reducing the incidence of these infections.

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Conflict of interest:
"No conflict of interest associated with this work".

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Table 1 : The prevalence of MSSA and MRSA among isolated coagulase positive (*S. aureus*) in Sana'a city, 2017

Organisms	**Number**	**Percentage**
MSSA	188	80.7
MRSA	45	19.3
Total *Staphylococcus aureus*	**233**	**100**

Table 2: The potential associated risk factors of MRSA in patients with *S.aureus* infection in Sana'a city, 2017

Factors	**MRSA**		**MSSA**		**OR**	**CI**	χ^2	***p***
	No.	**%**	**No.**	**%**				
In-patients n=103	**20**	**19.4**	**83**	**80.6**	**1.0**	**0.5-1.97**	**0.001**	**0.97**
Out-patients n=130	**25**	**19.2**	**105**	**80.8**	**0.98**	**0.5-1.9**	**0.001**	**0.97**
Age Median (24 years)								
<24 years n=104	**13**	**12.5**	**89**	**85.6**	**0.45**	**0.23-0.9**	**5**	**0.02**
>24 years n=129	**32**	**24.8**	**99**	**76.7**	**2.2**	**1.1-4.5**	**5**	**0.02**
Clinical specimens								
Surgical wound pus n=39	**9**	**23.1**	**30**	**76.9**	**1.3**	**0.6-3.2**	**0.6**	**0.43**
Nonsurgical wounds pus n=77	**13**	**16.9**	**64**	**83.1**	**0.78**	**0.36-1.6**	**0.43**	**0.51**
Pleural fluid n=5	**1**	**20**	**4**	**80**	**1.04**	**0.11-9.5**	**0.002**	**0.96**
Sputum n=23	**3**	**13**	**20**	**87**	**0.6**	**0.17-2.1**	**0.64**	**0.42**
Surgical site infections (SSI) Wound and pus n=21	**7**	**33.3**	**14**	**66.7**	**2.3**	**1.0-6**	**3.9**	**0.05**
Urine culture n=41	**6**	**14.6**	**35**	**85.4**	**0.67**	**0.26-1.7**	**0.69**	**0.4**
Bone pus n=27	**6**	**22.2**	**21**	**77.8**	**1.2**	**0.46-3.2**	**0.16**	**0.68**

OR Odds ratio >1 (at risk)
CI Confidence intervals
χ^2 Chi-square ≥3.9 (significant)
p Probability value ≤0.05 (significant)

Table 3: The association between prevalence of MRSA and type of hospital wards in Sana'a city**.**

In-patients Wards	MRSA		MSSA		OR	CI	χ^2	*p* v
	No.	%	No.	%				
Orthopedic ward n=34 (33%)	7	20.6	27	79.4	1.1	0.39-3.1	0.031	0.86
Urology wards n=14 (13.5%)	4	28.6	10	71.4	1.5	0.45-5.1	0.37	0.59
Internal Medical wards n=7 (6.8%)	1	14.3	6	85.7	0.67	0.07-5.9	0.12	0.72
Surgical wards n=48 (46.6%)	8	16.7	40	83.3	0.56	0.2-1.5	1.3	0.25

OR Odds ratio >1 (at risk)

CI Confidence intervals
χ^2 Chi-square ≥3.9 (significant)
p Probability value ≤0.05 (significant)

Table 4: The antibiotic sensitivity for isolated MRSA and MSSA for tested antibiotics in Sana'a city.

Antibiotics	MRSA n=45		MSSA n=188	
	% Sensitive	% Resistant	% Sensitive	% Resistant
Azithromycin	66.7	33.3	60.9	39.1
Carbinicillin	0	100	100	0
Cefotaxime	25	75	61	39
Cefepime	44.4	55.6	65.6	34.4
Ceftazidime	50	50	50	50
Ceftriaxone	100	0	61.2	34.8
Cefalexin	49	51	76.3	23.7
Nalidixic acid	50	50	46.7	53.3
Chloramphenicol	50	50	66.7	33.3
Ciprofloxacin	100	0	61.3	38.7
Clindamycin	0	100	33.3	66.7
Cotrimoxazole	0	100	80	20
Erythromycin	100	0	65.6	34.4
Gentamicin	50	50	87.5	12.5
Nitrofurantoin	0	100	71.4	28.6
Rifampicin	100	0	33.3	76.7
Tetracycline	40	60	22.2	77.8
Ticarcillin	42.9	57.1	9.5	90.5
Tobramycin	55	45	30	70
Vancomycin	60	40	20	80