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Research Article

Frequency of Methicillin Resistance Among *Staphylococcus aureus* Clinical Isolates in Khartoum State, Sudan

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) have emerged as an important cause of nosocomial and community-acquired infections ranging from mild to severe life-threatening infections. Therefore, a reliable detection of such strains is required for effective treatment.

Objectives: To determine the frequency and the antibiogram of MRSA among different clinical isolates.

Study Design: A cross-sectional, descriptive study.

Materials and Methods: Standard bacteriological methods, disk diffusion and PCR were performed to determine the frequency of MRSA among different clinical isolates.

Results: The overall results showed 96/210 (45.7%) of isolates were MRSA mostly recovered from wounds and blood stream. High percentage was detected in hospital-associated (HA) strains (64.2%) rather than community (CA) (37.1%) (*P*-value < 0.001). From the generated Antibiogram, Co-trimoxazole was the most active (80.2%), while Penicillin was the least one (6.2%).

Conclusion: As MRSA strains were mostly isolated from Hospitals, clinicians should be aware of such burden strains. Local frequency investigation of MRSA is recommended for perfect diagnosis and treatment.

Keywords: MRSA, *S. aureus*, *mecA*, Sudan

1. Introduction

The *Staphylococcus aureus* has been recognized as one of the major human pathogens. It has been reported as the cause of spectrum of infections in hospitals and throughout the communities [1]. Humans and animals are the natural reservoirs of *S. aureus*. It has been reported that from 10 to 40% of people not associated with hospitals are carriers of *S. aureus* and the predominant sites of its colonization were the throat and anterior nares [2]. It is considered as one of the most common causes of soft tissues and skin infections such as carbuncles, abscesses, furuncles, folliculitis, impetigo, cellulitis, and bullous impetigo [3]. Other than skin and soft tissue infections, *S. aureus* is known to cause bone and joint infections, pneumonia, endocarditis, and scalded skin syndrome [4]. Methicillin-resistant *S. aureus* (MRSA) was first reported from the United Kingdom (UK) in 1961 [5]. In the following decades, MRSA had been established as a major nosocomial pathogen worldwide with a significant economic impact on healthcare systems [6]. Several reports indicated that MRSA infections have been associated with high mortality, morbidity, and high treatment cost. Furthermore, failure to identify MRSA in patients results in therapy failure and thus increases overall morbidity and mortality as well as the cost effectiveness [7–9]. MRSA resistance to methicillin has been attributed to a number of mechanisms, but the chief factor is supposed to be its ability to produce specific penicillin-binding protein 2a (PBP-2a) that renders β-lactamase-resistant penicillin ineffective including all other β-lactam drugs [10]. The PBP-2a has shown a usual low binding affinity for almost all beta-lactam antibiotics as compared to native PBPs [11]. The PBP-2a is coded and induced by the *mecA* gene, a part of *Staphylococcal* cassette chromosome (*SCCmec*). The *SCCmec* is known to be present in MRSA but not in the methicillin-susceptible *S. aureus* (MSSA) strains [12–14]. According to their origin, MRSA strains were classified into two main types: community-associated MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA). However; the prevalence varies from country to country, where most MRSA strains have been hospital-associated (HA-MRSA) [15, 16]. For most countries affected by MRSA, there have been many years of debate about its relative virulence compared with methicillin-susceptible *S. aureus* (MSSA) and whether it could be controlled. MRSA is endemic in the majority of hospitals around the world, and it is now considered as an additional burden of healthcare-acquired infection [8]. In the current study, the presence of *mecA* gene was investigated in *S. aureus* strains isolated from three Khartoum major hospitals. This cross-sectional study was designed to determine the frequency of MRSA strains and to elucidate their distribution among community and hospital strains.

2. Methods

The current study was performed mainly in Khartoum state; during the period from 2013 to 2015. The specimens were collected from three Khartoum major hospitals – Khartoum teaching hospital, Alribat teaching hospital, and the Military hospital. Study subjects were patients presented with any *S. aureus*-suspected infections. Scientific and ethical approval of the study was obtained from the ethical committee of Tropical Medicine Research Institute (Sudan).

2.1. Bacterial identification

The isolation and identification of bacterial isolates was done as described previously by Cowan et al. (1993) [17] and Collee et al. (1996) [18]. Two hundred and ten *S. aureus* isolates were recovered from different sites of infections. *S. aureus* ATCC 25923 was used as control strain in all procedures.

2.1.1. Criteria for labeling isolates as community associated (CA) or hospital associated (HA)

The isolates were differentiated into community or hospital strains according to the CDC standardized definition [19].

2.2. Antibiotic susceptibility testing

Muller-Hinton agar was used to test the isolates to different antibiotics according to CLSI, (2006) [20].

2.2.1. Testing isolates for methicillin resistance

Standard disk diffusion was done using oxacillin 1μg to differentiate MRSA from MSSA strains. A zone size of $\geq$ 10 mm was considered resistant; a zone size of $\geq$13 mm was considered susceptible [20].

2.3. Molecular detection of mecA gene

2.3.1. DNA extraction

DNA extraction was performed using DNeasy kit (QIAGEN). Pretreatment of bacterial cells was done according to the manufacturer instructions.

2.3.2. PCR amplification

mecA specific primers were used (Metabion International-Germany) as follows: *mecA*-1 (5 -AAA ATC GAT GGT AAA GGT TGG C- 3), *mecA*-2 (5 -AGT TCT GCA GTA CCG GAT TTG C- 3) for detection of the methicillin-resistant gene. Single PCR amplifications were performed in 25 µl reaction mixture (Maxime PCR PreMix Kit i-Taq 5 µl) (INTRON Biotechnology, South Korea), containing 3µl of bacterial DNA template, 1 µl of 5 pmol/µl from each primers, and the mixture was completed with 15 µl of sterile distilled water in a 0.2 Eppendorf (PCR) tube. Amplifications were performed by using the thermalcycler as follows:

An initial denaturing step at 94ºC for 3 min followed by 30 cycles of amplification with 94ºC for 30 S, annealing at 55ºC for 30 S, and extension at 72ºC for 30 S, except for the final cycle which had an extension step of 4 min. The size of the PCR product was 532 bp [21].

3. Results

3.1. Oxacillin disk diffusion result

80/210 (38.1%) of the *S. aureus* isolates were found to be MRSA while the remainder 130 (61.9%) were found to be MSSA.

3.2. PCR result

It is observed that 96/210 (45.7%) of isolates were *mecA* positive (MRSA), while 114 (54.3%) were *mecA* negative (MSSA) (Figure 1).

3.3. Distribution of MRSA according to the site of infection

According to the anatomical site of infection, the distribution of the encountered MRSA isolates was as follows: Surgical wound infection 40 (63.5%), skin and soft tissues 15

(34.1), paronychia 10 (37.0%), bone 8 (44.4%), urinary tract 5 (45.5%), ear infection 3 (37.5%), blood stream 5, others 7 (53.8%) (Table 1).

3.4. Frequency of MRSA according to the source

Among the total isolated community strains (143), the *mecA* positive (CA-MRSA) were 53 (37.1%), while among the total isolated hospital strains (67), the *mecA* positive (HA-MRSA) were 43 (64.2%) with significant difference (*P*-value < 0.001) (Table 2).

3.5. Antibiotics sensitivity pattern of MRSA isolates

With regard to MRSA isolates, we found that the most active antibiotic was Co-trimoxazole (80.2%), while the Penicillin was the least active (6.2%) (Table 3).
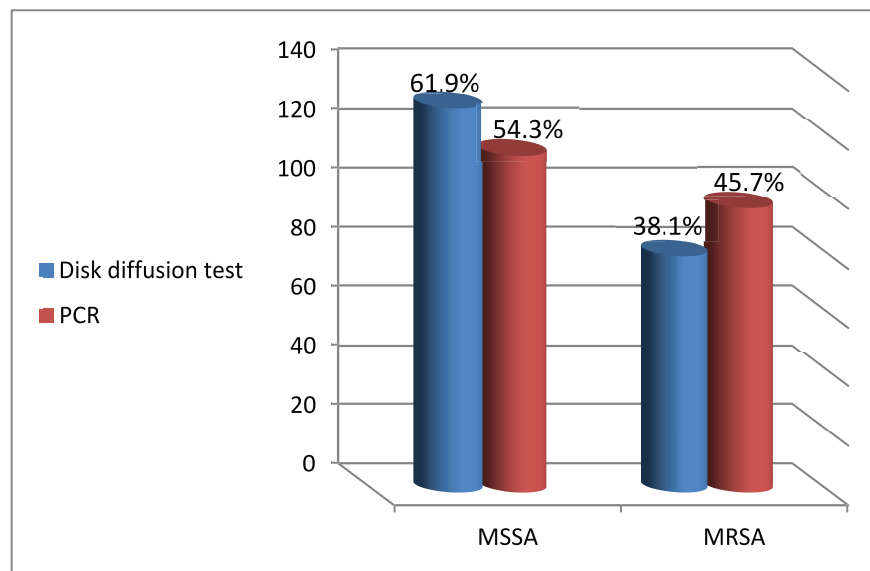

Figure 1: Number and percentage of detected MRSA and MSSA strains by disk diffusion and PCR (*mecA* detection).

4. Discussion

In the last few years MRSA have emerged as one of important medical pathogens. Hence, their rapid and accurate detection is of vital importance to formulate strategic interventions such as effective treatment regimens and sound control measures to combat and hinder their spread. However, the present study demonstrated that encountered MRSA strains are less frequent than MSSA while using standard disk diffusion and PCR procedures. This may be attributed to the more frequent MSSA
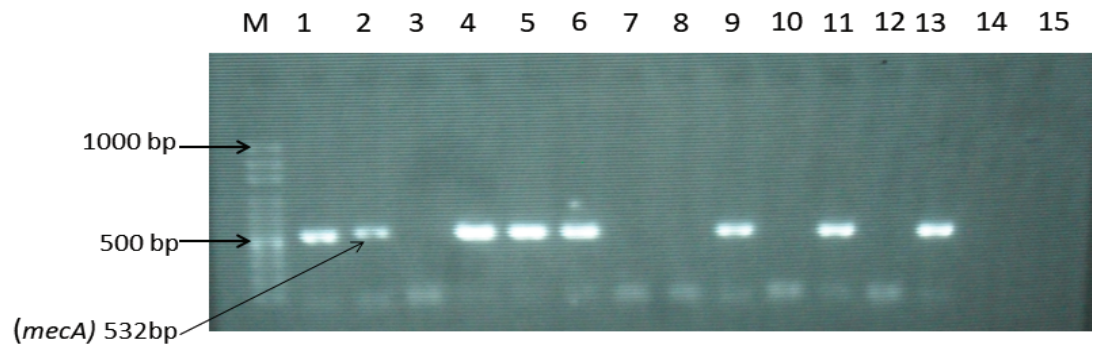
Figure 2: Detection of *mecA* gene by PCR in 1.5% agarose gel electrophoresis. M: 100 bp molecular ladder; lane (1 & 13): positive control; lane 2: isolate 131; lane 3: isolate 174; lane 4: isolate 175; lane 5: isolate 176; lane 6: isolate 177; lane 7: isolate 178; lane 8: isolate 179; lane 9: isolate 180; lane 10: isolate 181; lane 11: 182; lane 12: negative control. (Isolates 131, 175, 176, 177, 180, and 182 were *mecA* gene positive (MRSA) with product size 532 bp, while isolates 174, 178, 179 and 181 were *mecA* gene negative MSSA.)

TABLE 1: Distribution of isolated MRSA and MSSA strains according to the site of infection.

Site of Infection	Number (*n*)	MRSA (%)	MSSA (%)
Surgical wound	63	40 (63.5)	23 (36.5)
Skin and soft tissues	44	15 (34.1)	29 (65.9)
Paronychia	27	10 (37.0)	17 (63.0)
Bone	18	8 (44.4)	10 (55.6)
Urinary tract	11	5 (45.5)	6 (54.5)
Ear	8	3 (37.5)	5 (62.5)
*Aspiration	7	0	7 (100)
Blood stream	7	5 (71.4)	2 (28.6%)
Throat	5	3 (60.0)	2 (40.0%)
Nasal cavity	5	0	5 (100)
Respiratory tract	2	0	2 (100)
**Others	13	7 (53.8)	6 (46.2)
Total	210	96	114

Notes: *Aspiration include: Peritoneal (3), Pleural (2), Knee (2); **others include: tracheal swab (3), breast infection (2), umbilical swab (2), uterus swab (1), tissue biopsy (1), vaginal discharge (1), myositis (1), bacteremia (1) and semen (1).

TABLE 2: Frequency of MRSA and MSSA community (CA) and hospital (HA) strains.

Type of Isolate	CA–S. aureus (n = 143)	HA- S. aureus (n = 67)	*P-value
MRSA	53 (37.1%)	43 (64.2%)	< 0.001
MSSA	90 (62.9%)	24 (35.8%)	—

Notes: *p-value calculated using chi-square test; CA = community associated; HA= hospital associated.

strains rather than MRSA. Similar results were reported by different previous studies as that of Butt et al. (2004) and Durmaz et al. (1997) [22, 23]. Our finding showed that 16 isolates of the 130 MSSA isolates were phenotypically susceptible when tested by

TABLE 3: Antibiogram of some antibiotics used against MRSA isolates (total number 96 isolates).

Type of antibiotic	Susceptible isolates (%)	Resistant isolates (%)	Intermediate susceptible isolates (%)
Co-trimoxazole	77 (80.2%)	18 (18.8%)	1 (1%)
Ciprofloxacin	44 (45.9%)	23 (23.9%)	29 (30.2%)
Gentamicin	39 (40.6%)	49 (51.1%)	8 (8.3%)
Cefazolin	33 (34.4%)	50 (52.1%)	13 (13.5%)
*Amoxiclav	22 (22.9%)	74 (77.1%)	–
Penicillin G	3 (3.1%)	93 (96.9%)	–

Note: *Amoxiclav = amoxicillin clavulanic acid.

oxacillin disk diffusion, but when subjected to PCR they were found to be *mecA* gene positive. The remaining 80 isolates were detected as MRSA by both disk diffusion and PCR. No one of the phenotypically resistant isolates was found to be negative by PCR. However, the molecular assays now considered as standard method for genes detection including *mecA* gene and for discrimination of *S. aureus* from other species through multiplex PCR [24]. Even the PCR technique is used now as gold standard technique to evaluate the other conventional methods as done by Perez et al. [11]. Moreover, HA-MRSA (64.2%) were detected in a higher rate than the CA-MRSA strains significantly (*P*-value < 0.001) which supported our general impression. This finding was in consistence with that of Huang et al. (2006) who reported that MRSA strains were detected more frequently (55.1%) in hospital isolates [25]. On the other hand, the detected number of CA-MRSA strains may support the possibility that CA-MRS infections are in increase as observed in some other countries [19 26]. Furthermore, in the present investigation, MRSA infections were found to be distributed in different anatomical positions (Table 2). This may explain the increasing incidence of MRSA infections, which may be reflected by the increased duration of hospitalization and therefore increase in the overall treatment expenditure as stated by Cosgrove et al. [27]. With regard to antibiotics-susceptibility profile of MRSA, co-trimoxazole was found to be the most effective one (80.2%), followed by vancomycin (47%), ciprofloxacin (45.8%), and gentamicin (40.6%). These results explain why the co-trimoxazole was considered as one of the drugs used to treat MRSA infections [28–31]. For ciprofloxacin and gentamicin, the generated results are comparable to those reported in India where less than 30% were found to be resistant to ciprofloxacin, while about 40–50% were found to be resistant to gentamicin [32]. The susceptibility of MRSA isolates to β-lactam antibiotics was found to be 34.4%, 22.9%, and 3.1% for cefazolin, amoxicillin clavulanic acid, and penicillin, respectively. The high resistance

may be due to the production of additional β-lactamase by MRSA strains rendering them to resist improved antibiotics such as cefazolin and amoxicillin clavulanic acid. Schweizer et al. (2011) stated that cefazolin does not work in case of MRSA [33]. The current study had certain limitations. First, the study was done in only three major hospitals in the study area. Second, some sites of infection which can be infected by *S. aureus* were not represented here. In conclusion, this study reported significant high frequency of MRSA in hospital isolates. Moreover, these strains were mostly isolated from surgical wounds and blood stream. It is recommended that clinicians should be aware of the most active antibiotics against MRSA such as co-trimoxazole, vancomycin and ciprofloxacin.

Acknowledgement

Conflict of Interest

The authors have no competing interests.

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