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Molecular Detection of Methicillin Resistant *Staphylococcus aureus* in Patients With Urinary Tract Infections in Khartoum State

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ABSTRACT

Recent studies have reported the increasing prevalence of *Staphylococcus aureus* (*S. aureus*) in patients with urinary tract infections (UTIs). The resistance of *S. aureus* to commonly used antibiotics in different parts of the world has been widely reported. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been steadily increasing in the world. The study aimed to report the frequency of *S. aureus* and MRSA among urinary isolates and its antimicrobial resistance profile from patients with UTI in Khartoum state, Sudan. The study was carried out using 332 urinary bacterial isolates collected from different Khartoum hospitals. Identification of isolates was done by using conventional biochemical methods. All Gram positive bacteria were tested for their antimicrobial resistance to various antibiotics in vitro by the Kirby-Baur disk diffusion method. Using oxacillin screen agar test, all *S. aureus* isolates were screened for MRSA and confirmed by detecting the *mec*A gene by polymerase chain reaction (PCR). Out of 332 urinary isolates, 114(34.3%) were Gram positive bacteria. Fifty of the 332 (15%) were *S. aureus*. Among the 50 *S. aureus*, the number of MRSA isolates was 39 (78%). Gram positive isolates revealed that the maximum sensitivity was seen for vancomycin (90%) followed by norfloxacin (86%), cephalexin (74.6%), and tetracycline (66.7%). MRSA bacteria showed maximum resistance to nitrofurantoin (84.6%) followed by cefotaxime (71.8%) and gentamicin (64.1%) while minimum resistance was seen with vancomycin (0%) followed by norfloxacin (18%) and tetracycline (28.2%).

المستخلص

أشارت كثير من الدراسات الحديثة إلى زيادة انتشار التهاب المجاري البولية بسبب العنقوديات الذهبية وخاصة تلك المقاومة للميثيسيلين كما لوحظ ارتفاع معدل مقاومتها للمضادات الحيوية في عدة أنحاء من العالم. هدفت هذه الدراسة إلى معرفة معدل انتشار العنقوديات الذهبية وتلك المقاومة للمثيسيلين بين مرضى التهابات المجاري البولية بولاية الخرطوم كما هدفت إلى معرفة مقاومتها للمضادات الحيوية. تم جمع 332 سلالة معزولة من

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مرضى التهابات مجاري بولية من مختلف مستشفيات ولاية الخرطوم حيث تم التعرف عليها بالطرق التقليدية والكيموحيوية المعروفة. كذلك تم إجراء اختبارات الحساسية للمضادات الحيوية لكل البكتريا الموجبة الجرام بطريقة الانتشار القرصي (طريقة كيربي بوير). تم التعرف على العنقوديات الذهبية المقاومة للميثيسلين بطريقة آجار الاوكساسيلين وتم التأكد منها بطريقة اختبار التسلسلي التضاعفي لجين المك ايه (*mec*A). أظهرت النتائج أن نسبة البكتريا الموجبة الجرام كانت (34,4 %) وعدد العنقوديات الذهبية من بين ال332 سلالة كان 50 (15%). تسع و ثلاثون منها (78%) كانت مقاومة للميثسيلين. أعلى حساسية للبكتريا الموجبة الجرام كانت للفانكوماسين (90%) يليه النوروفلاكساسين (86%) يليه السيفالوكسين (74,6 %).أعلى نسبة مقاومة للعنقوديات الذهبية المقاومة للميثيسلين كانت ضد النيتروفونتوين (84,6 %) يليه السيفوتاكسيم (71,8%) ثم الجنتامايسين (64,1%). بينما أقل مقاومة كانت ضد الفانكوماسين(0 %) يليه النوروفلاكساسين (18%) ثم التتراسايكلين (28,2%).

***KEYWORDS*:** Staphylococcus aureus*, methicillin resistant, mecA gene, polymerase chain reaction, urinary tract infection*.

INTRODUCTION

UTIs are common infections among inpatients and outpatients, ranking second only to respiratory infections and thus, are a frequent cause for prescription of antibiotics[(1)]. UTIs are one of the most common bacterial infections in the general population, with an estimated overall incidence rate of 18 per 1000 person per year. In addition UTIs are a major cause of hospital admissions and are associated with significant morbidity and mortality as well as a high economic burden[(2)]. The vast majority of UTIs are due to the persons own fecal bacteria[(3)]. The common pathogens of UTIs are enteric Gram-negative bacteria and coagulase negative *Staphylococcus saprophyticus* [(4)]. *S. aureus* is a relatively infrequent urinary tract isolate in the general population. However, recent studies have reported the increasing prevalence of *S. aureus* in UTIs [(5-7)]. *S. aureus* is an important human pathogen in both nosocomial and community [(8)]. *S. aureus* is known to be notorious in the acquisition of resistance to new drugs and continues to defy attempts at medical control. The resistance of *S. aureus* isolates to commonly used antibiotics in different parts of the world has been widely reported [(4,6)]. MRSA is a type of Staphylococcus bacteria that is resistant to beta-lactams. These antibiotics include methicillin and other more common antibiotics such as oxacillin, penicillin, and amoxicillin. The prevalence of multi-drug (MDR) MRSA with very limited treatment choice is also on an increase. The first MRSA isolate was detected in the medical centers setting in the early 1960 [(9)]. Over the past decade, the percentage of *S. aureus* in clinical specimens that are methicillin-resistant has been steadily increasing in the world. Rates were highest (55.9%) in inpatient with lower respiratory specimens and lowest (37.6%) in

outpatient with skin and soft tissue specimens [10]. Many strains of *S. aureus* carry a wide variety of multi-drug resistant genes on plasmids which aid the spread of resistance even among different species [11]. The resistance of the organism is due to the acquisition of the methicillin resistance gene *mec*A coding for the low-affinity penicillin-binding protein PBP2A [12]. This changing spectrum of microorganisms involved in UTIs lead to the necessity of the need for continuous and regular antimicrobial resistance surveillance in these organisms in order to guide empirical therapy in UTIs. Most studies on UTIs have concentrated on the antimicrobial resistance profile of Gram-negative bacteria while prevalent rate of *S. aureus* in UTIs and its role in antibiotic resistance is in an increase rate. Thus this study investigates the antimicrobial resistance profile of *S. aureus* strains from patients with UTIs in Khartoum, Sudan.

MATERIALS and METHODS

The study was carried out using 332 urinary bacterial isolates collected from different hospitals in Khartoum State. The desired patient information were obtained from each patient and recorded in a special request form. The isolates were taken by sterile swabs then inserted in nutrient broth media and transported to the microbiology lab (research lab) at Sudan University for Sciences and Technology (SUST). To obtain pure culture, isolates were subcultured in Cysteine Lactose Electrolyte Deficient (CLED) agar and nutrient Agar, then identification of isolates was done by using conventional and biochemical methods. Identification of staphylococci was based on the colony morphology, Gram staining, catalase, coagulase tests and a latex slide agglutination Staphytect Plus test (OXOID).

Antibiotic Susceptibility Tests

All Gram positive bacteria were tested for their antimicrobial resistance to various antibiotics in vitro by the Kirby-Baur disk diffusion method. They were tested with cefotaxime(CTX) (30 µg), cephalexin (CFX) (30 µg), nitrofurantoin (NF) (300 µg). tetracycline(TE) (30 µg), vancomycin (V) (30 µg), norfloxacin (10 µg), amoxyclav(AMC) (20/10 µg), amikacin (AK)(30 µg), gentamicin (GN) (10 µg), cotrimoxazole(SXT) (1.25/23.75 µg) and azithromycin (AZM) (15 µg), were placed on the Mullar Hinton Agar (MHA) (Oxoid). After overnight at 37° incubation, the diameter of each zone of inhibition was measured in mm. The susceptibility testing results were noted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [13].

The Oxacillin Screen Agar Test

Muller-Hinton agar plates containing 4% NaCl and 6 µg/ml of oxacillin were prepared. To perform the oxacillin screen test, a swab which was dipped in a suspension of the isolate and then was deposited as a spot on the agar surface and it was incubated at 35°C for 24 h. The plates were observed carefully for any growth. Any growth after 24 hr was interpreted as oxacillin resistance [14].

Detection of *mec*A gene DNA Isolation

A single colony was taken from a nutrient agar plate (Oxoid) that had been incubated overnight and then emulsified into 50 µL of lysostaphin (100 mg/L; Sigma, Sydney, Australia). After incubation for 10 min at 37°C, 50 µL of proteinase K (100 mg/L; Sigma) and 150 µL of TE buffer (1 mM EDTA/10 mM Tris, pH 7.5) were added to the suspension and incubated for a further 20 min at 37°C then incubated at 95°C for 5 min to deactivate the proteinase K. DNA was stored at −20°C until reuse. Five microlitres were taken from the suspension and used directly for the PCR [15].

Amplification of DNA by PCR

All suspected MRSA isolates were screened for the resistance *mec*A gene by PCR, with specific primers MECA P4 (5' TCCAGATTACAACTTCACCAGG -3') and MECA P7 (5'-CCACTTCATATCTTGTAACG -3'). The mecA primers amplified at 162-bp fragment[16]. The primers were synthesized by IDT (Integrated DNA technologies, Interleucvenlaan, 12A,B 3001, Belgium).

PCR Conditions

A 50µl PCR mixture containing 8 µl of DNA template, 1µl (100 pmol) of each primer and a 25µl of Taq PCR Master Mix polymerase containing 100mM Tris-HCl, 500mM KCl at pH 8.3 at 20°C, 1.5 mM $MgCl_2$, 200M of each of deoxyribonucleoside triphosphate and 0.025U Taq polymerase (Qiagen, USA) was prepared. Then 1µl of template DNA from each isolate was added to each tube separately. Amplification of DNA was performed using Mastercycler PCR machine (Appendorf Co). The cycling program was performed with an initial denaturation for 5 min at 94°C, then 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 53°C and extension for 1 min at 72°C and final extension for 5 min at 72°C.

Agarose Gel Electrophoresis25 µl of PCR products were mixed with 2-8 µl of 10 x TBE buffer and 1-4 µl of bromophenol blue (0.25%). PCR mixtures were applied to 1% agarose gel (sigma, USA) and products were separated by electrophoresis for 35 minutes at 90 V using 5 x TBE running buffer (4.84 g/l Tris, 0.37 g/l EDTA, pH 8). A 100 bp DNA ladder (Qiagen, USA) was included in each run. DNA band was viewed under UVP BioDoct-It digital imaging system (UVP, Inc., Cambridge, UK) after staining of the gel by ethidium bromide (2 g/ml).

RESULTS

The present study showed that the antibiotic resistance patterns phenotype and MRSA genotyping of Gram positive uropathogens in Khartoum. Out of 332 urinary isolates, 114 (34.3%) were Gram positive bacteria while fifty (15%) were *S. aureus.* Among the 50 *S. aureus*, the number of MRSA isolates was 39 (78%). PCR was 100 % positive for *mec*A gene for all 39 MRSA isolates as shown in figure 1. Antibiotic susceptibility pattern of all Gram positive isolates (114) and antibacterial resistance of MRSA (39) bacteria are shown in (Table 1). The antibiotic sensitivity

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pattern of the Gram positive isolates revealed that the maximum sensitivity was seen for vancomycin (90%) followed by norfloxacin (86%), cephalexin (74.6%), and tetracycline (66.7%). The maximum resistance was seen against ceftazidime (74%), nitrofurantoin (55.3%) and gentamicin (37.8%). MRSA bacteria showed maximum resistance was to nitrofurantoin (84.6%) followed by cefotaxime (71.8%) and gentamicin (64.1%) while minimum resistance was seen with vancomycin (0%) followed by norfloxacin (18%) and tetracycline (28.2%).

Table 1: Antibiotic sensitivity pattern for the total urinary Gram positive isolates (n = 114) and MRSA (n= 39)

	Antibiotic Name	Sensitive %	Intermediate%	Resistant	
				MRSA (39)	Total (114)
1	AK (30 µg)	72 (82)	7 (8)	35.9 (14)	21 (24)
2	GN (10 µg)	44 (50)	18.4 (21)	64.1(25)	37.8 (43)
3	AZM (15 µg)	59.7 (68)	14 (16)	51.3 (20)	26.3 (30)
4	CTX (30 µg)	30.7 (35)	5.3(6)	71.8 (28)	64 (73)
5	V (30 µg)	90 (103)	1.8 (2)	0.0 (0)	8 (9)
6	CFX (30 µg)	74.6 (85)	4.4 (5)	33.3 (13)	21 (24)
7	NF (300 µg)	41.2 (47)	3.5 (4)	84.6 (33)	55.3 (63)
8	SXT (1.25/23.75 µg)	49.1 (56)	22 (25)	38.5 (15)	28.9 (33)
9	TE (30 µg)	66.7 (76)	15 (17)	28.2 (11)	18.4(21)
10	AMC (20/10 µg),	51.8 (59)	20.2(23)	43.6 (17)	28 (32)
11	NOR (10 µg)	86 (98)	2.6 (3)	18 (7)	11.4 (13)

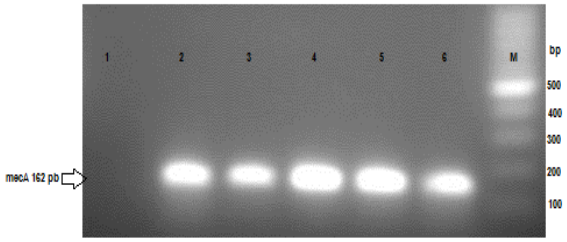

Figure 1. MecA gene after PCR on 1% Agarose gel electrophoresis. Lane 1: Negative control. lanes 2, 3, 4, and 5 positive mecA gene at (162bp). Lane 6: Positive control: Lane M: 100-bp DNA ladder

DISCUSSION

Worldwide Gram negative bacteria account for more than 80% of the culture positive cases of UTIs and 10 to 20% are caused by Gram positive bacteria. *S. aureus* is a relatively infrequent urinary tract isolate in the general population. However, there is an increasing prevalence of *S. aureus* as a UTI pathogen with an alarming

rate of developing antimicrobial resistance [4]. The present study revealed high rate of UTIs caused by *S. aureus* (15%). A near finding was reported by Al-Ruaily and Khalil in Saudi Arabia [17], Akerele *et al.* [18], Okonko *et al.* [19], in Nigeria and Manikandan *et al.* [20], in India reported near this finding. Their findings confirm that *S. aureus* has become an important etiologic agent of UTIs. MRSA is a significant pathogen that has emerged over the last decades causing both nosocomial and community-acquired infections. In this study the number of MRSA isolates was 39 (78%) which were confirmed by PCR. Molecular techniques remains to be the most sensitive method in detecting *S. aureus* and was found in some studies with 100% accuracy in detecting MRSA, when compared with the classical identification method [17]. In a similar study from Nigeria, Onanuga et al. [4] reported high rate of MDR-MRSA among urinary isolates. In the present study the antibiotic sensitivity pattern of the total Gram positive isolates showed maximum sensitivity to vancomycin (90%) followed by norfloxacin (86%), cephalexin (74.6%), and tetracycline (66.7%). Thus, these antibiotics were the only effective agents on most of the UTIs with *S. aureus.* and therefore they can be used in the empiric treatment of Gram positive UTIs. The good finding in this study was the diminished antibiotic resistant of MRSA to vancomycin (0%). Thus, vancomycin continues to be the drug of choice for treating most MRSA infections caused by multi-drug resistant strains. Recently in other countries, *S. aureus* with elevated vancomycin MICs have emerged. *S. aureus* with intermediate susceptibility to vancomycin (MIC 8 mg/L) were first identified in Japan in 1996 [21]. In this study, MRSA showed maximum resistance to nitrofurantoin (84.6%) followed by cefotaxime (71.8%) and gentamicin (64.1%), so the use of these agents in the empiric treatment of MRSA is useless and should be avoided. The situation in Sudan is of particular concern because self-medication and the use of antibiotics without medical guidance are largely facilitated by inadequate regulation of the distribution and sale of prescription drugs [22]. Rapid and accurate detection of methicillin resistance in *S. aureus* is important for the use of appropriate antimicrobial therapy and for the control of spread of MRSA strains. MRSA organisms should be identified quickly so that appropriate antibiotic usage and infection control measures can be implemented. For greater detection rates of MRSA, molecular methods have the shortest turn around time. Although, molecular testing remains expensive relative to conventional agar-based detection, there is an overall cost savings, especially if molecular testing is directed at high-risk populations.

CONCLUSION

The study revealed high rate of UTIs caused by *S. aureus* and high rate of MRSA confirmed by PCR in Khartoum. High prevalence of multi-drug methicillin-resistant *S. aureus* (MDR-MRSA) with very limited treatment choices is also on an increase

in Sudan. MRSA organisms should be identified quickly so that appropriate antibiotic usage and infection control measures can be implemented.

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