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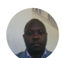
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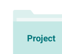
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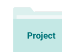
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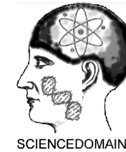

Prevalence and Antibiotic Susceptibility Patterns of Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* in a Tertiary Care Hospital in Western Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IJS participated in the planning of the study, data entry and drafting of manuscript; author FB supervised the laboratory work, authors HI, MN and MB reviewed the manuscript and participated in laboratory work, author JB participated in the planning of the study, drafting and critical review of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To determine the prevalence and antibiotic susceptibility patterns of clinical isolates of methicillin resistant *Staphylococcus aureus* isolated at Mbarara Regional Referral Hospital.
Method: A total of 400 *S. aureus* isolates recovered from various clinical specimens at Mbarara Regional Referral Hospital were included in this study. Phenotypic screening was performed using Oxacillin. Presence of *mecA* gene was studied using polymerase chain reaction (PCR). The *mecA* positive isolates were tested for susceptibility to, Vancomycin, Imipenem, Fusidic acid, Trimethoprim/Sulfamethoxazole, Clindamycin and Linezolid using the Kirby Bauer technique.
Results: Of the 300 isolates of *S. aureus* 31.3% (94) were phenotypically MRSA and 38% (114) had the mecA gene. All the MRSA isolates were susceptible to vancomycin and linezolid but were highly resistant to trimethoprim/sulfamethoxazole (70.2%). Of the 114

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MRSA isolates 19.3% (22) were multi-drug resistant *S. aureus* (MDR-MRSA). The study found that there was a significant difference between genotypic and phenotypic detection methods ($p < 0.001$).
Conclusion: The prevalence of MRSA in Mbarara is high (38%) with a high resistance to trimethoprim/sulfamethoxazole. The detection of *mecA* gene is a good predictor of methicillin resistance in *S. aureus.* There is a worrying prevalence of MDR MRSA among the clinical isolates of *S. aureus* in South Western Uganda.

Keywords: Staphylococcus aureus, MRSA (Methicillin Resistant Staphylococcus aureus), Antibiotic, Resistant.

1. INTRODUCTION

Staphylococcus aureus causes diseases ranging from mild skin infection to life-threatening infections [1]. In the pre-antibiotic era, mortality from *S. aureus* disease was high and introduction of penicillin had a dramatic impact, which was short-lived due to the emergence of penicillinase-producing *S. aureus* [2]. Methicillin was introduced in 1959, and methicillin-resistant *Staphylococcus aureus* (MRSA) strains rapidly emerged and became a major problem in hospitals. First identified in the 1960s, MRSA was initially considered a nosocomial pathogen. However, in recent years, increasing numbers of MRSA strains have been isolated worldwide from patients with community-acquired infections [3,4]. Two main types of MRSA now circulate in the community: (i) hospital-acquired strains (HA-MRSA) that infect patients with risk factors such as recent hospitalization, surgery, underlying chronic diseases, or immunosuppression and (ii) strains arising in the community (CA-MRSA) and infecting patients with no established risk factors. CA-MRSA strains tend to be susceptible to more antibiotics than HA-MRSA strains [5]. The genome of methicillin-resistant Staphylococci contains a 21- to 67-kb heterologous mobile genetic element, termed staphylococcal cassette chromosome mec (SCCmec), harbouring the *mecA* gene and other resistance determinants. Methicillin resistance is mediated by production of an altered penicillin-binding protein, PBP-2a, encoded by the mecA gene [6,7].

SCCmec characterization relies on variations of the *mec* gene complex, cassette chromosome recombinase (ccr) complex, and junkyard regions [8].

There is limited data on Methicillin resistant *Staphylococcus aureus* (MRSA) in Uganda and use of PCR for MRSA detection is not affordable. We aimed to determine the prevalence and antibiotic susceptibility patterns of MRSA among *S. aureus* isolates in MRRH, and compare the performance of oxacillin disc diffusion with *mecA* PCR for the detection of MRSA.

2. MATERIALS AND METHODS

A total of 400 consecutive isolates of *S. aureus* from clinical specimens that were collected between June 2012 and June 2013 in the Microbiology laboratory of Mbarara University of Science and Technology were included in the study. All the isolates were identified as *S. aureus* by cultural characteristics and biochemical tests which included free and bound coagulase, DNAase, chromogenic agar, and Mannital Salt Agar (Oxoid).

The biochemical tests were carried out as per the standard procedure performed routinely in our laboratory [9].

Phenotypic screening for methicillin resistance was performed using oxacillin disc (1µg) on Mueller-Hinton agar (Oxoid). Mueller Hinton agar plate containing 2% NaCl was inoculated with 10 µL of 0.5 McFarland suspension of the isolate, the disc was placed on the medium and the set up incubated at 37ºC for 24 hours. Methicillin resistant *Staphylococcus aureus* population tends to prefer a high salt concentration as contained in Mueller Hinton agar with 2% NaCl [10] An inhibition zone diameter of ≤ 10 mm was considered resistant for Oxacillin.

Genotypic detection of MRSA was performed using PCR. A loopful of an overnight pure bacterial culture was collected using a sterile inoculation loop and suspended in 100µl of sterile PCR water, vortexed and boiled at 100ºC for 15 min in a heat block. The products of boiling were left to cool for 5 min and then centrifuged at maximum speed (14,000 rpm) for 15 minutes and 2 µl of supernatant used for PCR. A 162-bp fragment of the *mecA* gene was amplified using the primers 5' TCC AGA TTA CAA CTT CAC CAG G-3' (Forward) and 5' CCA CTT CAT ATC TTG TAA CG-3' (Reverse) from Integrated DNA Technologies (IDT).

Antimicrobial susceptibility testing was performed by using the Kirby-Bauer method according to the Clinical laboratory standard Institute 2011 and incubated for 18 hours at 37ºC after which the results were recorded. The following drug were tested; Trimethoprim/sulfamethoxazole (1.25µg/23.75µg), Vancomycin (30µg), Clindamycin (2µg), Linezolid (10µg), Imipenem (10µg) and Fusidic acid (10µg). The radius of the zone of inhibition was measured using a ruler and reported as sensitive or resistant in accordance to the CLSI guidelines in order to establish the susceptibility pattern [11]. *S. aureus* ATCC 25923 (*mecA* negative) and ATCC 43300 (*mecA* positive) – were used for quality control. PCR water was used as a negative control during PCR. All molecular work was carried out at MBN Clinical laboratories.

3. RESULTS

The study included 400 isolates from different clinical samples from all the wards of the hospital.

Of the 400 clinical isolates only 300 isolates were clearly identified as *S. aureus* using our identification schemes. The study included 400 clinical isolates from various clinical specimens sent to the microbiology laboratory for culture and sensitivity.

As shown in Fig. 1 below, of 300 isolates tested by disc diffusion method using oxacillin, 94 (31.3%) were identified as MRSA. PCR identified 114 (38%) out of 300 isolates as MRSA.

A total of 59 isolates were resistant to oxacillin and confirmed as MRSA based on the detection of the *mecA* gene (Table 1). Phenotypically 94 (35%) of the isolates were positive and 206 (68.5%) were negative. Gentoypically 114 (38%) of the isolates were positive and 186 (62%) were negative.

Table 1. Comparison of phenotypic and genotypic methods for detection of *mecA* in *S. aureus* isolates (n=300)

PCR *mecA*	Phenotypic detection		No. of isolates
	Sensitive	Resistant	
Positive	55 (18.3%)	59 (19.7%)	114 (38%)
Negative	151 (50.3%)	35 (11.7%)	186 (62%)
Total	206 (68.5%)	94 (31.5%)	300 (100%)

(X^2= 35.64; p = 0.000).

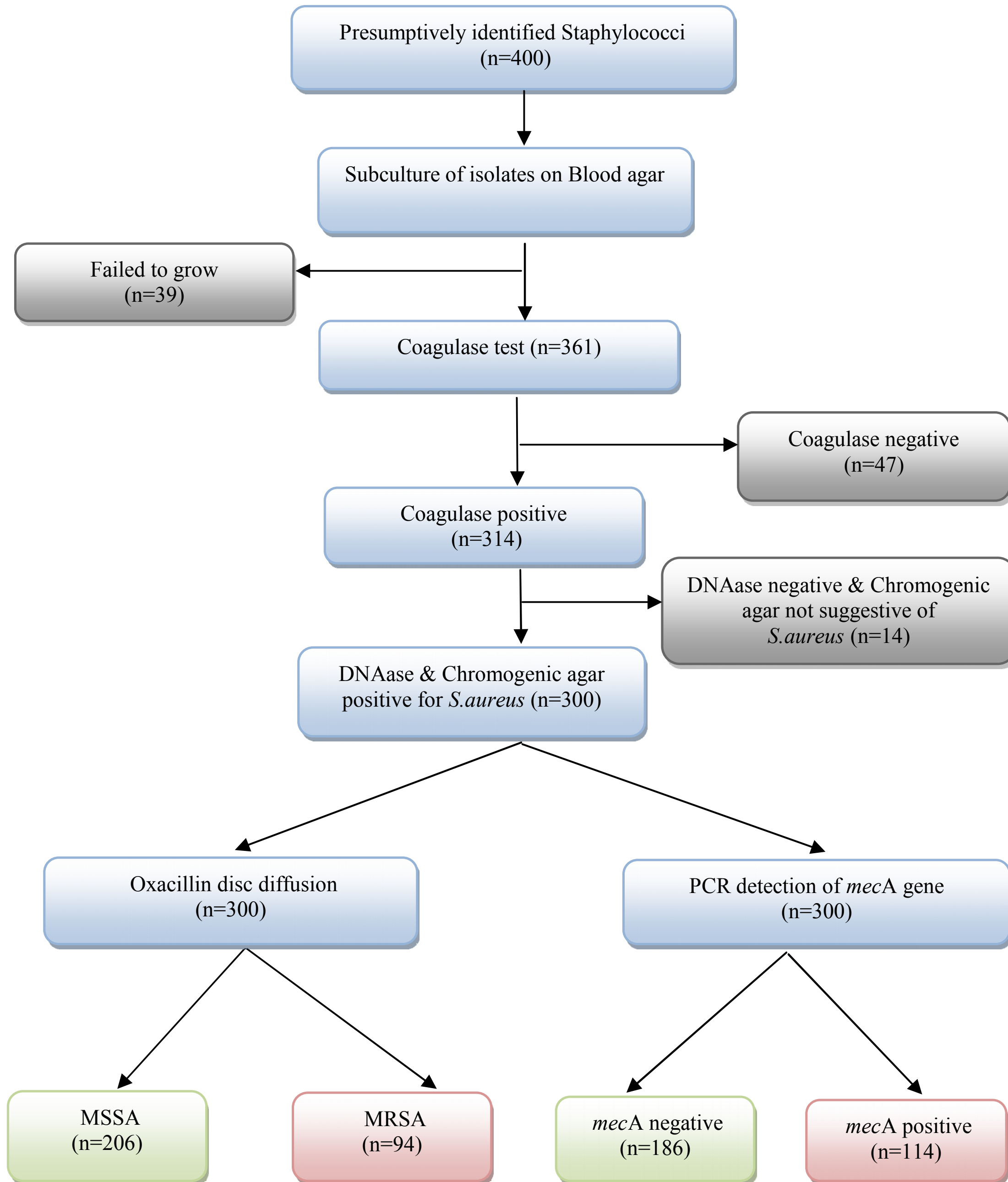

Fig. 1. The study profile

3.1 Antibiotic Resistance Patterns

The 114 *S. aureus* isolates that were positive for *mecA* gene were analysed for antimicrobial resistance (Fig. 2).

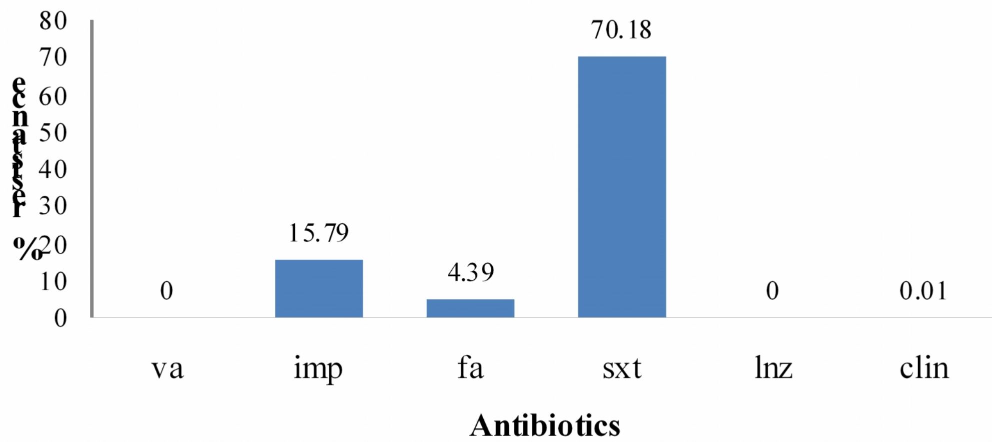

Fig. 2. Antibiotic resistance patterns of MRSA isolates

Antibiotics used: va= vancomycin, imp= imipenem, fa= fucidic acid, sxt= trimethoprim/sulfamethoxazole lnz= linezolid and clin=clindamycin.

As shown in Fig. 2 above, all the isolates were susceptible to vancomycin and linezolid. However most isolates were resistant to trimethoprim/sulfamethoxazole. In this study 19.3% of the MRSA showed co-resistance to two or more classes of antibiotics and are referred to as multi-drug resistant *S. aureus* (MDR-MRSA) as shown in Fig. 3 below.

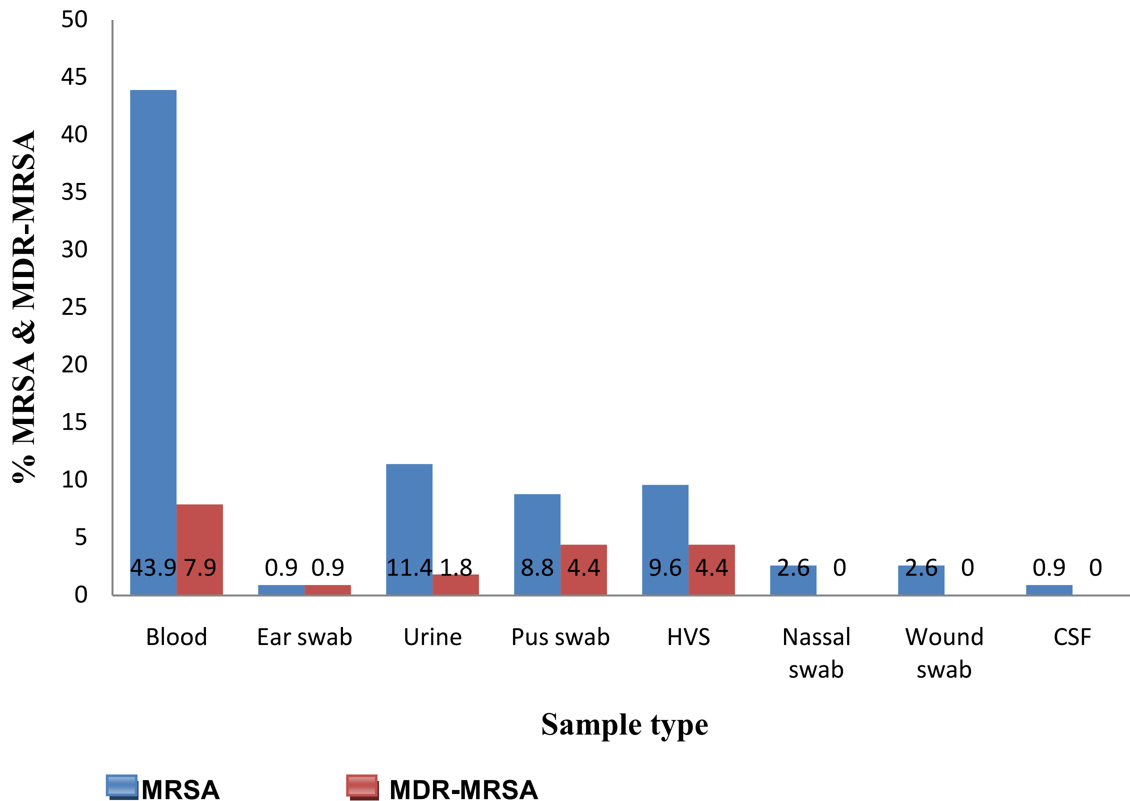

Fig. 3. Distribution of MRSA by Sample type

Blood had the highest proportion of MDR-MRSA and ear swabs had the lowest proportion where as nasal swabs, wound swabs and CSF has no MDR-MRSA as shown in table 2 below.

Table 2. Distribution of MRSA by ward

Ward	MRSA total cases (%)	MDR-MRSA total cases (%)	Total cases (%)
ENT	1(0.9)	1(0.9)	2(2)
OPD	26(22.8)	3(2.6)	29(25)
Paediatrics	39(34.2)	8(7.0)	47(41)
Obs/Gyn	7(6.1)	9(7.9)	16(14)
Medical	14(12.3)	1(0.9)	15(13)
Dermatology	4(3.5)	0(0)	4(4)
ICU	0(0)	0(0)	0(0)
Surgery	0(0)	0(0)	0(0)
Emergency Ward	1(0.9)	0(0)	1(1)
Total	92(80.7)	22(19.3)	114(100)

ENT= Ear, Norse and Throat, OPD = Out Patient Department, Obs/Gyn = Obstetrics/Gynecology, ICU = Intensive Care Unit.

The Pediatrics ward had the highest proportion of MRSA, ENT and Emergency ward had the lowest proportion while ICU and Surgery had no MRSA. This study registered the highest distribution of MDR-MRSA in Obstetrics/Gynecology ward and Paediatics ward, Medical ward had the lowest proportion of MDR-MRSA where as Dermatology, ICU, Surgery and Emergency ward had no MDR-MRSA.

4. DISCUSSION

We screened all isolates phenotypically and genotypically using Oxacillin and *mecA* gene by PCR respectively. The 38% prevalence in our study as determined by *mecA* PCR is comparable to that reported [12] in Kampala - Central Uganda at 37.5%, [13] India at 36.4% and 37.7% [14] in shiraz-Iran. Similar rates have been reported in USA. Studies from tertiary care centers all over the world [13,15,16] supported this high incidence which was found in our study. In neighbouring Tanzania the prevalence of MRSA was 16% reported in 2008) and rates of 21–30% were noted in Nigeria, Kenya, and Cameroon [17-19].

The phenotypic susceptibility testing showed that 31.3% of isolates of *S. aureus* were MRSA a finding similar to 31.5% reported in Kampala Central Uganda [20] using the same methods. The findings of our study carried out in South Western Uganda being similar to finding of the study in central Uganda may imply that the MRSA is quite well spread in Uganda.

The sensitivity and specificity of the oxacillin disc diffusion method of 62.8% and 73.3% respectively, reported in this study is much less than 90% and 100% reported by Pramodhini et al. [13]. A number of studies have reported, sensitivity and specificity of disc diffusion method of between 61.3 - 100% and 50 - 99.1% respectively [21-26]. This could be due to the heterogeneous nature of resistance showed by MRSA which limits the sensitivity and specificity of oxacillin disc diffusion method for detection of MRSA.

PCR identified 55 (18.3%) isolates that were sensitive to oxacillin as MRSA. The high prevalence of *mecA* gene among *S. aureus* isolates shows the weakness of phenotypic detection method for MRSA implying that genotypic method is superior to phenotypic method of detection of MRSA.

All the isolates in this study were susceptible to vancomycin and Linezolid. This finding is similar to the study conducted in Shiraz, Bangalore, Pakistan and South Africa [14,27-29] and indicates that these drugs can still be effectively used in our setting. The high resistance to trimethoprim/sulfamethoxazole in this study is similar to what has been reported by Ojulong [30] in Central Uganda and many other researchers, a finding similar to that by Kumar et al. [31]. This could be due the fact that this drug is very commonly available in our setting and is also used for prophylaxis by all HIV positive individuals.

In this study the isolates from blood had the highest MRSA and MDR MRSA rates. This differs from the study by Carnicer-Pont et al. [32] and Terry et al. [18] where a high proportion of MRSA were isolated from indwelling devices and endocervical swabs, while low rate of isolation of MRSA had been found in blood. Most isolates in this study came from blood (54.7%) and this could explain the high rate of MRSA in blood. Multi-resistant MRSA has been reported to be relatively high in African countries including Morocco, Kenya, Nigeria and Cameroon [33].

The Pediatrics ward had the highest proportion of MRSA with low rates in ENT and Emergency ward and no MRSA in ICU.

The Obstetrics/Gynecology ward and Paediatics ward had the highest prevalence of MDR MRSA. The high prevalence of MDR-MRSA could be attributed to the wide use of antibiotics for prophylaxis after birth or caesarean section and common use in children.

5. CONCLUSION

The prevalence of MRSA as detected by *mecA* gene in South Western Uganda is high with a high proportion of the MRSA isolates being multidrug resistant. The MRSA isolates are highly susceptible to Linezolid and vancomycin but highly resistant to trimethoprem/sulfamethoxazole. Genotypic detection of MRSA was better than phenotypic detection of MRSA.

6. RECOMMENDATION

Complete eradication of MRSA may not be an easy goal to achieve but simple methods like hand washing coupled with regular surveillance of hospital-associated infection and monitoring of antibiotic sensitivity pattern is widely recognized as effective in reducing MRSA prevalence.

ETHICAL APPROVAL

Permission was sought from Department of Microbiology, Mbarara University of Science and Technology faculty of Medicine Research and Ethics committee, Institutional review committee and National Council for Sciences and Technology Institutional Review Committee.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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