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Methicillin-Resistant *Staphylococcus aureus* among Patients with Skin and Soft Tissue Infections: A Cross-Sectional Study at a Tertiary Hospital in Bushenyi, Western, Uganda

Theophilus Pius[1*], Reagan Irege[1], Danladi Makeri[2], Andrew Tamale[3]

[1]Department of Medical Laboratory Sciences, School of Allied Health Sciences, Kampala International University Western Campus, Kampala, Uganda
[2]Department of Microbiology and Immunology, Kampala International University Western Campus, Kampala, Uganda
[3]Department of Public Health, Kampala International University Western Campus, Kampala, Uganda
Email: *piustheophillus@kiu.ac.ug

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Abstract

Background: *Staphylococcus aureus* is the most common isolate from Skin and Soft Tissue Infections (SSTIs) especially, strains of Methicillin-resistant *Staphylococcus aureus* (MRSA) worldwide. The treatment of SSTIs caused by MRSA has become more unsolved and a public health concern. **Methods:** A pre-designed questionnaire was used to collect data on predisposing factors for MRSA among patients with SSTIs. A sterile swab stick was used and pus was collected from the affected site of the skin. Samples were cultured immediately following standard laboratory procedures. *Staphylococcus aureus* isolated were identified culturally, morphologically, and biochemically. MRSA was detected using an oxacillin disc (1 μg) and a methicillin disc (30 mcg). Data were analyzed using Statistical Package for Social Science (SPSS). **Results:** Out of a total of 78 *Staphylococcus aureus* isolates, 42 (53.8%) were MRSA. 39 (50%) were among participants with no formal education and 40 (51.28%) among females. Bivariate analysis showed prolonged hospitalization (P < 0.04), long-term on antibiotics for months (P < 0.03), and having surgery in the previous 6 months (P < 0.04) were significantly associated with MRSA in SSTIs. MRSA was sensitive to imipenem (97.44%), and chloramphenicol (51.28%). Resistance was found mainly with erythromycin (87.1%), and ampicillin (98.7%). **Conclusion:** Prolonged hospital stay after surgery was significantly associated with MRSA among SSTIs. Healthcare facilities should intensify measures for infection control and ensure regular washing of beddings. Prescribers should take a pathogen-specific approach to treatment with susceptible confirmed antibiotics to address the burden of antibiotic resistance.

Subject Areas

Public Health

Keywords

Methicillin-Resistant, *Staphylococcus aureus*, Skin, and Soft Tissue, Infection, Predisposing Factors

1. Introduction

Skin and soft tissue infections (SSTIs) are known to be caused by the invasion of microorganisms into the skin layers and underlying soft tissues through an opened skin or direct contact [1] [2]. The practice guidelines of the Infectious Diseases Society of America (IDSA) for the diagnosis and management of skin and soft tissue infections classified SSTIs into five categories, including superficial (folliculitis, furuncle, carbuncle), uncomplicated infection (impetigo, erysipelas, and cellulitis), necrotizing infection, infections associated with bites and animal contact, surgical site infections (SSIs) and infections in the immune-compromised hosts [3]. In an immunocompetent host, the vast majority of SSTIs are due to *Staphylococcus aureus* and *β*-hemolytic *Streptococci*, primarily *Streptococcus pyogenes*, while in patients with compromised immune systems, other, less-virulent *β*-hemolytic streptococci (for example *Streptococcus agalactiae*), and even Gram-negative pathogens, may cause SSTIs [4]. *S. aureus* is the most common cause of SSTIs worldwide [5] [6]. Infections due to methicillin-resistant *S. aureus* (MRSA) have increased worldwide during the past twenty years [7], and a considerable rate of SSTIs is associated with MRSA [8], thus, treatment of SSTIs has become more challenging over the last 2 decades [1].

Worldwide, MRSA is frequently isolated from complicated SSTIs, and cases of MRSA are increasing among outpatients [9], and it accounted for more than 80,000 severe infections in the United States of America (USA) alone in 2011. Also, more than one-half of hospital-related *Staphylococcus aureus* infections have been reported in most Asian countries [10]. In 2016, the percentage of MRSA in invasive *Staphylococcus aureus* infections in Europe reached its lowest level of about (13.7%) since the European Centre for Disease Prevention and Control (ECDC) first presented population-weighted data for the European Union in 2009, but large inter-country variations of (1.2% to 50.5%) remained static [11]. Between 2015 and 2016, global surveillance found the lowest rate of MRSA in Asia (56.5%), and the highest rate in Oceania (62.7%), with Africa having a rate of 60.1% [12]. A study [13] reported MRSA prevalence ranging from 2.6% - 4.0% in East Africa. Meanwhile, a few studies [14] and [15] in Uganda have reported on the prevalence of MRSA only in one category of SSTIs which is surgical site infections (SSIs), with none of the studies reporting on the prevalence of MRSA and associated risk factors among patients with SSTIs, therefore this study aimed at understanding the epidemiology of MRSA, antibiotic therapy of *S. aureus*, and identifying the risk factors associated with these infections (MRSA) among patients with SSTIs at KIU-TH, Bushenyi-Western Uganda.

2. Methods

2.1. Study Design, Study Area and Study Population

A cross-sectional study was adopted and data was collected on predisposing factors associated with methicillin-resistant *Staphylococcus aureus* (MRSA) in patients with SSTIs at Kampala International University Teaching Hospital (KIUTH) Located in Bushenyi district in South Western region of Uganda. Bushenyi has an estimated population of about 234,440 according to the Uganda Bureau of Statistics [16], with most households practicing subsistence farming as their occupation. KIU-TH was chosen as the study site because it is a referral health care provider for both outpatients and inpatients in the district and neighboring districts and villages (Bugalamba, Bushenyi, Ishaka, Kashenyi, Kitozho, Kyamumali, Makota, and others) with an about 700-bed capacity and has a microbiology laboratory with the facilities to perform culture and sensitivity test.

2.2. Sample Size and Sampling Technique

The Cochran [17] formula was used to estimate the sample size as described below:

$$n_o = \frac{t^2 p(1-p)}{d^2}$$

n_o = required sample size;

t = confidence level at 95% (standard value of 1.96);

p = prevalence of MRSA. The prevalence of MRSA in South Western Uganda according to Ayebare *et al.* (2019), 5.4% was used in the calculation;

d = margin of error at 5% (standard value of 0.05).

Substituting in the formula;

$$n_o = \frac{1.96^2 \times 0.054(1-0.054)}{0.05^2} = 78$$

Thus 78 *S. aureus* isolates from patients with SSTIs were used in this study.

2.3. Sample Collection, Processing and Identification of *Staphylococcus aureus*

Initial clinical diagnosis of SSTIs was performed by a clinician and samples were obtained using sterile swab sticks from the wound and skin lesions of the patients. The samples were labeled with a code number and immediately transferred to the Microbiology Laboratory of KIU-TH for processing. Samples were cultured by streaking on the Blood and chocolate agar surface of the medium with the help of a sterile wire loop. The inoculated media were incubated at 37˚C for 18 - 24 hours. Colonies' characteristics were observed after 24 hours of incubation. *Staphylococcus species* produced yellow to cream and white colonies in diameter [18]. It was also observed in some colonies clear and colorless zones appeared around the colonies, indicating a breakdown of red blood cells due to the *β*-hemolysin released by some strains of *Staphylococcus aureus*. Gram stain was done to identify the morphology of the bacteria isolates, and colonies identified as Gram-positive cocci were subjected to catalase test for presumptive identification of *Staphylococcus species* (spp.). Catalase-positive *Staphylococcus spp.* were further inoculated on mannitol salt agar (MSA) and incubated at 37˚C for 24 hours. *Staphylococcus aureus* grew as yellow colonies which indicated fermentation of mannitol from the MSA media. Seventy-eight (78) isolates were identified as *Staphylococcus aureus*. MRSA was identified using methicillin and oxacillin disc [18].

2.4. Antibiotics Susceptibility Test

Antibiotic susceptibility test was done on Muller Hinton agar by disc diffusion method (modified Kirby-Bauer's method) using selected antibiotics (oxacillin 1 μg, clindamycin 2 μg, ampicillin 10 μg, ciprofloxacin 5 μg, erythromycin 15 μg, chloramphenicol 30 μg, imipenem 10 μg, and vancomycin 30 μg) [Bioanalyse Türkiye]. 4 - 5 colonies were prepared with sterile nutrient broth and compared to 0.5 McFarland turbidity standard. A sterile swab stick was used to inoculate the Muller Hinton agar surface and was allowed on the bench for 20 - 30 minutes. Antibiotic discs were placed on the agar surface with the help of sterile forceps. All inoculated plates were inverted and incubated at 37˚C for 18 - 24 hours. The zone of inhibition for all antibiotics discs was measured with a ruler in millimeters and compared with the antibiotics standard chart, which aided in the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and categorized the susceptibility as Sensitive, Intermediate, and Resistance as compared with the chart [19]. Data analysis was done using a statistical package for social sciences (SPSS) software version 20. Bivariate analysis was done to determine the association of predisposing factors of MRSA in patients with SSTIs. A significant value of <0.05 was taken as significantly associated with the predisposing factors.

2.5. Quality Control

All standard laboratory procedures were strictly observed during the collection of samples, handling, and processing to ensure there was no contamination of the samples and culture media. The disposal of culture media followed the guidelines of good clinical laboratory practice for safety purposes and prevention of the transfer of pathogenic organisms back into the environment. All culture media were decontaminated after processing.

3. Results

The study revealed a prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) among patients with Skin and Soft Tissue Infections (SSTIs) to be 53.9%. (Figure 1)

Table 1 shows *Staphylococcus aureus* was isolated more among females 40 (51.28%) while 38 (48.72%) among males. The age group with the highest number of isolates is age group 0 - 20 (30), 21 - 41 (25), and 63 - 83 (13) respectively while the age group with the least number of isolates is 42 - 62 (09) and 84 (01).

Table 1. Demographic of participants.

	Frequency	Percentage
GENDER		
Female	40	51.28
Male	38	48.72
AGE		
0 - 20	30	38.46
21 - 41	25	32.05
42 - 62	9	11.54
63 - 83	13	16.67
84	1	1.28
EDUCATION		
None	39	50.00
Nursery	4	5.13
Primary	15	19.23
Secondary	12	15.38
University	8	10.26
OCCUPATION		
Boda-boda (motorcyclist)	4	5.13
Car driver	2	2.56
Farmer	10	12.82
Health personnel	3	3.84
Market attendant	5	6.41
None	28	35.90
Other	6	7.69
Pupil	8	10.26
Shop attendant	5	6.41
Student	7	8.97

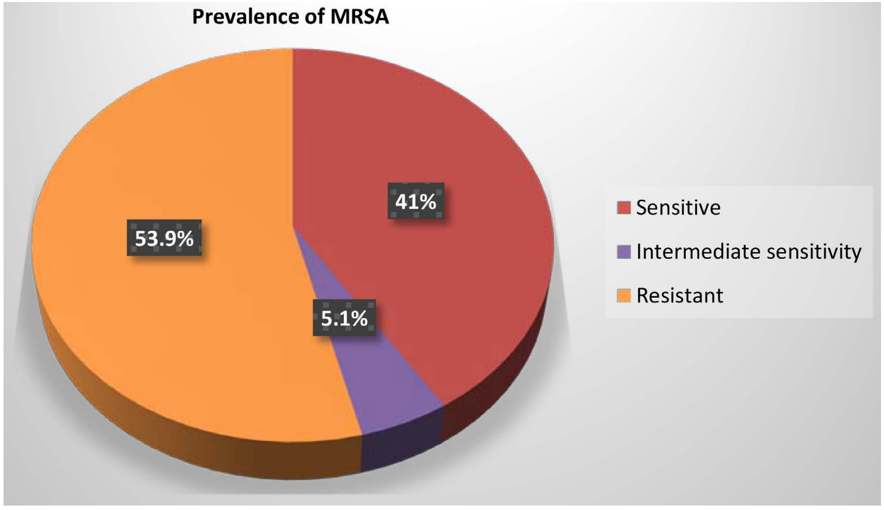

Figure 1. Prevalence of MRSA among patients with SSTIs at KIU-TH.

On education, 39 of the isolates were from participants with no formal education, (15) from primary education level, (12) from secondary education level, (8) from University level, and the least isolates (04) were from Nursery level. On occupation, the highest isolates of *Staphylococcus aureus* were recorded with Non-occupation 28 (35.90%) while the least was recorded with car drivers 2 (2.56%) and health personnel 3 (3.84%) respectively.

Table 2 showed the predisposing factors significantly associated with MRSA amongst patients with SSTIs. Being hospitalized in the last 6 months (P-value < 0.04), taking antibiotics in the last 3 months, (P-value < 0.03), and having had surgery in the previous 3 months (P-value < 0.04).

The multivariate analysis below indicated those who had surgery in the previous 3 months with SSTIs, were significantly associated with MRSA infection at P-value < 0.04. (Table 3)

Table 4 showing results for antimicrobial susceptibility pattern for *S. aureus.*

Table 4 indicates *S. aureus* was most susceptible to imipenem (97.44%) and least susceptible to ampicillin (0%). *S. aureus* also showed high resistance to most of the antibiotics used in the study with the highest resistance exhibited against ampicillin (98.72%), erythromycin (87.18%), and oxacillin (53.9%).

4. Discussion

In this study, 78 *Staphylococcus aureus* were isolated from 148 samples, and 42 were found to be methicillin-resistant *Staphylococcus aureus* (MRSA) among patients with SSTIs which recorded a prevalence of 53.8%. The higher prevalence of MRSA in this study is attributed to the indiscriminate use of antibiotics which are not based on evidence of laboratory investigations (culture and sensitivity) tests that aid in the isolation of bacteria and testing for antibiotics sensitivity. It is also due to a lack of adequate resources and facilities for laboratory diagnosis especially in rural communities where the majority of the patients with SSTIs in this study originated from. The findings in this study were in agreement

Table 2. Bivariate analysis of factors associated with MRSA among patients with SSTIs.

Variables	MRSA Infection n = 78		P-value (<0.05)	RR
	Yes	No		
Having been hospitalized in the last 6 months	30	14	**0.04***	**1.9**
	12	22		
Antibiotics therapy in the last 3 months	39	24	**0.03***	**3.1**
	3	12		
Having surgery in the previous 3 months	20	6	**0.04***	**1.8**
	22	30		
Having had SSTI in the past	18	15	0.916	1.0
	24	21		
Having had contact with another person with SSTI	10	9	0.903	0.97
	32	27		
Having a chronic condition/disease	11	7	0.481	1.2
	31	29		
Lack of access to running water	30	26	0.938	1.0
	12	10		
Having an open wound	40	35	0.65	0.8
	2	1		
Having contact with pigs	5	4	0.913	1.0
	37	32		

Key: * = Significant P-value, **RR** = Relative Risk.

Table 3. Multivariate analysis of factors associated with MRSA among patients with SSTIs.

	Presence of MRSA Infection		P-value	OR (95% CI)
	MRSA Infection	Non-MRSA Infection		
Having been hospitalized in the last 6 months	12	22	0.40	3.928 (0.538 - 5.492)
Antibiotics in the last 3 months	3	12	0.11	6.5 (0.755 - 18.097)
Have surgery in the previous 3 months	22	30	**0.04**	4.545 (1.070 - 10.4555)

Table 4. Antibiotics susceptibility pattern of *S. aureus* among patients with SSTIs at KIU-TH.

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Oxacillin (1 μg)	32 (41.0)	4 (5.1)	42 (53.8)
Imipenem (10 μg)	76 (97.44)	2 (2.56)	0
Ciprofloxacin (5 μg)	32 (41.03)	19 (24.35)	27 (34.61)
Clindamycin (2 μg)	37 (47.44)	20 (25.64)	21 (26.92)
Chloramphenicol (30 μg)	40 (51.28)	21 (26.92)	17 (21.8)
Vancomycin (30 μg)	20 (25.64)	25 (32.05)	33 (42.31)
Erythromycin (15 μg)	1 (1.28)	9 (11.54)	68 (87.18)
Ampicillin (10 μg)	0	1 (1.28)	77 (98.72)
Methicillin (30 μg)	36 (46)	0	42 (53.8)

with research done in Kenya by [10] who reported a similar prevalence as found in this study but less than a higher prevalence of 65.9% reported by [14] in rural Eastern Uganda. The prevalence in this study is in contrast with the one reported by [1] in the United States, 22.6% in Botswana [8], and 21.7% in Egypt [12] who all reported a lower prevalence of MRSA among patients with SSTIs.

The univariate analysis of demographic showed *Staphylococcus aureus* was isolated more among females than males (**Table 1**). Also, the age groups with the highest number of *Staphylococcus aureus* isolates are age group 0 - 20 years and 21 - 41 years old while the age groups with the least isolates of *Staphylococcus aureus* are 42 - 62 years and 84 years respectively. Most of the *Staphylococcus aureus* isolates were isolated from participants with no formal education. The higher number of *Staphylococcus aureus* isolates among participants with no formal education may be because they find it hard to process and understand health education information compared to those who have gone through formal education. We also observed in this study that the highest isolates of *Staphylococcus aureus* were recorded with participants with No occupation while the least was recorded with car drivers and health personnel respectively (**Table 1**).

A bivariate analysis of factors associated with MRSA indicated three factors (having been hospitalized in the past six months, being on antibiotics in the last three months, and having surgery previously in the past three months were significantly associated with MRSA among patients with SSTIs) (**Table 2**). Previous studies observed that patients who had been on admission for long had developed resistance to most common antibiotics in use which is due to the nature of the environment surrounded with nosocomial bacteria which is resistant to most antibiotics. The findings in this study are in agreement with studies done by [1] [5] [20] [21]. In other studies, contrast reports were observed by [9] [10] [22] which in their report revealed there was no significant association between previous hospitalization with MRSA infection.

A study by [23] in Uganda reported a high percentage of self-medication as a reason for antibiotic resistance recorded in the country. Other studies that are in agreement with the link of prolonged antibiotics medication with MRSA infection are [9] [20] [24]. But contrary to our study, their study [25] revealed no association between previous antibiotic therapy with MRSA infection. Additionally, we observed in this study that those who had surgical procedures in the last 3 months and participated in this study showed a significant association with MRSA infections (**Table 2**). These findings agreed with reports by [20] [22] [26]. Further analysis with multivariate showed those who had surgery in the previous 3 months were significantly associated with MRSA infection and this could be due to prolonged hospital stay after surgery which exposed them more to constant contact with health personnel and caretakers during care. This could also be due to a lack of frequent cleaning of beddings and stringent adherence to hand hygiene by health personnel. The other two significant factors (being hospitalized in the past six months and being on antibiotics in the past three months) from the bivariate analysis showed no significance in the multivariate analysis (**Table 3**). These findings are in agreement with a study done in North-East Ethiopia which indicated recent surgery history as a factor associated with MRSA infection [25].

Antibiotics susceptibility pattern from this study indicated that *S. aureus* isolates were susceptible to imipenem and chloramphenicol (**Table 4**), this is because these antibiotics are not frequently prescribed to patients for treatment of bacterial infections. In addition, imipenem is expensive in this locality and most patients cannot afford it for the duration of the treatment. We observed resistance with the following antibiotics in this study ciprofloxacin, clindamycin, vancomycin, erythromycin, and ampicillin (**Table 4**). The resistance to the above-named antibiotics agreed with another report on antibiotics resistance in Uganda by [27], although, the high resistance against vancomycin (**Table 4**) calls for concern as it has been considered the best choice against *S. aureus* and for the treatment of MRSA infection. The resistance to many antibiotics reported in this study can be attributed to the increased usage of over-the-counter self-medication even without evidence-based test reports that justified its need for the treatment of bacterial infection [23].

5. Conclusions

We observed in this study a high prevalence of MRSA among patients with SSTIs. We also observed a significant risk factor for MRSA infection among those who had surgery in the previous six (6) months. *Staphylococcus aureus* was sensitive to imipenem but showed high resistance to ampicillin, erythromycin, and vancomycin.

Healthcare facilities should intensify measures for infection control and ensure regular washing of beddings. Prescribers should take a pathogen-specific approach to treatment with susceptible confirmed antibiotics to address the burden of antibiotic resistance.

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Conflicts of Interest

The authors declare no conflict of interest whatsoever in regards to this study.

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Abbreviations and Acronyms

ECDC: European Center for disease prevention control, IDSA: Infectious disease society of America, KIUTH: Kampala International University Teaching Hospital, MSA: Mannitol salt agar, MRSA: Methicillin resistance *Staphylococcus aureus*, REC: Research and ethics committee, SSTIs: Skin and soft tissue infections, SSIs: Surgical site infections, SPSS: Statistical package for social sciences, USA: United States of America.