

Prevalence of *Staphylococcus aureus* among clinical isolates and their responses to selected antibiotics at Centre Hospitalier Universitaire de Kigali (CHUK)

Abstract

Staphylococcus aureus is one of the major causes of bacteremia associated with higher morbidity and mortality, compared to the bacteremia caused by other pathogens. The burden of *S. aureus* bacteremia, particularly penicillin-resistant *S. aureus*, in terms of cost and resource use is high. *S. aureus*, a mainly acquired hospital infection is responsible for many suppurative lesions and has demonstrated the ability of developing resistance to many antimicrobial agents leading to life threatening infections and long hospital stay. A cross-sectional study was carried out at CHUK to determine the prevalence and antibiotic susceptibility profiles of *S. aureus* isolates from blood and pus samples. After gram staining, cultural techniques on Mannitol Salt Agar (MSA) and blood agar were performed. *Staphylococcus aureus* was isolated based on the colonial characteristics and confirmed by Catalase and Coagulase tests. The antibiotic susceptibility test was done on Mueller Hinton agar (MHA) by disc diffusion method.

A total of 300 specimens were collected over a period of three months. Out of 300 specimens, 30(10%) and 270(90%) proved to be positive and negative respectively for *S. aureus*. Among the 30 positive for *S. aureus*, 11(36.7%) and 19(63.3%) were from blood and pus respectively. The sensitivity pattern for the 6 antibiotics tested was: Vancomycin (8.5%), Penicillin (4.9%), Erythromycin (18.3%), Oxacillin (10.97%), Clindamycin (32.9%) and Tetracycline (24.4%). The resistance at CHUK was high for the penicillin 24 (27%) and vancomycin 21(24%).

There is a higher risk of acquiring multidrug resistant *Staphylococcus aureus* infection in inpatients attending CHUK, thus strategies need to be adopted in order to stop an increasing resistance of *S. aureus* to different antibiotics due to high cost and time in developing new antibiotics.

Keywords: prevalence, *Staphylococcus aureus*, antibiotic susceptibility test, cultural and Biochemical tests

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Abbreviations: MSA, mannitol salt agar; MHA, mueller hinton agar; MRSA, methicillin-resistant *S. aureus*; AST, antimicrobial susceptibility testing

Introduction

S. aureus is an aerobic gram positive coccus and ubiquitous commensal that periodically lives on the skin and anterior nares of about one third of the healthy human population without causing illness.¹⁻³ *S. aureus* is a leading cause of diseases like skin and soft tissue infections, pneumonia, bloodstream infections, osteomyelitis and endocarditis, as well as toxin-mediated syndromes like toxic shock and food poisoning in many developed countries.^{4,5} *S. aureus* has been the major cause of morbidity and mortality, and is among the ten main causes of death worldwide and the basic cause of death in 1% of cases.⁶

Antimicrobial agents have been used extensively to combat *S. aureus* infections but the increasing level of resistance of *S. aureus* to many antibiotics is complicating the treatment of serious infections caused by this pathogen.⁷ Antibiotics exert a selective pressure which plays a central role on the acquisition, selection, persistence and transmission of resistant pathogens.

S. aureus represents a prototype for drug resistance, especially to β -lactam antibiotics. Although this bug has been naturally susceptible to almost every antibiotic developed so far, it frequently gains resistance by gene mutations and horizontal gene transfer, that protect the bug under antibiotic selection pressure, and has been implicated in episodes of epidemic and pandemic proportions.^{1,8}

Two years after the massive use of penicillin to treat bacterial infections in the 1940s, strains of *S. aureus* able to produce penicillinase were selected and in 1960, almost 100% of strains were already resistant to penicillin. The discovery of semisynthetic penicillins (Methicillin and oxacillin) resistant to β -lactamase hydrolysis was achieved and the two drugs were used to treat Staphylococcal infections in the early 1960s.^{9,10} Unfortunately, after one year, resistance to methicillin was noticed in Europe and North America, and then worldwide.¹¹

Adult patients without serious complications can be given trimethoprim-sulfamethoxazole (TMP-SMX), minocycline, doxycycline, or clindamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Erythromycin, Gentamycin, Methicillin, Tetracycline and Co-trimoxazole.¹²

The rise of drug-resistant and virulent strains of *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA) is a serious

problem in the treatment and control of Staphylococcal infections.¹³ Efforts to eradicate carriage of *S. aureus* are one of the strategies adopted by control programs against the spread of MRSA.¹⁴ The knowledge of antimicrobial susceptibility profile of *S. aureus* in a particular area is important as this can contribute to rational choice and use of antimicrobial agents.¹⁵

Material and methods

A total of 300 non-duplicate Specimens were collected from CHUK between April and July 2014. The clinical specimens used were from blood and pus samples collected using sterile cotton swabs impregnated with sterile normal saline solution.

All isolates were characterized and identified by Gram staining, Catalase and coagulase tests. Cultural characteristics including golden yellow colonies of *S. aureus* on Mannitol Salt Agar (MSA) were used in *S. aureus* identification. After inoculation, plates were incubated at 37°C for 24 hours and then read.^{16,17} At 100X, *S. aureus* appears as purple gram-positive cocci in clusters.^{16,18,19}

Slide coagulase test was performed by emulsifying few pure colonies of Staphylococci from Blood agar with undiluted plasma. Agglutination within seconds indicated a positive result. Staphylococcal isolates negative by slide coagulase test were again

checked for tube coagulase test by diluting the plasma with normal saline (1:6) and the tubes were examined after four hours. If negative, the tubes were further incubated overnight at room temperature. Clotting of the plasma represents positive result.²⁰

The antimicrobial susceptibility testing (AST) was done using the Kirby-Bauer disc agar diffusion method on MHA with commercially available antibiotic sensitivity discs (AbtekBiologicals, Ltd, UK). The procedure of the AST was done as described by Ekundayo et al.²¹ After incubation, the plates were examined and the zone of inhibition was measured. The results were interpreted as per CLSI standards (CLSI, 2011). The data were presented in form of tables and complimented with columns and analyzed using Microsoft Office to get the prevalence and AST of *S. aureus* isolates during the study period.

Results

Results were presented based on age. The following table summarizes results based on presence or absence of *S. aureus* and the age of the patient. Table 1 shows that the interval with both higher positive and negative results is²⁰⁻²⁹ with 43.3% and 32.5% respectively. The Table 1 also shows that 30 patients had *S. aureus* infection and 270 were free from it. In addition, results were presented based on gender of patients as follow (Table 2).

Table 1 Prevalence of *S. aureus* based on age

Results Ages (yrs)	Positive		Negative		Total	%
	Number	%	Number	%		
< 10	0	0	4	1.5	4	1.5
11-20	11	36.7	80	30	91	30.3
21-30	13	43.3	88	32.5	101	33.6
31-40	1	3.3	75	27.7	76	25.3
41-50	4	13.3	23	8.5	27	9
<50	1	3.3	0	0	1	0.3
Total	30	100	270	100	300	100

Table 2 Prevalence of *S. aureus* based on gender

Results sex	Positive		Negative		Total	%
	Number	%	Number	%		
Female	11	36.7	111	41.1	122	40.7
Male	19	63.3	159	58.9	178	59.3
Total	30	100	270	100	300	100

Table 3 Distribution of results based on type of specimen

Results specimen	Positive		Negative		%	Total
	Number	%	Number	%		
Blood culture	11	36.7	179	66.3	63	190
Pus samples	19	63.3	91	33.7	37	110
Total	30	100	270	100	100	300

Table 4 Antibiotic susceptibility pattern of *S. aureus* isolates

Results antibiotics	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Erythromycin	15	18.3	0	0	14	16
Tetracycline	20	24.4	0	0	9	10
Vancomycin	7	8.5	1	33.3	21	24
Penicillin	4	4.9	1	33.3	24	27
Oxacillin	9	10.97	1	33.3	19	21
Clindamycin	27	32.9	0	0	2	2
Total	82	100	3	100	87	100

Of the 300 patients enrolled, 178(59.3%) and 122(40.7%) were males and females respectively. Males and females with *S. aureus* were 19(63.3%) and 11(36.7%) respectively (Figure 1). Furthermore, results were also presented based on the type of Specimen (Table 3). Of the 300 specimens collected, 190(63.0%) and 110(37.0%) were collected from blood culture and pus samples respectively. The Specimens with *S. aureus* infection were 11(36.7%) in blood and 19(63.3%) in pus samples (Figure 2). Specimens were tested for the susceptibility to different antibiotics. The following table gives a summary of the results obtained (Table 4).

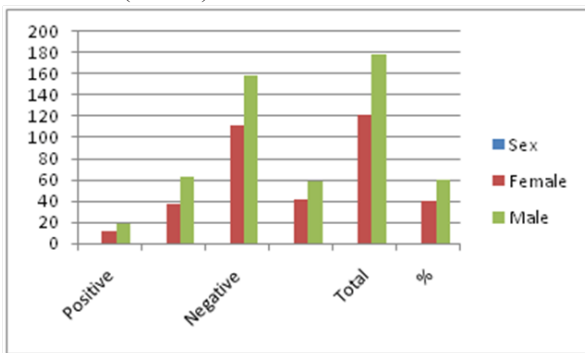


Figure 1 Prevalence of *S. aureus* based on gender.

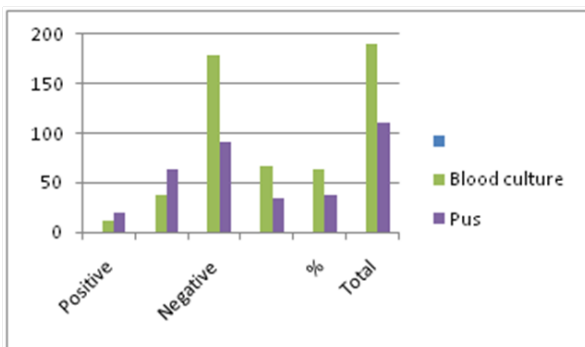


Figure 2 Distribution of results based on specimen.

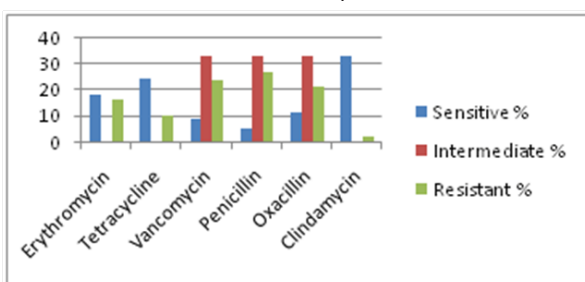


Figure 3 Antibiotic susceptibility pattern of *S. aureus* isolates.

The results showed that *S. aureus* isolates were sensitive to clindamycin with 27(32.9%), followed by tetracycline with 20.0(24.4%) and the erythromycin with 15(18.3%). The above table also showed that the isolates were resistant to Penicillin with 24(27%) followed by Vancomycin with 21(24%), and then Oxacillin 19(21%). Note that the Antibiotic Susceptibility Test was done for 29 isolates and not 30 (Figure 3).

Discussion

The study showed that the overall prevalence of *S. aureus* was 10% which is less than the results of earlier studies with a prevalence of 40.5%²² and 24.5%.²³ Although the results of this study showed

a lower prevalence of *S. aureus* when compared to other studies, *S. aureus* should be considered as a pathogen of great concern.

Of the 30 isolates positive for *S. aureus*, 11(36.7%) and 19(63.3%) were from Blood and pus swabs respectively which concurs with 1(6.7%) and 10(29.4%).²³ The resistance of *S. aureus* to several antimicrobial agents represents a serious concern. The organisms exhibit remarkable versatility in their behavior towards antibiotics, with some strains having become resistant to most commonly used antibiotics.²⁴

Antimicrobial resistance patterns of *S. aureus* infection in the present study showed that *S. aureus* isolates were sensitive to tetracycline with 20.0(24.4%) which is in accordance with 16(69.6%)²⁴ but contrasts with 0(0%).²⁵ Furthermore, our results showed that 15(18.3%) *S. aureus* isolates were sensitive to Erythromycin which concurs with 49(47.1%).²³

On the other hand, our results proved that 24(27%), 19(21%) were resistant to Penicillin and Oxacillin respectively which is in accordance with 66(90%), 32(43.8%)²³ and 462(100), 462 (100) for Penicillin and Oxacillin respectively.²⁵ High rate of resistance to penicillin and other β -lactam antibiotics has been reported in parts of South-western Nigeria.²⁶⁻²⁸ The high level of resistance to the two antibiotics may be attributed to overuse of them as empirical treatment. The high frequency of resistance observed in these antibiotics could be attributed to their use in treatment of diseases in animals and humans. The low activity of these antibiotics can also be attributed in part to earlier exposure of the isolates to these drugs, which may have enhanced resistance development.²⁴ No one of the isolates was susceptible to all of the tested antibiotics and also none of the *S. aureus* isolates was resistant to all the tested antibiotics.

Conclusion

The present study showed that there is a high prevalence of *S. aureus* and an alarming level of resistance to commonly used antibiotics. Thus, adequate preventative and control measures are needed to reduce transmission and infections caused by resistant strains of *S. aureus*. There is need to carry out periodic monitoring of AST of important pathogens including *S. aureus*. It is also time to install greater control and rational use of antibiotics in order to slow down the rate of resistance development and spread of resistant organisms in the community.

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Conflicts of interest

The authors declare there is no conflict of interest.

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