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Original Research Article

Prevalence and antibiotic susceptibility profiles of bacteria isolated from out-patients presenting with septic wounds in Kajiado county, Kenya

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ABSTRACT

Background: The emergence of antibiotic resistance amongst bacteria colonizing wounds remains a serious global public health concern. There is a need for surveillance data on antimicrobial resistance, as this is critical in influencing policy on disease management.

Methods: The study utilized a cross-sectional study design to select 182 patients with septic wounds attending three level 3 hospitals within Kajiado County. Samples from abscesses, burns, surgical septic wounds, and lesions from patients were collected using sterile swabs, placed in transport media and transported to the lab in cold. The samples were subjected to standard bacteriological methods for isolation, characterization and identification of bacteria isolates. Antimicrobial susceptibility test was performed using the Kirby-Bauer method.

Results: The predominant bacteria isolated were Coagulase-negative *Staphylococci* (35.71%), *S. aureus* (31.69%) and *E. faecalis* (30.77%), *P. mirabilis* (19.78%), *Pantoea* species (14.84%) and *A. viridans* (12.09%). The *S. aureus* exhibited varied resistance to amoxicillin (20-91.9%) and ceftazidime (62.22-94.00%) but was highly susceptible to ciprofloxacin (82-97.30%) across different hospitals.

Conclusions: *Staphylococcus* spp. were the most prevalent bacteria isolated from patients in the study area and exhibited varying patterns of antibiotic resistance, with high resistance to amoxicillin and ceftazidime and high susceptibility to Ciprofloxacin. Further studies are needed to determine the factors associated with the occurrence of antibiotic resistance in bacteria in order to develop strategies to limit the emergence and spread of resistant pathogens.

Keywords: Prevalence, Antibiotic susceptibility, Kajiado county

INTRODUCTION

Approximately 8.2 million people across the world sustain septic wound infections annually, leading to a treatment cost of between US $28.1-$96.8 billion.[1] Wound infections occur due to colonisation of the exposure surface leading to sepsis. The most common microorganisms found in wounds are *S. aureus, S. pyogenes, P. aeruginosa, E. coli,* and *Enterococci* spp., among others.[2-4] *S. aureus* is a versatile pathogen that can colonize mucous membranes and skin, where it can cause severe, toxin-mediated disease or invasive pyogenic infections in humans and animals.[5,6] The bacteria cause a wide range of diseases, from mild superficial skin infections to life-threatening diseases such as bacteraemia, pneumonia, infective endocarditis, deep-seated abscess, and toxic shock syndrome. In Kenya and other countries, *S. aureus* has been observed as the most prevalent pathogen associated with surgical site infection.[6,7] Further, a previous study reported that the prolonged hospital stays and dirty wounds are some of the risks associated with postsurgical sepsis.[8] In a recent study, the current investigators reported a high burden of wounds in patients attending hospitals in Kajiado County, Kenya, and the occurrences of the wounds were associated with road accidents, amongst other causes.[9]

In recent years, there has been a concern about the worldwide emergence of *S. aureus* resistant to commonly used antibiotics. For example, in Africa, the prevalence of Methicillin-resistant *S. aureus* is heterogeneous, with a mean prevalence of 34.6% reported in Cameroon, 47% in Ethiopia and 31.5% in Uganda.[10-12] A study undertaken in Ghana found the high prevalence of multidrug-resistant *S. aureus,* with 17% of isolates being resistant to oxacillin, 13% resistant to ciprofloxacin, 9% resistant to tetracycline, 28% to streptomycin, and 89% of the *S. aureus* isolates to erythromycin.[13] In Kenya, an earlier study conducted in Agha Khan Hospital reported the prevalence of *S. aureus* with antibiotic-resistant characteristics ranging from 10% to 60%.[6]

Resistant bacteria cause severe infections that are expensive to diagnose and difficult to treat.[6] The mechanism by which resistance develops is complex and can result in multidrug-resistant bacterial strains due to the simultaneous development of resistance to several antibiotics. Thus, a study asserted that determining local bacterial sensitivity patterns to antibiotics is important in providing a guide for antibiotic selection to be used in the treatment of patients.[6] Amongst the few studies done in Africa and elsewhere majority of the strains with MRSA were prevalent in inpatients with burns on the skin.[14] However, none of the existing studies in the Kenyan context has been conducted in any clinical or non-clinical settings of Kajiado County, which a large population of pastoralists with cattle having a high burden of resistant bacteria.[9] Whether the similar burden of AR is also common in the local human beings is not clear. Further, the county has a high population of people living in the suburbs of Nairobi city, where wound risks could be quite high.

Therefore, this study aimed at determining the prevalence of bacteria in wounds of patients attending local hospitals in Kajiado County, Kenya, and thereafter assessed the susceptibility patterns of *S. aureus* isolated from the patients.

METHODS

Description of study area and hospitals

The study was carried out from February 2023 to December 2023 in three main public hospitals in Kajiado County, Kenya: namely Kajiado County Referral Hospital (KCRH), Ongata Rongai Sub-County Hospital (OSCH), and Ngong Sub-County Hospital (NSCH). These are the major hospitals serving the county with a population of approximately 680,000 people in an area of 12,292.7 km^2 [KNBS, 2019]. KCRH is located in Kajiado town and has a bed capacity of 200 and serves an average of 400 patients daily, with a total of 150 inpatients and 250 outpatients. NSCH is located in Ngong Town with a bed capacity of 80 and serves an average of 250 patients daily-50 inpatients and 200 outpatients. OSCH is located in Ongata Rongai town, has a bed capacity of 40, and serves an average of 250 outpatients daily.

Study design and sample size determination

A cross-sectional study design was used to investigate the prevalence of bacteria present in wounds and antimicrobial sensitivity of *S. aureus* infections among 182 patients with septic wounds in KCRH, ORSCH, and NSCH. The number of participants included in the study was established based on the method described earlier.[14] This approach led to the recruitment of 75 patients from KCRH, 46 from ORSCH, and 61 from NSCH. The process of recruitment of the patients is described in a previous article.[14]

Collection of samples from wounds.

Each wound was cleansed using sterile normal saline to remove slough, necrotic tissue and dried exudate in order to aseptically collect swabs that were placed in Stuart transport medium (Oxoid, Basingstoke, Hampshire, England).[6] All swabs in Stuart transport media were coded with identification details, packed in a cooler box containing ice packs and transported to Microbiology laboratory at Kenya Institute of Primate Research for bacteria isolation and identification.

Culturing and identification of bacteria

Culturing and identification of bacteria were undertaken according to standard methods.[6,15] Briefly, the clinical specimens were inoculated to sheep blood agar SBA (Oxoid, Basingstoke, Hampshire, England) and mannitol salt agar (MSA) (Oxoid, Basingstoke, Hampshire, England) plates and incubated aerobically at 37°C for 24 hours. Characteristic bacterial colonies; beta-haemolytic on SBA or yellow on MSA were sub-cultured onto Tryptone Soy Agar (TSA) plate to obtain pure isolates for identification based on Gram staining and biochemical tests reactions including catalase and coagulation assays.[15] Pure *S. aureus* were stored at -80^{o}C for subsequent analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the standardized Kirby-Bauer disk diffusion method on the Muller-Hinton Agar (MHA) (Oxoid, Basingstoke, England) plates following the recommendations of the Clinical and Laboratory Standards Institute guidelines [CSLI, 2022]. Pure *S. aureus* isolates were suspended in sterile normal saline to produce a turbidity equivalent to 0.5 McFarland standards and evenly spread on MHA plate before carefully placing antibiotic disks; amoxicillin, amoxicillin/clavulanate (20/10 µg), oxacillin

(1 µg), ciprofloxacin (30 µg), clindamycin (2 µg), gentamycin (10 µg), streptomycin (10 µg), erythromycin (15 µg), vancomycin (20 µg), tetracycline (30 µg), ceftriaxone (30 µg) and ceftazidime (30 µg) (Oxoid, Basingstoke, England) firmly on the media surface. Inoculated plates containing antibiotic disks were incubated at 37ºC for 24 hours. Diameters of inhibition zones surrounding each antimicrobial was measured and interpreted as resistant (R), intermediate (I), or sensitive (S) and interpreted according to the CLSI guidelines (CLSI, 2022).

Statistical method and tools used

The collected data were entered into MS excel (Microsoft) and then exported to statistical package of social science (SPSS) 25.0 version for statistical analysis (IBM, United States). Descriptive statistics were used to describe the analysed data.

RESULTS

Prevalence of bacteria found in wounds by hospital

The prevalence of different bacteria isolated from 182 wound patients across the three hospitals in Kajiado is presented in Table 1. The most prevalent bacteria were Coagulase-negative *Staphylococci* (CoNS) (35.71%), followed by *S. aureus* (31.69%) and *E. faecalis* (30.77%). Other bacteria with moderate prevalence included *P. mirabilis* (19.78%), *Pantoea* spp. (14.84%) and *A. viridans* (12.09%), *E. cloacae* (8.80%), *K. oxytoca* (7.69%), and *K. pneumoniae* (7.14%). The other bacteria had low prevalences (below 5%) (Table 1).

Susceptibility of S. aureus to antibiotics

The results of susceptibility of *S. aureus* to various antibiotics are presented in the Table 2. The bacteria had higher resistance against amoxicillin in patients from KCRH (91.89%) compared to those from ORSCH (88.89%) and NSCH (20.00%). There was higher resistance to amoxicillin/clavulanic acid in bacteria isolated from patients from NSCH (72.00%) compared to high susceptibility in those from KCRH (83.78%) and ORSCH (57.78%).

There was higher resistance to oxacillin in isolates from patients attending NSCH (78.00%) and KCRH (70.27%) compared to even distribution between susceptibility (20.00%) and resistivity (24.44%) in those from the ORSCH.

The *S. aureus* were highly susceptible to ciprofloxacin in bacteria isolated from patients attending ORSCH (88.89%), NSCH (82.00%) and KCRH (97.30%). Clindamycin recorded high susceptibility in isolates from patients attending ORSCH (66.67%) and KCRH (70.27%) but susceptibility was low (42.00%) in isolates obtained from patients attending NSCH. There was higher susceptibility of bacteria to gentamycin in isolates obtained from patients attending KCRH (94.59%) compared to those from ORSCH (62.22%) and NSCH (56.00%). The susceptibility of the isolates to Streptomycin was low in patients attending NSCH (62.00%), KCRH (48.65%) and ORSCH (26.67%).

Low susceptibility against erythromycin was also observed in isolates obtained in patients attending NSCH (44.00%) and KCRH (43.24%) and ORSCH (20.00%). The *S. aureus* isolates were highly susceptible to vancomycin in patients from KCRH (81.08%) compared to those from ORSCH (64.00%) and NSCH (55.56%). Isolates were also susceptible to ceftriaxone in percentages ranging from 45.95% to 68.89% in patients from different hospitals. Lastly, the isolates exhibited high resistance to ceftazidime in patients attending NSCH (94.00%), KCRH (89.19%) and ORSCH (62.22%).

Table 1: Prevalence (%) of bacteria found in wounds of patients seeking treatment at three hospitals in Kajiado County, Kenya.

Isolates	KCRH, (n=75)		Hospitals NSH, (n=46)		ORSCH, (n=61)		Total (n=182)	
	N	%	N	%	N	%	N	%
CoNS	14	18.67	20	43.48	31	50.82	65	35.71
S. aureus	16	21.33	14	30.43	28	45.90	58	31.69
***Micrococcus* spp.**	1	1.33	1	2.17	0	0.00	2	1.10
E. faecalis	18	24.00	18	39.13	20	32.79	56	30.77
E. faecium	8	10.67	0	0.00	0	0.00	8	4.40
E. avium	2	2.67	0	0.00	0	0.00	2	1.10
A. viridans	9	12.00	5	10.87	8	13.11	22	12.09
G. haemolysans	3	4.00	0	0.00	0	0.00	3	1.65
***Lactococcus* spp.**	1	1.33	0	0.00	0	0.00	1	0.55
***Corynebacterium* spp.**	7	9.33	0	0.00	1	1.64	8	4.40
***Bacillus* spp.**	2	2.67	2	4.35	0	0.00	4	2.20
***Clostridium* spp**	1	1.33	0	0.00	1	1.64	2	1.10
***Lactobacillus* spp**	0	0.00	1	2.17	4	6.56	5	2.75
Proteus mirabilis	3	4.00	9	19.57	24	39.34	36	19.78
Proteus vulgaris	0	0.00	1	2.17	0	0.00	1	0.55

Continued.

Isolates	KCRH, (n=75)		Hospitals NSH, (n=46)		ORSCH, (n=61)		Total (n=182)	
	N	%	N	%	N	%	N	%
P. alcalifasciens	0	0.00	0	0.00	4	6.56	4	2.20
K. pneumoniae	6	8.00	0	0.00	7	11.48	13	7.14
K. oxytoca	1	1.33	0	0.00	13	21.31	14	7.69
E. cloacae	5	6.67	5	10.87	6	9.84	16	8.80
***Pantoea* spp.**	3	4.00	10	21.74	14	22.95	27	14.84
***Pseudomonas* spp.**	1	1.33	4	8.70	4	6.56	9	4.95
***Kluyvera* spp.**	0	0.00	0	0.00	1	1.64	1	0.55
A. hydrophila	1	1.33	0	0.00	0	0.00	1	0.55
R. ornithinolytica	0	0.00	1	2.173	0	0.00	1	0.55
C. koseri	0	0.00	0	0.00	1	1.64	1	0.55
S. mercescins	0	0.00	0	0.00	1	1.64	1	0.55

*Key: CoNS=Coagulase negative *Staphylococci*, KCRH=Kajiado County Hospital, OSCH=Ongata Rongai Sub-County Hospital, NSCH=Ngong Sub-County Hospital

Table 2: Susceptibility of *S. aureus* isolated from patients attending to various hospitals to common antibiotics.

Antibiotics	ORSCH			NSCH			KCRH		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Amoxicillin	4.44	0.00	88.89	0.00	0.00	20.00	8.11	0.00	91.89
Amoxy/ Clav	57.78	0.00	28.89	28.00	0.00	72.00	83.78	0.00	16.22
Oxacillin	20.00	20.00	24.44	0.00	0.00	78.00	16.22	13.51	70.27
Ciprofloxacin	88.89	4.44	4.44	82.00	4.00	6.00	97.30	2.70	0.00
Clindamycin	66.67	20.00	13.33	42.00	26.00	32.00	70.27	2.70	27.03
Gentamycin	62.22	31.11	6.67	56.00	16.00	28.00	94.59	0.00	5.41
Streptomycin	26.67	37.78	11.11	62.00	18.00	20.00	48.65	35.14	16.22
Erythromycin	4.44	73.33	20.00	6.00	50.00	44.00	0.00	56.76	43.24
Vancomycin	55.56	42.22	0.00	64.00	0.00	30.00	81.08	0.00	18.92
Tetracycline	75.56	24.44	0.00	52.00	18.00	30.00	72.97	2.70	24.32
Ceftriaxone	68.89	22.22	8.89	32.00	0.00	68.00	45.95	45.95	8.11
Ceftazidime	6.67	17.78	62.22	6.00	0.00	94.00	5.41	5.41	89.19

*Key: S: Susceptible, I: Intermediate, R: Resistance, KCRH=Kajiado County Hospital, OSCH=Ongata Rongai Sub-County Hospital, NSCH=Ngong Sub-County Hospital.

DISCUSSION

The current study builds on previous work undertaken by the current investigators in patients attending major hospitals in Kajiado County, Kenya.[9] The study sought to undertake surveillance of AMR in wound infections in a region which have previously not well studied in Kenya. Our study identified a diverse array of bacteria with the most prevalent being CoNS, *S. aureus* and *E. faecalis*. The reported prevalence for *S. aureus* was relatively close to other studies on wound infection undertaken elsewhere (34.7%), (21.6%) and (34.34%).[16-18] However, the prevalence was higher than reported by others (19.96%), (27%).[19,20] The differences could be due to variation of hygiene practices and healthcare in different settings.[21, 22]

The prevalence of *E. faecalis*, reported in our study is higher than that reported in similar studies in Kenya, 10-15% and this could be attributed to variation in differences in hospital hygiene practices or patient demographics.[23,24] The predominance of CoNS and *S. aureus* in our findings is particularly significant. In wound management, CoNS are often overlooked, but in recent years have emerged as key pathogens in hospital-acquired infections.[25] On the other hand, *S. aureus*, known for its virulence and adaptability, presents a substantial challenge in wound management, especially with the increasing prevalence of methicillin-resistant strains.[10,25] The moderate prevalences of *P. mirabilis, Pantoea* spp. and *A. viridans* in wound infections suggest the involvement of environmental or endogenous sources of contamination.[26,27] *P. mirabilis* is a Gram-negative bacterium that can cause urinary tract infections and wound infections, especially in patients with indwelling catheters or foreign bodies.[28,29] *Pantoea* spp. are Gram-negative bacteria that are widely distributed in soil, water and plants and can cause wound infections, especially in immunocompromised patients or those with traumatic injuries.[30,31] Their presence in wound samples, as noted by a previous study, suggests environmental exposure.[24,26] On the other hand, *A. viridans* is a Gram-positive bacterium that is normally found in the oral cavity and can cause wound infections, especially in patients with dental procedures or poor oral hygiene.[27,28]

The other gram-negative bacteria found in our study included *E. cloacae, K. oxytoca* and *K. pneumoniae* are known to cause wound infections, especially in patients with diabetes, burns and chronic ulcers.[29] The bacteria, while being part of normal gut flora, are primarily recognized for causing hospital-acquired infections, including serious wound infections.[24,25,28] They have been reported to have capacity to develop resistance against a range of antibiotics.[25,28]

Our findings showed that the isolated *S. aureus* had high resistance to amoxicillin, particularly in KCRH which is a referral hospital. The figure was higher than that reported in other studies in Kenya.[9,14,18,30] A study also showed high resistance rates (77%) of *S. aureus* against amoxicillin in Ethiopia.[15] Such discrepancies in AMR rates might be attributed to local prescription practices and the wide over-the-counter availability of specific antibiotics.[14] Over the counter sales of antibiotics without prescription and their extra-label use has been shown to be a major cause for emergence of AMR.[14] Further, a study noted that widespread use of broad-spectrum treatments, along with the promotion of polypharmacy potentially contributes to the escalation of drug resistance. The occurrence of such practises in the study area was not subject of this study.[31]

The observed resistances to oxacillin, an indicator of MRSA, particularly in patients attending NSCH and KCRH were high when compared to global reports of (21·8%), 57.8%, (41.66%).[18,25,34] In Botswana, during the period from 2000 to 2007, a study indicated that the prevalence of MRSA ranged from 23% to 44%.[25] This could suggest a regional widespread occurrence of MRSA. Ciprofloxacin showed high susceptibility in patients from all three hospitals and is consistent to the findings by others (80%).[34] The high susceptibility to ciprofloxacin across all hospitals in our study is encouraging when compared to lower susceptibility rates reported in other African countries, often below 70%.[34] This shows the drug can be considered for management of wounds in patients in the study area. The disparity in different regions could be due to variations in antibiotic usage policies, local prescribing habits, and patient compliance.[25]

The isolated *S. aureus* from patients attending ORSCH had high susceptibility to Clindamycin and this is comparable to the findings of 81.8% and 78.6%.[25,35] The isolated from KCRH had high susceptibility to Gentamycin compared to those from ORSCH and NSCH. This aligns other studies who reported that Gentamycin's effectiveness can vary significantly across different healthcare settings, often influenced by localized bacterial resistance patterns. However, this susceptibility was higher than those reported in other areas (27·7%), (29%), (40.4%) and (69.8%).[34,35,37]

In the current study, the *S. aureus* isolates had low susceptibility to streptomycin and erythromycin across all the three hospitals. The low susceptibility in isolates from NSCH aligns with the work done by others (60%) and is consistent with the broader trend of increasing resistance to these older antibiotics, as documented by other studies (43.6%) and (38.1%).[18,32,37] Vancomycin susceptibility was higher in isolates from KCRH compared to those from ORSCH and NSCH. This is somewhat atypical as Vancomycin resistance is generally low in several studies, including those by others (87.64%), (94.4%) and (100%).[37,38] The observed low susceptibility of the isolates to Ceftriaxone mirrors the findings of isolates from elsewhere where prevalences of (52%) and (60.9%) have been noted.[18,35]

***Limitation*s**

This study was conducted to investigate the prevalence of bacteria present in wounds and antimicrobial sensitivity of *S. aureus* infections among 182 patients with septic wounds in KCRH, ORSCH, and NSCH. It was limited to Kajiado County. The limitations that were encountered were lack of time for the questionnaire by the busy and migrating pastoralist farmers and language barrier.

CONCLUSION

In conclusion, our study sheds light on the complex landscape of bacterial prevalence and AMR microbes isolated from septic wounds in three hospitals in Kajiado County, Kenya. The high prevalence of CoNS, *S. aureus*, and *E. faecalis*, coupled with the occurrence of significant *S. aureus*, having resistance to commonly used antibiotics like amoxicillin and oxacillin, underscores the need for robust antibiotic stewardship and tailored approaches in wound management. There is need to determine the risk factors which could be associated with emergence and spread of AMR in the study area. Ongoing surveillance and targeted research are essential in mitigating the challenge of AMR in wound care and management.

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