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Full Length Research Paper

Biofilm production and antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from HIV and AIDS patients in the Limpopo Province, South Africa

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Staphylococcus aureus **is a common pathogen associated with nosocomial as well as community acquired infections. Despite multiple reports on the severity and recurrent nature of *S. aureus* infection, the pathogenesis as well as antibiotic susceptibility profiles of *S. aureus* infecting HIV and AIDS patients has not been well studied in Limpopo Province, South Africa. Hence, the study was aimed at determining the biofilm producing capability and antibiotic resistance profiles of the *S. aureus* isolated from drinking water and clinical samples from HIV patients in the Limpopo Province. *S. aureus* strains isolated from clinical samples including urine, sputum, and stools obtained from HIV and AIDS patients as well as their drinking water samples were analysed for biofilm production using the microtitration plate method, haemolytic activities, antibiotic susceptibility, methicillin resistance and β-lactamase production. Methicillin resistance was tested from all the *S. aureus* isolates by the oxacillin agar plate. Overall, 140 *S. aureus* were isolated. Sixty (60) were from stools, 48 from urine, 15 from sputum and 17 from water. Of all the isolates, 67 (48%) produced biofilm of which 14 (10%) were strong biofilm producers and 53 (38%) moderate biofilm producers. Biofilm production was the highest among the urine isolates (62.5%) (χ^2 = 6.276; p = 0.01) and the water isolates (70.6%) (χ^2 = 4.006; p = 0.040). However, biofilm was not associated with sputum samples (p = 0.571) nor stool samples (p = 0.763). Isolates were highly resistant to most antibiotics tested and 125 (90%) isolates were resistant to more than 3 antibiotics. Higher resistance was observed against ampicillin (92%) while the most active antibiotic was ciprofloxacin with 89% susceptibility. Eighteen (14%) of the isolates were identified as MRSA and showed high resistance against vancomycin (28%) compared to MSSA among which vancomycin resistance was 14%. This is the first study on staphylococcal isolates from HIV patients in the Limpopo Province. The level of resistance to vancomycin was high and further attention is needed from the health system for more stringent measures of infection control. Biofilm production appeared to be a factor fuelling the increased in antibiotic resistance as well as pathogenicity in these strains. Furthermore, water could be a transmission vector of staphylococcal UTIs among HIV and AIDS patients in this region. However, further studies are needed to confirm these hypotheses.**

Key words: Antibiotic susceptibility, biofilm, diarrhea, urinary tract infections, pulmonary infections, epidemiology, HIV and AIDS, *Staphylococcus aureus*, venda, South Africa.

INTRODUCTION

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) is a major health problem in South Africa and HIV and AIDS patients are vulnerable to several infections termed opportunistic infections because of their weakened immune system. Over the past two decades, *Staphylococcus aureus* has emerged as a significant opportunistic pathogen among HIV and AIDS patients in both nosocomial and community settings, and recent studies have shown

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Abbreviations: MRSA, Methicillin-resistant *Staphylococcus aureus*; **MSSA,** Methicillin-sensitive *Staphylococcus aureus*.

greater frequency and morbidity of this organism among HIV positive individuals (Chacko et al., 2009; Hidron et al., 2010). However, there is little data on the occurrence, pathogenicity and antibiotic resistance of this organism among HIV positive patients in the Limpopo Province, South Africa.

S. aureus normally localize in the skin and mucous membranes in the nose of healthy humans and about 30% of the normal healthy population are transiently colonised by the organism (Liu, 2009) which has been associated with several syndromes such as skin infections, osteomyelitis, bacteraemia, septicaemia, diarrhea, pneumonia and urinary tract infections (Tumbarello et al., 1996; Franzetti et al., 2006; Flemming and Ackermann, 2007; Muder et al., 2006; Baba-Moussa et al., 2007; Shigemura et al., 2005; Huggan et al., 2008). These syndromes have been mostly described among HIV and AIDS patients and are responsible for high rates of morbidity and mortality. In South Africa, few literatures exist on the different types of *S. aureus* infections, particularly among HIV positive patients. Developing countries are at risk of getting waterborne disease as a result of consuming drinking water which is contaminated with pathogenic microbes of faecal origin. Death resulting from water-borne diarrhoeal disease is estimated at over 3 million annually, especially among infants and young children in poor communities in Africa, Asia and South America (Battu and Reddy, 2009). Normally, *S. aureus* should not be present in drinking water, but many studies have isolated high rates of *S. aureus* in water (Mihdhair, 2009). Residential care centres, communities and hospitals are often supplied with contaminated water and results in the distribution of infection among patients, particularly those with weaken immune system (Johnson et al., 2009).

Biofilm formation has been described as a possible attribute to the resistance and major contributor to nosocomial infections. Biofilm impairs the action of both host immune system and antimicrobial activities. Biofilm production is an important virulence factor of *S. aureus* (Dhanawade et al., 2009). The formation of biofilms is an example of a phenotypic change in *S. aureus* to adapt to its surroundings in the presence of environmental challenges (Jain and Agarwal, 2009), and is a recognized method of some organisms' ability to establish and maintain certain infections, and a way which increases its persistence and boosts its levels of antimicrobial resistance (Monre, 2007). Diseases such as endocarditis, osteomyelitis and medical-device related infections are caused by *S. aureus* biofilms and are not readily treatable with antibiotics.

Although, there is limited data on the prevalence of staphylococcal infection in Africa, one of the earliest case of MRSA was reported in South Africa (Shittu and Lin, 2006). Baseline data from Pan-European antimicrobial resistance using local surveillance (PEARLS) study of 2001 to 2002 showed that South Africa had MRSA prevalence of 33.3% with 23% MRSA in patient around Johannesburg (Marais et al., 2009). The prevalence of MRSA was lower, about 10% in Tunisia, Malta, and Algeria and around 15% in Kenya, as compared to the high prevalence of 21 to 30% in Cameroon and Nigeria when many African hospitals were studied (Kesah et al., 2003). In Uganda, about 10% of the surgical procedures become septic which account for an increasing morbidity and mortality with the commonest organism isolated being *S. aureus* (Ojulong et al., 2009). Data from the national nosocomial infection surveillance network (USA) have shown that MRSA present more than 50% of *S. aureus* strains causing infection in patients in intensive care units (Hidron et al., 2005). In Europe, MRSA prevalence ranges from over 50% in Portugal and Italy to below 2% in Switzerland and the Netherlands, where infection control measures have been shown to work (Ojulong et al., 2009). In Asia, the prevalence lies around 50%, with extremely high rates in Hong Kong (75%) and Japan (70%) (Saikia et al., 2009). The prevalence of MRSA in the Northern part of South Africa however, is not known.

Antimicrobial resistance is an increasing problem and a challenge worldwide (Uneke et al., 2010). The prevalence of resistance in *S. aureus* also is increasing globally. Staphylococcal resistance to penicillin is mediated by penicillinase production: an enzyme which breaks down the β-lactam ring of the penicillin molecule (Bassetti et al., 2009). The identification of possible associations between biofilm production and pathogenesis as well as antibiotic susceptibility profiles of infecting *S. aureus* could provide better control measures particularly among immunocompromised individuals. This study determined the prevalence of biofilm formation among *S. aureus* isolates from clinical and water samples in relation to antibiotic susceptibility and haemolytic activity.

MATERIALS AND METHODS

Ethical considerations

Ethical clearance of the study was obtained from the University of Venda Health Safety and Ethics Committee. Authorization to conduct the study was obtained from the Department of Health, Limpopo in Polokwane. Ethical clearance and authorization was also obtained from the ethical committees of the Donald Fraser and Tshilidzini Hospitals. The objectives of the study were explained to the patients in their mother tongue (Tshi-Venda, Tsi-Pedi or Tsi-Tsonga) and their right to say no to participate in this study was explained to them. Once the patients had agreed to participate in the study they were requested to sign a consent form. To preserve their privacy, the patients were given a code and were referred to by that code. As such, different samples including sputum, urine, mouth wash and stools were collected whenever it was possible to do so. Patients with HIV positive, visiting major hospitals in the Vhembe district, situated in the Northern part of South Africa.

Bacterial isolation and identification

The different samples were transported to the laboratory within 4 h of collection and were inoculated onto freshly prepared mannitol

salt agar and incubated at 37°C for 24 h. Golden yellow colonies were presumptively identified as *S. aureus*. Then a single golden yellow colony from the plate was subcultured on freshly prepared nutrient agar and incubated at 37°C for 24 h. The nutrient agar plates were then stored in the fridge for further analysis. As a result, a total of 140 isolates were obtained and used in this study.

Confirmation of the isolates by polymerase chain reaction (PCR)

Genomic DNA was extracted from the isolates using the protocol described by Kumar et al. (2008) and the quality of the DNA was checked by agarose gel electrophoresis. The DNA was stored in the freezer at -20°C until further analysis.

The identification of *S. aureus* by polymerase chain reaction was done through the detection of *tuf*-gene from the genomic DNA extracted from the isolates according to Jimenez et al. (2008) with some slight modifications. The primer set used included *tuf*-g (5´-GGTGTACCAGCATTAGT-3´), *tuf*-e (5´-TTCGTGCATACCGATGA) and *tuf*-a (5´TTCAGTATGTGGTGTAA-3´). The reaction was done in a multiplex PCR format to allow the identification of *Staphylococcus epidermidis* strain from isolates if present. The specificity of the assay was confirmed using a negative control of 5 µl of nuclease free water. The PCR amplicons were detected using 1% agarose gel electrophoresis stained with ethydium bromide.

Phenotypic characterization of *Staphylococcus aureus*

Haemolytic activity

The haemolytic activity testing of the *S. aureus* isolates was performed according to the method previously described by Jimenez et al. (2008). Briefly, Colombia agar was prepared and supplemented with 5% horse blood. The microorganisms were inoculated on Columbia blood agar and incubated at 37°C. The plates were analyzed after 72 h and isolates were classified as non-haemolytic when no lysis was observed, beta (β) hemolytic when moderate lysis was observed and alpha (α) hemolytic when complete lysis of the blood in the media was observed. For better visibility, about 8 microorganisms were tested in a plate.

Biofilm assay

In order to test for biofilm production by the different strains of *S. aureus* isolated from clinical and water samples, two different methods were used namely; the microtitre plate method (Kwon et al., 2008) and the Congo red agar based method (Freeman et al., 1989).

Microtitre plate method

The assay was performed using U shaped polystyrene microtitre plates with brain heart infusion broth (BHI) supplemented with 1% sucrose (Kwon et al., 2008). Prior to inoculation on the plates the microorganisms were grown in BHI for 2 to 4 h. 199 µl of the 1% sucrose BHI was added to each well of the microtitre plate and 1 µl of the microbial suspension was added. All the reactions were done in triplicate (that is, using three wells for each strain). The plate was covered and incubated at 37°C for 24 h. The next day, the plates containing the culture were emptied and washed with phosphate buffered saline (PBS) five times to remove any unfixed microbial cell and leave only those fixed in the well within a biofilm matrice. Then 175 µl of 1% crystal violet was added and incubated at room temperature for 15 min.

The plates were further washed 5 times with PBS and dried for 30 min at room temperature. 200 µl of ethanol-acetone (80 to 20%) were added to solubilise the microorganism and the crystal violet and incubated at room temp for 25 min. The optical density was recorded using an ELISA reader (Bio-Tek Instruments, INC Winooski, VT, USA) at the wavelength of 490 nm. The strains that gave an OD value less than 0.120 were recorded as non-biofilm producers, those with OD values between 0.120 and 0.240 were considered as moderate producers and those with OD values more than 0.240 were considered as strong producers. The reaction was repeated at two different occasions.

The Congo red agar based method

The agar medium used was prepared by adding 37 g of the BHI powder, 50 g of sucrose and 10 g of agar No 1 in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121°C. Once the agar solution has cooled down to about 50°C, a solution of Congo red (8 g/L) was added and mixed again and then the media was poured into the Petri plates and allowed to solidify. Once the media had settled, the plates were inoculated with the microorganisms and incubated at 37°C for 24 h. The plates were read the next day and the organisms were considered positive (biofilm-producers) when they produced black colonies on the agar and negative (non-biofilm producers) when they produced pink, or red-orange colonies on the Congo red agar (Freeman et al., 1989).

Antibiotic susceptibility profiles of *Staphylococcus aureus*

The disc diffusion method was used in order to determine the antibiotic susceptibility profiles of the strains. The microorganisms were prepared in Mueller-Hinton broth (MHB) by directly suspending one colony in a tube of sterile MHB. Prior to testing for antibiotic susceptibility, a 0.5 McFarland standard of the organism was prepared in MHB. The test was conducted on Mueller Hinton agar plates using a swab and the 0.5 MacFarland culture. The cultures were inoculated into the plates using sterile swabs and left for about 10 min and the antibiotics discs were placed on the culture and incubated at 35 to 36.5°C for 24 h. The zone of inhibition was measured the following day and the results were interpreted according to CLSI (previously known as NCCL) standards (CLSI, 2007). The antibiotics tested included amoxycillin (AML 10), ampicillin (AMP 10), cefepime (FEP 30), ceftriaxone (CRO 30), cefoxitin (FOX 30), chloramphenicol (C 30), erythromycin (E 15), kanamycin (K 30), meropenem (MEM 10), penicillin G (P 10), polymyxin B (PB 300), streptomycin (S 10), tetracycline (30) and vancomycin (VA 30).

β-Lactamase test

The β-lactamase test was performed using the tube based iodometric method as previously described (Catlin, 1975; Thornberry et al., 1974).

Detection of methicillin-resistant *Staphylococcus aureus* by the agar method

Mannitol salt agar was prepared and cooled to about 55°C. The media was supplemented with 2 mg/L of oxacillin. Microorganisms were inoculated into the oxacillin agar plate and incubated at 37°C for 42 h. Growth of isolates was identified as positive. Eight isolates were tested per plate (Arora et al., 2010).

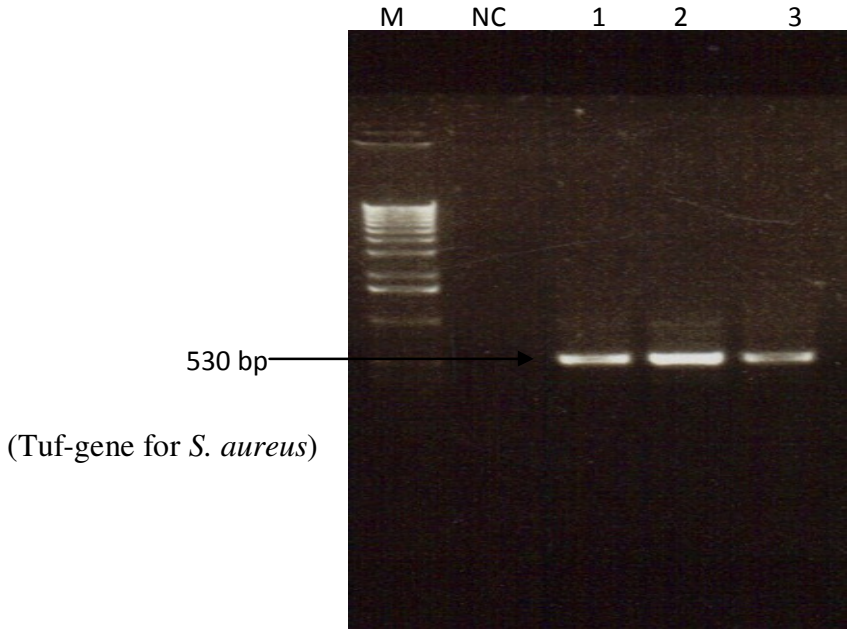

Figure 1. Agarose gel electrophoresis pattern showing the 530 bp of the amplification product for *S. aureus tuf*- gene. Lanes: M DNA molecular Marker (1000 bp ladder: Marker X, from Invitrogen, CA, USA); lanes 1 to 4 *tuf*-gene positive amplicon products, NC is negative control (*E. coli* DNA amplification).

Table 1. The overall distribution of haemolytic activity of the isolates by sample type.

Haemolysis type	Sputum (%)	Stool (%)	Urine (%)	Water (%)	All sample (%)
α –haemolysis	6 (40)	32 (54.2)	17 (40.5)	5 (33.3)	60 (45.8)
β-haemolysis	5 (33.3)	22 (37.3)	17 (40.5)	7 (46.7)	51 (39)
γ –haemolysis	4 (26.7)	5 (8.5)	8 (19)	3 (20.0)	20 (15.2)
Total	15 (100)	59 (100)	42 (100)	15 (100)	131 (100)

Statistical analysis

All data was entered into a Microsoft excel sheet. The analysis was conducted using the statistical package for social sciences (SPSS) program, version 17.1. Chi-square test was used for comparison of the different variables and the correlation between all the tests performed. A p value of <0.05 was considered to be statistically significant.

RESULTS

Bacterial isolates used in this study

A total of 140 strains of *S. aureus* were isolated from different types of samples (from a total of about 650 samples). All the isolates were confirmed to be *S. aureus* by PCR by the observation of the specific bands as shown in Figure 1. Of these, 48 (34%) were from urine, 60 (43%) from stools and 15 (11%) from sputum. The remaining 17 (12%) isolates were from water samples. Of all the isolates, 79 (64%) were from females, while 44 isolates were from males. The age of the patients varied between 18 and 81 years.

Haemolytic activity of the isolates

131 isolates were tested for haemolysis. Of these, 60 (45.8%) presented alpha (α) haemolysis phenotype, 51 (39%) presented beta (β) haemolysis phenotype while 20 (15.2%) were gamma (γ) or non haemolytic. The stool samples had the highest prevalence of α haemolytic strain (54.2%), while beta (β) haemolytic strains were higher among the water isolates (46.7%), (Table 1). There was no significant difference between sample type and haemolytic activity, ($p>0.05$).

Biofilm production

The microtitre plate method (MTP) was compared to the Congo red agar based method for the detection of biofilm

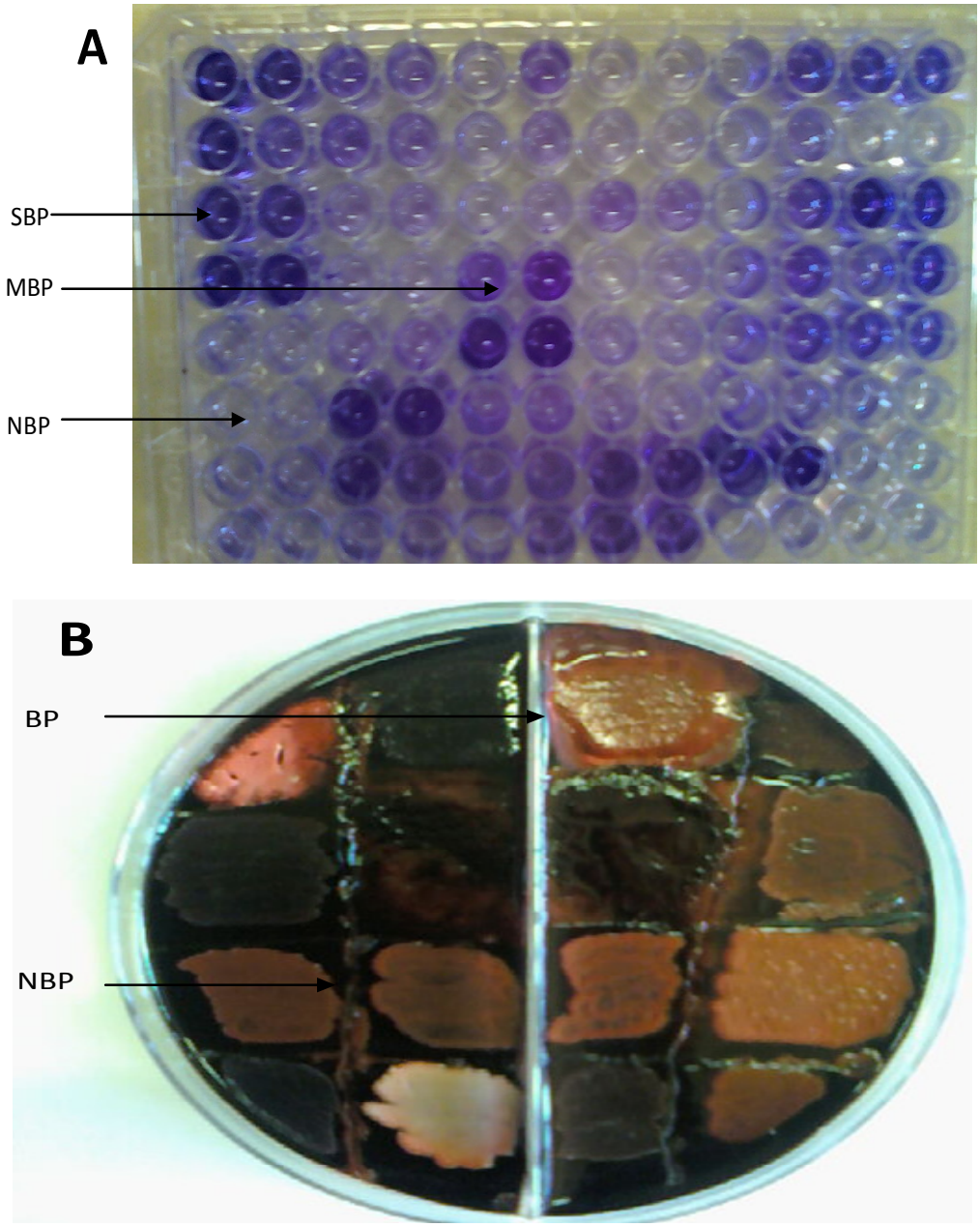

Figure 2. (A) Microtitre plate indicating some biofilm positive and negative isolates. (B) Congo red agar plate indicating some positive and negative isolates, Note: BP = Biofilm producer, NBP = Non-Biofilm producers, SBP = Strong Biofilm producer, MBP = Moderate Biofilm producers.

production. It was easy to discriminate between strong biofilm producers, moderate and non producers when using the MTP (Figures 2A), while the CRA could only identify microorganism as producers and non producers (Figures 2B). There was a good correlation between the two methods, ($p<0.05$), but the MTP methods was the one used for further analysis, because it was found to be more sensitive and easy to read, although, both methods were easy to perform. With the polystyrene microtitre plate, 14 (10%) were strong producers, 53 (38%) were moderate biofilm producers while 73 (52%) were non biofilm producers. With the Congo red methods, 53 (37.3%) biofilm producers and 87 (62.1%) were non-biofilm producers. Figure 3 shows a scatter plot of the OD values obtained from the biofilm producers. The microtitre plate method was more sensitive as compared to the Congo red agar method, and it was the one used for further analysis in this study.

Organisms from water samples, followed by those from urine samples had the highest rate of biofilm production. However, water samples had the highest percentage of strong producers (17.6%), followed by those from stools

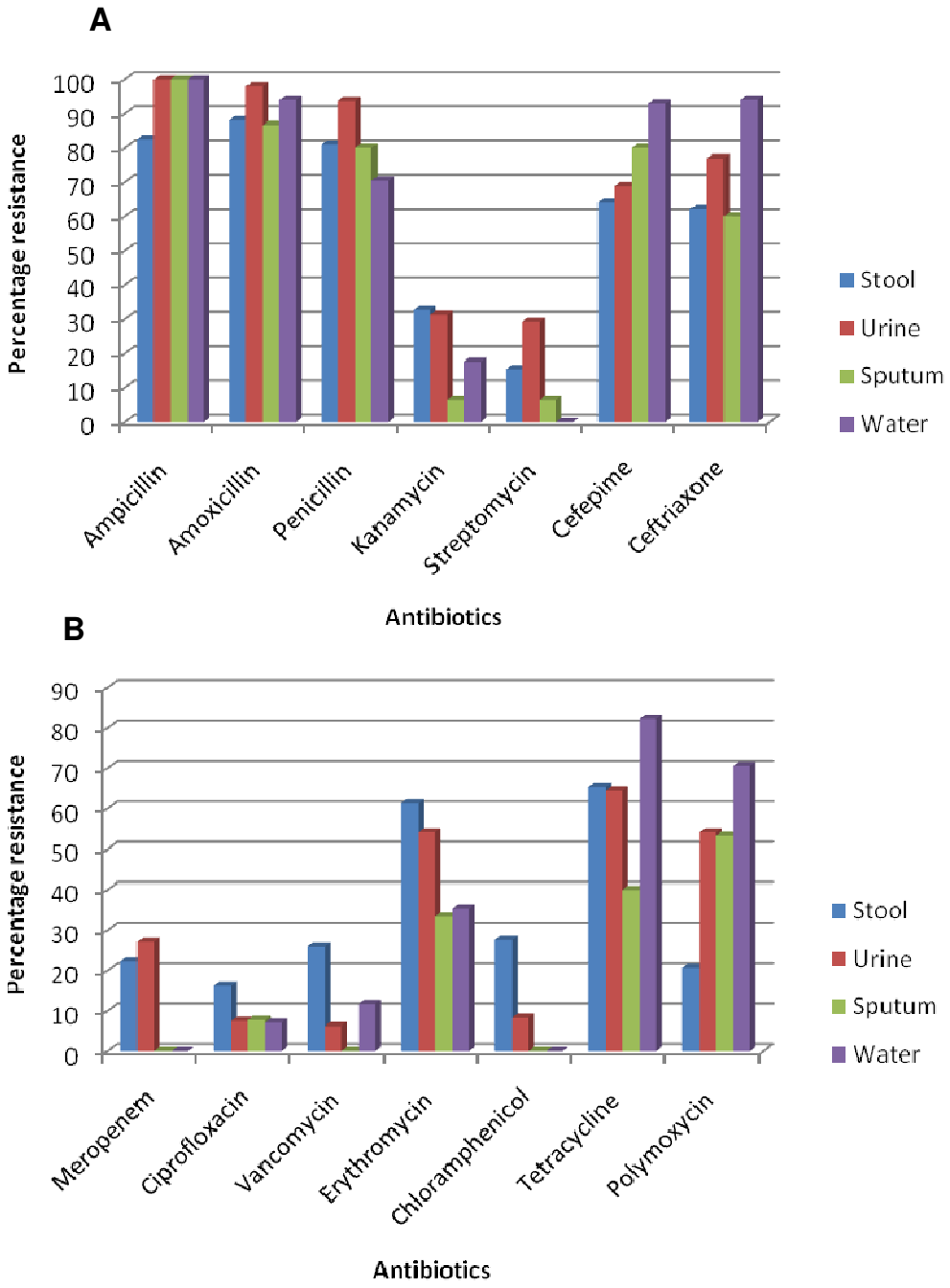

Figure 3. Antibiotic resistance profile of the isolates by sample type.

(10%) compared to the isolates from other samples. Most organisms from urine samples showed moderate biofilm production phenotype (56.3%) (Table 2). There was a significant difference between sample origin and biofilm production ($p<0.05$).

Antibiotic susceptibility/resistance profiles

High resistance was observed against the penicillin group and cephalosporines, while less resistance was observed against the carbapenems, fluoroquinolones and glycol-peptides groups. None of the isolates was completely susceptible to all the tested antibiotics. Resistance to ampicillin, cefepime, tetracycline and erythromycin were 92, 76, 63.5 and 52% respectively. In contrast, there was less resistance to meropenem (18.8%), chloramphenicol (14.5%), vancomycin (14.5%) and ciprofloxacin (11.4%). Table 3 summarizes the antibiotic susceptibility profiles of the isolates against 14 antimicrobial agents.

Table 2. Biofilm production by organisms from different types of sample.

Sample	Strong producer (%)	Moderate producer (%)	Non producer (%)	Total (%)	P value
Stool	6 (10)	12 (20)	42 (70)	60 (100)	0.581
Sputum	1 7)	5 (33.3)	9 (60)	15 (100)	0.571
Urine	4 (8.3)	27 (56.3)	17 (35.4)	48 (100)	0.010
Water	3 (17.6)	9 (53)	5 (29.4)	17 (100)	0.040
Total	N = 14 (10)	N = 53 (38)	N = 73 (52)	140	

Table 3. Antibiotic resistance of the *S. aureus* isolates from HIV patients in the Limpopo province.

Family of antibiotic	Antibiotic (unit)	Resistance (%)	Susceptible (%)	Breakpoint* (mm)
Penicillins	Ampicillin (AMP 10 µg)	115 (92)	10 (8)	≥29
	Amoxicillin (AML10 µg)	128 (92.1)	12 (7.9)	≥29
	Penicillin G (P 10 µg)	122 (88.4)	16 (11.6)	≥29
Aminoglycosides	Kanamycin (K15 µg)	38 (27.5)	100 (72.5)	≥18
	Streptomycin (S10 µg)	24 (17.4)	114 (82.6)	≥18
Cephalosporines	Cefepime (Fep 30 µg)	94 (76.4)	29 (23.5)	≥18
	Ceftriaxone (CRO 30 µg)	90 (72)	35 (28)	≥21
Carbapenems	Meropenem (MEM10 µg)	26 (18.8)	112 (81.2)	≥16
Fluroquinolones	Ciprofloxacin (CIP 15 µg)	14 (11.4)	109 (86.4)	≥21
Glycopeptides	Vancomycin (VA 30 µg)	20 (14.5)	118 (85.5)	≥15
Macrolides	Erythromycin (15 µg)	72 (52.6)	65 (47.4)	≥23
Phenicols	Chloramphenicol (C 30 µg)	20 (14.5)	118 (85.5)	≥18
Tetracyclines	Tetracycline (TE 30 µg)	89 (64.5)	49 (35.5)	≥19
Polymyxin	Polymoxycin (PB 300 µg)	58 (42)	80 (58)	≥

*CLSI 2006, M2-A9 and M7-A7 were used for determining the zone of inhibition breakpoint.

Antibiotic resistance and sample types

Isolates from all type of samples showed high resistance to the penicillin group with 100% resistance to ampicillin observed from sputum and water isolates. Within the aminoglycosides group, 32.8% of urine isolates were resistant to kanamycin as compared to only 6.7% of the sputum isolates. Twenty nine percent of urine isolates were resistant to streptomycin, while all water isolates were susceptible (0% resistance) to the same antibiotic. Furthermore, both meropenem and chloramphenicol were 100% active against the water and sputum isolates, although, 27.1 and 26.4% resistance were observed among the urine and stool isolates respectively. Vancomycin was 100% active against sputum isolates with low resistance in the urine isolates (6.3%), and high resistance 26.9% in the stool isolates (Figure 3A and B).

Multiple drug resistance among the isolates obtained from clinical and water samples from HIV positive patients in the Limpopo Province

Multiple drug resistance (MDR) was defined as resistance to 3 or more antibiotics (ATB). From 140 isolates tested against 14 antibiotics, 125 (90%) of the isolates were resistant to at least 3 or more ATB. None of the isolate was susceptible to all antibiotics. The highest rate of multiple drug resistance was observed against 7 antibiotics with 26 (18.7%) isolates, followed by isolates resistant to 6 antibiotics 24 (17.3%), while 16 (11.5%) were resistant to 8 antibiotics at a time. None of the isolates were resistant to 13 and14 antibiotics, while 1 (0.7%) was resistant to 12 antibiotics.

Multiple drug resistance and sample type

The occurrence of multiple drug resistance among the isolates was compared between sample types. From those that resisted to the 7 antibiotics, higher resistance rate was observed among the water isolates (29.4%) as compared to 13% among sputum. Four (23.5%) of the water isolates were resistant to 9 antibiotics with only 6.25% among urine isolates and none among sputum. Six (12.5%) of the urine isolates were resistant to 10 antibiotics, while none of the sputum and water isolate was resistant to 10 antibiotics at a time (Figure 4).

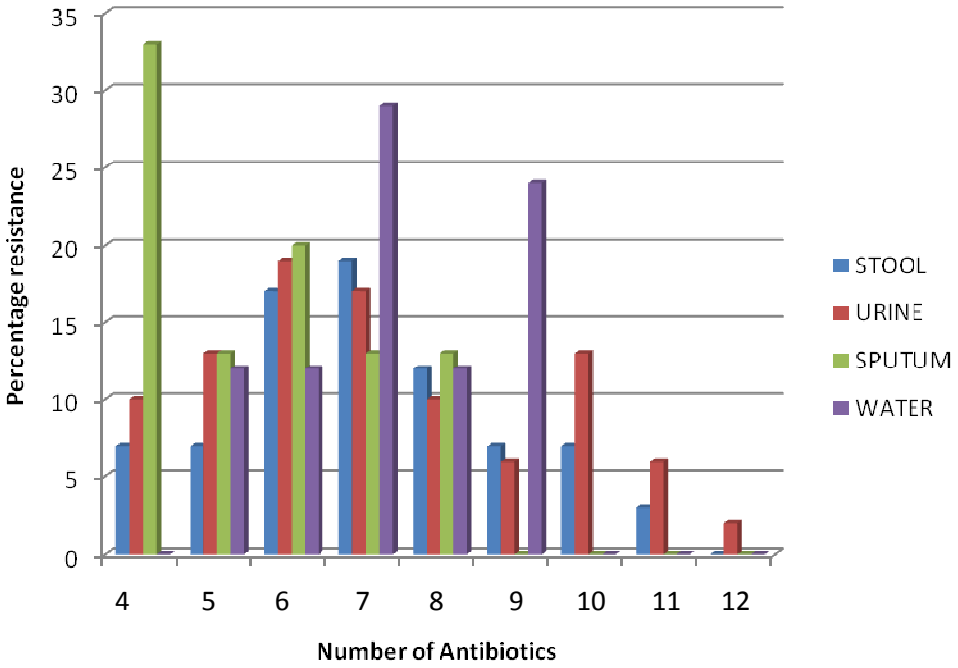

Figure 4. Pattern of multiple drug resistance of *S. aureus* by sample type.

Antibiotic resistance and gender

The rate of penicillin resistance among female was higher compared to the resistance rate in males (86 and 81% respectively), however, the difference was not statistically significant ($p>0.05$). The resistance profile to most antibiotics used was generally similar among females and males, although, slight differences were observed with the aminoglycoside, 26% of the isolate from females were resistant to kanamycin compared to 30% for the isolates obtained from males, while 20% females' isolates were resistant to streptomycin compared to 12% of the males. A difference was observed with the vancomycin in which 23% isolates from female displayed resistance with only 6% among the strains isolated from male patients.

Multiple drug resistance and gender

60 (92%) of the isolates from female were multi-drug resistant as compared to 28 (84.8%) in males. Among the isolates from female, higher rate of MDR was observed against 6 ATB (23%); while only 9% was observe among the strains isolated from males against the same number of antibiotics. In some cases, there was high rate of MDR by male isolates as compared to female isolates. While the rate of MDR to 4 and 8 antibiotics was 18 and 15.1% respectively among the male isolates, the rate was only 9.2 and 9.2% for the female isolates. None of the male isolates were resistant to 11 antibiotics at a time, while 4.6% of female isolates displayed resistance to 11 ATB at a time.

β-Lactamase production

Comparison of the two methods

All samples were tested for the β-lactamase activity using the two methods. With the tube method, 55 (39.6%) were β-lactamase positive, while 84 (61.4%) were β-lactamase negative. With the agar method, 53 (38%) were positive and 86 (62%) were negative. Among the 55 positive with the tube, 40 were also positive with the agar while the rest were negative. The tube method was the one selected and used for further analysis.

β-Lactamase distribution according to sample types

Out of the 139 isolates tested, for β-lactamase activity, 55 (39.6%) were positive, whereas 84 (60.4%) were negative. Water isolates were more likely to be β-lactamase positive with 65% of the isolates from water producing β-lactamase ($\chi^2 = 5.118$; $p = 0.024$). The prevalence of β-lactamase production among the isolates from urine, stool and sputum isolates was 33.3, 39 and 33.3% respectively.

Association between β-lactamase and resistance to other antibiotics

β-Lactamase was associated with resistance of isolate to other antibiotics. β-Lactamase positive isolates showed 92 and 94% resistance to penicillin and ampicillin

Table 4. Characteristics of MRSA positive samples from HIV/AIDS patients in the Limpopo Province.

S/N	MRSA agar	Origin	Biofilm	Black	Antibiotic resistance profile	Hemolysis
1.	Positive	Sputum	Positive	Negative	AMP, Cro, E, PB	β
2.	Positive	Sputum	Negative	Negative	AMP, AMI, P	β
3.	Positive	Urine	Positive	Negative	AMI, P	α
4.	Positive	Urine	Negative	Negative	AMP, AMI, P, S, Fep, E, T, PB	β
5.	Positive	Urine	Positive	Negative	AMP, AMI, P, Cro	α
6.	Positive	Urine	Positive	Negative	AMP, AMI, P, Fep, T	γ
7.	Positive	Urine	Positive	Negative	AMP, AMI, P, Fep, T	α
8.	Positive	Urine	Negative	Negative	AMP, AMI, P, K, Fep, Cro, MEM, E, T, PB	β
9.	Positive	Sputum	Positive	Positive	AMP, AMI, P, T, PB, Fep, Cro	α
10.	Positive	Sputum	Negative	Negative	AMP, AMI, P, Fep, T	β
11.	Positive	Urine	Positive	Positive	AMP, AMI, P, Fep, Cro, Cip	β
12.	Positive	Stool	Positive	Positive	AMP, AMI, P, K, Fep, MEM, E, T	β
13.	Positive	Stool	Positive	Positive	AMP, AMI, P, Cro, MEM, Cip, VA, E, T	γ
14.	Positive	Stool	Negative	Positive	AMP, AMI, P, K, S, Fep, Cip, VA, E, C, T	β
15.	Positive	Stool	Negative	Negative	AMP, AMI, P, T, PB, Fep, Cro	β
16.	Positive	Stool	Negative	Positive	AMI, P, K, VA, E, T	α
17.	Positive	Stool	Positive	Positive	AMP, AMI, P, VA, E, T	β
18.	Positive	Stool	Positive	Positive	AMP, AMI, P, K, S, VA, Cro, E, C, T	α
19.	Negative	Stool	Negative	Negative	AMP, AMI, Fep, K, MEM, E, T	α

AMP = Ampicillin, AML = Amoxicillin, MEM = Meropenem, K = Kanamycin, P = Penicillin, S = Streptomycin, E = Erythromycin, FEP = Cefepime, CRO = Ceftriaxone, T = Tetracycline, CIP = Ciprofloxacin, VA = Vancomycin, PB = Polymoxycin, C = Chloramphenicol.

respectively. β-Lactamase positive isolates showed least association with resistance to chloramphenicol (14.5%). B-Lactamase was significantly associated with resistance to ciprofloxacin (χ^2 = 7.383; p = 0.008). Although, vancomycin resistance was higher among beta lactamase positive isolates, the difference was not statistically significant (p = 0.224).

β-lactamase and multiple drug resistance

β-Lactamase production of the isolates was also compared with multiple drug resistance. Highest rate of MDR was observed among the β-lactamase producing isolates (93%) compared to 88% among the β-lactamase negative isolates. High β-lactamase MDR to 7 antibiotics was observed among positive isolates (21.1%) compared to 16.7% among negative isolates. The only isolate that was resistance to 12 antibiotics was a β-lactamase positive isolates (1.8%) (Table 4). β-Lactamase production rate was higher among female (51%) compared to males (26%).

Overall antibiotic susceptibility profile of the biofilm producing and non producing isolates

In general, there was no significant difference between antibiotic resistance among biofilm producing strains and non-producing strains. However, biofilm producing strains were more resistant to meropenem with 42.8% resistance compared to only 13.7% resistance among moderate biofilm producers and 17.8% resistance among non-biofilm producing strains (χ^2 = 6.394; p = 0.042). Also, 92.8% strong producers were resistance to ceftriaxone, while 64 and 63% of moderate and non-producers showed resistance to this antibiotic (Table 5).

Impact of biofilm production on β-lactamase and methicillin resistance

Biofilm production was also correlated with β-lactamase production. Out of the 14 strong producers, 9 (64.3%) were β-lactamase positive compared to 40% and 34.2% of the moderate and non-producers respectively (χ^2 = 3.978; p = 0.045). Fifty-six (56%) biofilm producing strains were MRSA positive compared to 48% among the MSSA isolates. Moreover, when isolates were classified based on biofilm producing capacity, 37.8% of the strong biofilm producers were MRSA positive (χ^2 = 6.964; p = 0.021).

DISCUSSION

Over the past decade, there has been an increase in the rate of infection and diseases caused by *S. aureus* particularly MRSA throughout the world (Sadaka et al., 2009). The situation is even more alarming among

Table 5. Antibiotic susceptibility profile of the biofilm producer and non biofilm producers.

Antibiotic	Strong producers (n = 14) (%)	Moderate (n = 53) (%)	Non-producer (n = 73) (%)
Ampicillin (10 µg)	12 (92.3)	45 (85)	58 (79)
Amoxicillin (10 µg)	12 (85.7)	51 (96)	65 (89)
Penicillin (10 µg)	12 (85.7)	49 (92.4)	59 (80)
Kanamycin (15 µg)	5 (35.75)	14 (23.4)	19 (26)
Streptomycin (10 µg)	3 (21)	10 (18.8)	11 (15)
Cefepime (30 µg)	9 (69)	39 (73.5)	46 (63)
Ceftriaxone (30 µg)	13 (92.8)	34 (64)	46 (63)
Meropenem (10 µg)	6 (42.8)	7 (13.7)	13 (17.8)
Ciprofloxacin (15 µg)	2 (14.28)	2 (4)	10 (13.6)
Vancomycin (30 µg)	2 (14.2)	8 (15)	10 (13.6)
Erythromycin (15 µg)	9 (64.3)	26 (49)	37 (50.6)
Chloramphenicol (30 µg)	3 (21.4)	7 (13.2)	10 (13.6)
Tetracycine (30 µg)	10 (71.4)	33 (62.3)	46 (63)
Polymoxycin (300 µg)	3 (21.4)	23 (23.3)	32 (43.8)

patients with reduced immunity such as those undergoing chemotherapy or surgery, children, elders and patients with HIV and AIDS. *Staphylococcus* is an important cause of both community and hospital acquired infections resulting in high morbidity and mortality in tropical Africa. Currently, there is little data available in South Africa about this pathogen particularly among HIV and AIDS patients. Understanding the correlation between biofilm, antibiotic resistance and pathogenesis of *S. aureus* strains infecting HIV and AIDS patients in the Limpopo Province is important for design of treatment and control strategies for the concerned patients.

Although, resistance profiles would be expected to vary in different communities, many studies conducted in Africa have reported high antibiotic resistance among *S. aureus* isolates (Onanuga et al., 2005; Essa et al., 2009; Marais et al., 2009). In this study, we found resistance rates above 90% to a number of antibiotics among the isolates obtained from HIV/AIDS patients. None of the tested isolate was 100% susceptible to all the antibiotics tested. There was a high susceptibility to ciprofloxacin (89%), however, strains that produced β-lactamase were much more resistant to this antibiotic. These findings are consistent with those obtained by Onanuga et al. (2005).

It appears that the site of infection plays an important role in relation to antibiotic resistance. Meropenem and chloramphenicol were among the most effective antibiotics against *S. aureus* observed in this study, particularly against sputum and water isolates with 100% susceptibility to both antibiotics and could be recommended for the treatment of respiratory and waterborne diseases caused by *S. aureus*. However, the treatment outcomes might not be similar against urinary tract infection and gastrointestinal involvement, because stool and urine isolates showed high resistance against both antibiotics (22% among stool isolates and 27% among urine isolates were resistant to meropenem; 27 and 8% against chloramphenicol respectively). In a study by Stefani and Veraldo (2003), methicillin resistant *S. aureus* was much more resistant to meropenem compared to methicillin susceptible strains.

Vancomycin is recommended as an active drug against *S. aureus* in South Africa when considering its effectiveness as reported from previous studies on MRSA in South Africa (Marais et al., 2009). Our study seems to be the first to report on vancomycin resistance in South Africa, implicating that the resistance pattern of *S. aureus* might differ with region. Similar findings were described in Italy were the resistance of MRSA stains to vancomycin varied between 19 and 35% (Campanile et al., 2009).

S. aureus can be identified as MRSA if it has an oxacillin MIC of ≥4 µg/ml in an oxacillin salt agar screen test (Almer et al., 2002). The prevalence of MRSA (14%) identified in this study was lower than that identified in Kwazulu-Natal Province and in other major cities in South Africa such as Johannesburg (33%) and Cape Town (43%) (Shittu and Lin, 2006). MRSA identified in this study also displayed high resistance to erythromycin (50%) and tetracycline (61%) similar to those reported in Gauteng Province in South Africa about 500 km away from our study site (Marais et al., 2009). The resistance of MRSA and MSSA to the ciprofloxacin were (17% vs. 9%), findings similar to those observed in Kwa-Zulu Natal, indicating the likelihood of MRSA being more resistant compared to the MSSA isolates to fluoroquinolones (Shitu and Lin, 2006). The prevalence of MRSA in males (12%) and females (12%) were similar, unlike in the study done in Uganda where male were twice (76%) more likely to get MRSA compared to the females (23.5%) (Ojulong et al., 2009). Thus, HIV positive patients in the Limpopo Province have equal chance of contracting MRSA regardless of gender and it could be recommended that both male and female be treated with same antibiotics. There was no statistically

significant difference between distribution of MRSA and sample origin (p = 0.495) except for water.

Bacteria use biofilm mechanism as a way of causing chronic infection to human (Leid et al., 2002). Biofilm are also well suited for resistance to antibiotics and evasion of immune system's defences. The development of biofilm by *S. aureus* might be a mode of adaptation of these organisms to live in different environments. Furthermore, biofilm-mediated infections in the hospital environment have a significant negative impact on patient's health and place an enormous burden on the resources of the health services (Smith et al., 2008). The study characterized most strong biofilm producers from water with 17.6% as compared to clinical isolates. Householders should be advised on their water storage and a call for best water treatment methods. From the clinical isolate those from stools were also strong biofilm producers (10%) as compared to sputum and urine, thus, it should be important to look at the association between drinking water, biofilm and diarrhoea which this study could not clarify. There was a significant difference between biofilm production by the clinical isolates ($p<0.05$) and is in contrast to previous studies (Critchley et al., 2002). Although, biofilm formation in water have been identified as a major problem in drinking water and can results in food intoxication, biofilm production from urine pose a major public health burden for people requiring indwelling medical devices. The ability of nosocomial pathogens including *S. aureus*, to form biofilms is of significant clinical interest, since biofilm formation influences the efficacy of antimicrobial therapy, the subsequent outcome of an infection, increased prevalence of antibiotic resistance and induce resistance also to vancomycin (Götz, 2002). However, in our study, there was no clear relationship between biofilm production and vancomycin although strong producers were significantly resistant to meropenem.

Conclusions

This study has shown that strong biofilm production by *S. aureus* strains isolated from HIV and AIDS patients was predictive of meropenem resistance and β-lactamase production. Although, we detected low MRSA compared to other province, the high antibiotic resistance and multidrug resistance observed among these isolates is of serious concern for the treatment of these infections in HIV and AIDS patients. S. aureus and particularly MRSA appear to be responsible for urinary tract infections as well as respiratory infections among HIV and AIDS patients in the Limpopo Province. Drinking water seems to be a source of transmission of pathogenic strains of *S. aureus.* However, the pathogenicity of these strains needs to be confirmed by using larger number of patients in comparison with HIV negative patients. Further studies are needed in order to identify the genetic features contributing to biofilm formation among these isolates for better management of *S. aureus* infections among the HIV and AIDS patients and to reduce the level of morbidity and mortality among patients suffering from *S. aureus* infections. There is also a need to maintain surveillance control of MRSA infection in South Africa because the majority of MRSA are multidrug resistant as identified in our study and studies outside of South Africa.

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