African Journal of Microbiology Research Vol. 5(30), pp. 5498-5506, 16 December, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR11.871

*Full Length Research Paper*

# Molecular detection of methicillin resistance gene (*mec* A gene) and pathogenic genes among *Staphylococcus aureus* isolates from clinical and drinking water samples of HIV and AIDS patients in Limpopo Province, South Africa

**Samie A. and Shivambu N.**

University of Venda, Department of Microbiology, Private Bag X5050, Thohoyandou 0950, Limpopo, South Africa.

Accepted 3 November, 2011

**In the present study, *Staphylococcus aureus* strains isolated from de-identified clinical samples including urine, sputum, and stools obtained from Human Immunodeficiency virus (HIV) and Acquired immunodeficiency syndrome (AIDS) patients as well as their drinking water samples were analysed. Antibiotic resistance was tested by the disc diffusion while biofilm was tested using the microplate method. Methicillin resistance was tested from all the *S. aureus* isolates by the oxacillin agar plate and the *mec* A gene was detected by the polymerase chain reaction (PCR) method. A subset of 50 isolates randomly selected were analysed for the presence of 5 different *S. aureus* enterotoxins (SEA-SEE) using a multiplex PCR procedure, of the 140 *S. aureus* isolates from 478 samples, 18 (14%) were *methicillin-resistant S. aureus* (*MRSA*) and 3 out of the 18 showed resistance to vancomycin (17%) compared to methicillin sensitive *S. aureus* (MSSA) among which vancomycin resistance was 11%. Methicillin resistance was more common among the sputum isolates 4 (27%) compared to 7 (15.5%) and 7 (12%) from urine and stool isolates. None of the 17 water isolates was positive for MRSA. Pathogenic genes were detected in 13 (26%) isolates. *Staphylococcus* enterotoxin A (SEA) was the most commonly detected gene 12 (24%) and was more prevalent among organisms isolated from urine 8 (35%) ($\chi^2$ = 8.196; p = 0.042). The present study indicated that *Staphylococcus* enterotoxin A is the most common pathogenic gene. Based on the results obtained, it can be hypothesised that pathogenic *S. aureus* (producing biofilm and staphylococcal enterotoxin A) are responsible for urinary tract infections among HIV patients in the Limpopo Province. 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:** HIV and AIDS, *Staphylococcus aureus*, biofilm, antibiotic susceptibility, epidemiology, Venda, South Africa, toxins, *mec* A.

## INTRODUCTION

Over the past decade, *Staphylococcus aureus* has evolved from being mostly nosocomial to become even community acquired indicating the increase in the pathogenesis of this organism (Moussa et al., 2011). As an aggressive pathogen, *S. aureus* poses a significant public health threat because it is associated with nosocomial infections as well as community acquired infections (Zetola et al., 2005). Human Immunodeficiency virus (HIV) and Acquired immunodeficiency syndrome (AIDS) is the major leading health problems in South

*Corresponding author. E- mail: samieamidou@yahoo.com or samie.amidou@univen.ac.za. Tel: +27159628186 or +27760952916. Fax: 27 15 962 4749.

Africa and patients are vulnerable to several infections termed opportunistic infections. *S. aureus* as an opportunistic pathogen in AIDS patients lead to high morbidity and mortality among these patients (Chacko et al., 2009).

Since 1954, *S. aureus* was suspected to cause anti-biotic associated diarrhoea (AAD). Now there is enough data available on the occurrence of *methicillin-resistant S. aureus* (*MRSA*) in stool of patients with AAD and as a major cause of nosocomial diarrhoea (Baba-Moussa et al., 2010; Gravet et al, 1999), although many cases remain undiagnosed (Flemming, 2007). *S. aureus* is one of the causative agents of urinary tract infections (UTI) and is often isolated from urine samples obtained from long-term care patients (Muder et al., 2002). The prevalence of *S. aureus* is reported to be higher in developing countries particularly in West Africa (Baba-Moussa et al., 2008). Immunocompromised patients and those with indwelling catheter have high incidence of methicillin-*resistant S. aureus* (*MRSA*) -induced UTI. *S. aureus* is responsible for a wide variety of diseases ranging in severity from slight skin infection, to more severe diseases such as, osteomyelitis, bacteraemia and septicaemia through the production of virulence factors which can cause particular diseases (Lowy, 1998). Example of toxins include, toxic shock syndrome toxin-1 (TSST-1), which causes toxic shock syndrome (TSS), and staphylococcal enterotoxins (SEs) (SEA, SEB, SEC, etc.), which cause food poisoning (Mehrotra et al., 2000).

*S. aureus* has been identified as one of the most common causes of urinary tract infection diagnosed in outpatients and hospitalised patients (Shigemura et al., 2005). The urinary track infection can also lead to secondary infection of bacteraemia (Huggan et al*.,* 2008). The incidence of MRSA-induced urinary tract infection (UTI) is getting higher these days, especially for inpatients with an indwelling urinary catheter or those who are immunocompromised. In these patients, cross-infection might be involved in spreading MRSA in the ward, so that molecular epidemiological study plays an important role in the investigation and control of outbreaks (Baba-Moussa et al*.,* 2008). It has been reported that MRSA isolates from urine rarely caused serious infectious symptoms, but MRSA can be the cause of bacteremia and can be fatal. On the other hand, no clear mechanism has been elucidated about pathogenicity in MRSA-induced UTI (Araki et al*.,* 2002).

Numerous outbreak of food poisoning have been reported and *S. aureus* was identified as the causative agent (Bremer et al., 2004). *S. aureus* can arise from contaminated water resulting to food poisoning in both immunocompromised and immunocompetent individuals. In drinking water *S. aureus* may also serve as a source for colonizing residents exposed to contaminated water (Lechevallier and Seidler, 1980).

The pathgenicity of *S. aureus* is due to its ability to encode enterotoxin, exotoxin, leukocidins and leukotoxin which damage the host cells (Dinges et al., 2000). Six *staphylococcal* enterotoxin (SE) groups have been identified according to serological classification, which are the main cause of intensive intestinal peristalsis and food poisoning when release onto the food (Mehrotra et al*.,* 2000). Biochemical properties such as carotenoids, catalase production enhance the survival of *S. aureus* in phagocytes. Membrane-damaging toxins that lyse eukaryotic cell membrane such as hemolysins, leukotoxin and leukocidin have been described in *S. aureus* (Baba-Moussa et al*.,* 1999). Exotoxins that damage host tissues and trigger symptoms of disease (*Staphylococcus* enterotoxin A-G, toxic shock syndrome toxin (TSST), exfoliative toxin (ET) have also been documented (Mehrotra et al*.,* 2000). The objectives of this study were to detect gene(s) associated with methicillin resistance and pathogenesis of *S. aureus* using multiplex polymerase chain reaction (PCR), to detect methicillin resistant *S. aureus* among the isolates using the agar method and to determine the possible relationship between the different pathogenic features and the genetic characteristics of *S. aureus* isolated from HIV positive patients in the Limpopo Province.

## MATERIALS AND METHODS

### Ethical considerations

The study was approved by 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 and their right to say no to participate in the 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. The patients in the community were also requested to sign a consent form after the study has been explained to them. Whenever possible, different samples including sputum, urine, mouth wash and stools were collected. Water samples were collected from the households of patients from whom clinical samples have been collected and who have allowed the research team to visit them at home.

### Bacterial isolation and identification

The different samples were transported to the laboratory within 4 hours of collection and were inoculated onto freshly prepared mannitol salt agar and incubated at 35°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 35°C for 24 h. The nutrient agar plates were then stored in the fridge for further analysis. The haemolytic activities of the isolates were determined using the standard blood agar plate as previously described (Mueller and Whitman, 1931).

### Molecular analysis of the isolates

Molecular methods were used to confirm the identity of *S. aureus*,

**Table 1.** List of primers used in the study for the specific detection and identification of *S. aureus* entrerotoxic genes and Mec A gene (Mehrotra et al., 2000).

| Gene | Primer name | Oligonucleotide sequence(5'-3') | size of amplified product |
|---|---|---|---|
| SEA | GSEAR-1 | GGTTATCAATGTGCGGGTGG | 102 |
| | GSEAR-2 | CGGCACTTTTTTCTCTTCGG | |
| SEB | GSEBR-1 | GTATGGTGGTGTAACTGAGC | 164 |
| | GSEBR-2 | CCAAATAGTGACGAGTTAGG | |
| SEC | GSECR-1 | AGATGAAGTAGTTGATGTGTATGG | 451 |
| | GSECR-2 | CACACTTTTAGAATCAACCG | |
| SED | GSEDR-1 | CCAATAATAGGAGAAAATAAAAG | 278 |
| | GSEDR-2 | ATTGGTATTTTTTTTCGTTC | |
| SEE | GSEER-1 | AGGTTTTTTCACAGGTCATCC | 209 |
| | GSEER-2 | CTTTTTTTTCTTCGGTCAATC | |
| mecA | GMECAR-1 | ACTGCTATCCACCCTCAAAC | 163 |
| | GMECAR-2 | CTGGTGAAGTTGTAATCTGG | |

test for the presence of genes associated with pathogenicity of the isolates and to determine the presence of *mec* A responsible for the methicillin resistance phenotype of certain isolates. A subset of 50 isolates was randomly selected for the detection of pathogenic genes.

**DNA purification from the *S. aureus* isolates**

DNA was extracted using the protocol described by Kumar et al. (2008) with some slight modifications. In brief, two loop full of microorganisms from overnight culture of *S. aureus* were re-suspended in 1 ml of sterile distilled water and phenol-chloroform-isoamyl (25:24:1) alcohol was used. Two hundred µl of TE buffer was to resuspend the DNA. To check the quality of DNA, samples were run on 1% agarose gel. The DNA was stored in the freezer at -20°C until further analysis.

**Polymerase chain reaction for identification of *S. aureus***

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 suspected to be a *S. aureus*. The PCR reaction was done according to Jimenez et al. (2008) with some slight modifications. The Dream Taq master mix (Fermenters, Burlington, Canada), was used in a final volume of 25 µl in the PCR reaction tubes. 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.

**Detection of methicillin-resistant *S. 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 onto 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.

**Detection of *mec* A gene by PCR**

Out of the 140 isolates, 131 were used for the detection of *mec* A gene, since some of the cultures died out during subculturing process. A protocol previously described by Mehrotra et al. (2000), with slight modifications was used for the detection of *mec* A gene. The primers used are listed in Table 1. The reaction mixture was composed of 12.5 µl Dream Taq, 0.5 µl of each primer and 2 µl of the DNA template. The volume of the mixture was adjusted to 25 µl with nuclease free water. The PCR conditions were initial denaturation at 94°C for 5 min, followed by 35 cycles amplification with (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, extension at 72°C for 1 min) and final extension at 72°C for 7 min. The PCR amplicons were observed using 2% agarose gel electrophoresis stained with ethidium bromide.

**Detection of pathogenic genes from the *S. aureus* isolates**

Out of the 140 isolates, 50 were randomly selected for further molecular analysis. All the 140 isolates were confirmed to be *S. aureus* by the *tuf* gene. A multiplex PCR protocol previously described by Mehrotra et al. (2000), with slight modifications was used for the detection of *S. aureus* pathogenic genes including the *Staphylococcu*s enterotoxin gene A, B, C, D and E. All the primers used are listed in the Table 1. The reaction mixture was composed of 12.5 µl dream taq, 0.5 µl of each primer and 2 µl of the DNA template. The volume of the mixture was adjusted to 25 µl with 1.5 µl nuclease free water. The PCR conditions were initial denaturation at 94°C for 5 min, followed by 35 cycles amplification with (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, extension at 72°C for 1 min) and final extension at 72°C for 7 min. The PCR amplicons were observed using 2% agarose gel electrophoresis stained with ethidium bromide.

**Statistical analysis**

All data was entered in Microsoft Excel sheet. The analysis was

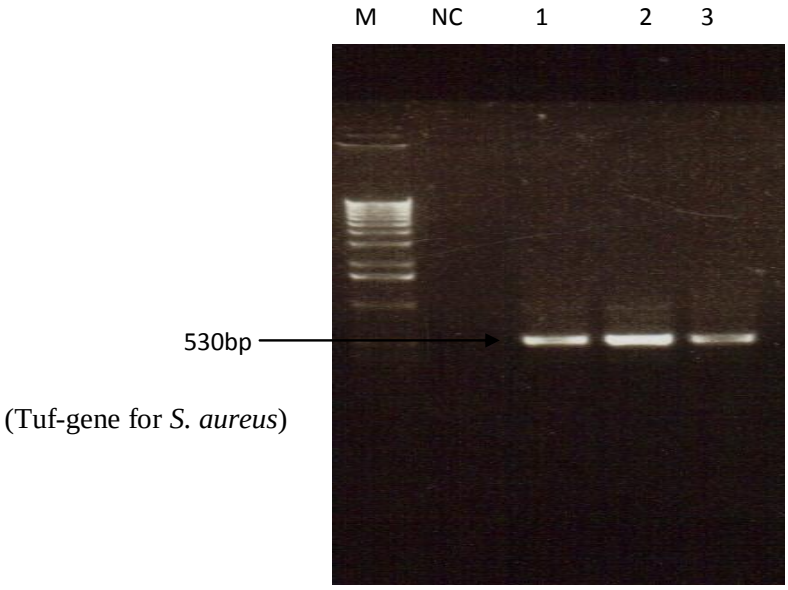


**Figure 1.** Agarose gel electrophoresis pattern showing 530 bp of the amplification product for *S.aureus tuf*- gene. Lanes: M= DNA molecular maker (1000 bp ladder (Marker X); NC = Negative control; lanes 1 to 4 = *tuf*-gene positive amplicon products.

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 the present study

A total of 140 strains of *S. aureus* were isolated from the different types of samples including 480 clinical samples and 220 water samples. Of the 140 isolates, 48 (34%) were from urine, 60 (43%) from stools and 15 (11%) from sputum. The remaining 17 (12%) isolates were from water samples. Sixty-five (65) isolates were from females and 33 were from males.

### DNA isolation and species identification by PCR

DNA was isolated from the isolates, and the PCR using *tuf*-gene produced band of 530bp indicating that all isolates were *S. aureus* (Figure 1).

### Methicillin resistance: Phenotypic analysis and PCR detection of *mec* A gene

After amplification of the *mecA* gene by PCR, the expected band of 163 bp was obtained for the positive samples (Figure 2). A total of 131 isolates tested with PCR were also tested by the oxacillin agar method for MRSA. When using the oxacillin agar test, 18 (14%) of the 131 tested isolates were positive for MRSA compared to 14 (11%) for the *mecA* gene with the PCR. Thirteen (13) of the *mec* A positive isolates were among the 18 positive with the agar methods, while the remaining isolates were negative by both the agar and the PCR methods. Table 2 shows the overall results obtained by both methods. In the present study, the results of the oxacillin agar method were used for further analysis based on its higher sensitivity.

### Prevalence of methicillin resistance by sample origin and sex distribution

There was no difference in the overall prevalence of MRSA among male and female participants in this study. From 63 isolates obtained from females 8 (12.7%) were MRSA positive, while 4 (12.9%) were positive among the 31 isolates from males. MRSA was higher among the sputum isolates 4 (27%) compared to 7 (15.5%) and 7 (12%) from urine and stool isolates. None of the water isolates was positive for MRSA.

### Methicillin resistance and other antibiotic susceptibility profile

From the 18 MRSA strains, higher resistance was observed to the penicillin (100%) and ampicillin (100%) resistance compared to MSSA with (90.5%) and (88%) to

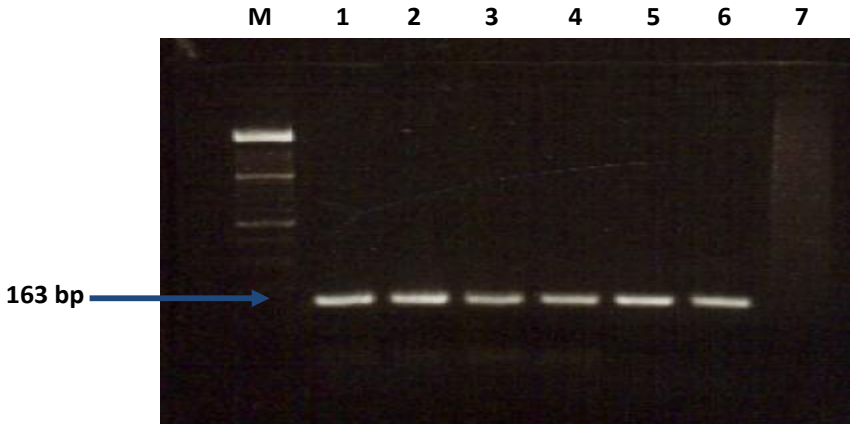


**Figure 2.** Agarose gel electrophoresis pattern showing 163 bp of the amplification product for *S. aureus mec*A gene. Lanes: M, DNA Molecular marker (100 bp ladder); lanes 1 to 7 MecA positive amplicon product; lane 8 MecA negative isolate.

**Table 2.** Comparison of the two MRSA test methods used.

| Oxacillin agar method | *mec* A gene detection method by PCR | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Negative | 112 | 1 | 113 |
| Positive | 5 | 13 | 18 |
| Total | 127 | 14 | 131 |

**Table 3.** Correlation between MRSA and MSSA distribution and resistance to other antibiotics.

| Antibiotics (µg) | Resistant among the MRSA (n=18) (%) | Resistant among the MSSA (n=113) (%) |
|---|---|---|
| Ampicillin 10 | 16(100) | 96(90.5) |
| Amoxicillin 10 | 17(94) | 104 (92) |
| Penicillin10 | 18(100) | 100 (88) |
| Kanamycin 15 | 5(28) | 33(29) |
| Streptomycin 10 | 4(22) | 22(19) |
| Cefepime 30 | 10(62.5) | 84 (79) |
| Cefriaxone30 | 8(44) | 75(66) |
| Meropenem10 | 3(17) | 22(19) |
| Ciprofloxacin 15 | 3(19) | 11(10) |
| Vancomycin 30 | 5(28) | 15(13) |
| Erythromycin15 | 10(55.5) | 61(54) |
| Chloramphenicol 30 | 2(11) | 18(16) |
| Tetracycline30 ) | 11(61) | 73(65) |
| (Polymoxycin 300 ) | 5(28) | 48(42) |

Only 16 MRSA and 106 MSSA isolates were tested against Ampicillin, Cefepime and Ciprofloxacin.

both antibiotics respectively. There was no significant difference in the resistant of the MRSA and MSSA isolates to most tested antibiotics. MRSA isolates showed high susceptibility to chloramphenicol (89%) and meropenem (84%) compared to 85 and 81% among the MSSA to the same antibiotics. Methicillin resistant strains were more commonly resistant to vancomycin (17%) compare to MSSA (11%). Table 3 shows the overall susceptibility of MRSA and MSSA to other antibiotics. There was 89% multiple drug resistance within the MRSA positive strains. MRSA isolate had high MDR to 4 (22%) and 10 (11%) antibiotics compared to MSSA with 10 and

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

| MRSA agar | MecA gene | Origin | Biofilm | Blac | Antibiotic resistance profiles | Path genes | Hemolysis |
|---|---|---|---|---|---|---|---|
| Pos | Neg | Sputum | Pos | Neg | AMP, Cro, E, PB | | β |
| Pos | Neg | Sputum | Neg | Neg | AMP, AMI, P | | β |
| Pos | Pos | Urine | Pos | Neg | AMI, P | SEA | α |
| Pos | Neg | Urine | Neg | Neg | AMP, AMI, P, S, Fep, E, T, PB | | β |
| Pos | Pos | Urine | Pos | Neg | AMP, AMI, P, Cro | | α |
| Pos | Pos | Urine | Pos | Neg | AMP, AMI, P, Fep, T | | γ |
| Pos | Pos | Urine | Pos | Neg | AMP, AMI, P, Fep, T | | α |
| Pos | Pos | Urine | Neg | Neg | AMP, AMI, P, K, Fep, Cro, MEM, E, T, PB | | β |
| Pos | Pos | Sputum | Pos | Pos | AMP, AMI, P, T, PB, Fep, Cro | | α |
| Pos | Pos | Sputum | Neg | Neg | AMP, AMI, P, Fep, T | | β |
| Pos | Pos | Urine | Pos | Pos | AMP, AMI, P, Fep, Cro, Cip | SEA | β |
| Pos | Pos | Stool | Pos | Pos | AMP, AMI, P, K, Fep, MEM, E, T | | β |
| Pos | Pos | Stool | Pos | Pos | AMP, AMI, P, Cro, MEM, Cip, VA, E, T | | γ |
| Pos | Neg | Stool | Neg | Pos | AMP, AMI, P, K, S, Fep, Cip, VA, E, C, T | | β |
| Pos | Pos | Stool | Neg | Neg | AMP, AMI, P, T, PB, Fep, Cro | | β |
| Pos | Pos | Stool | Neg | Pos | AMI, P, K, VA, E, T | | α |
| Pos | Pos | Stool | Pos | Pos | AMP, AMI, P, VA, E, T | | β |
| Pos | Neg | Stool | Pos | Pos | AMP, AMI, P, K, S, VA, Cro, E, C, T | | α |
| Neg | Pos | Stool | Neg | Neg | AMP, AMI, Fep, K, MEM, E, T | | α |

18% MDR to the same antibiotics. Table 4 shows some characteristics of MRSA positive samples identified in HIV patients in the Limpopo province. There was no difference in terms of beta lactamase production among the MRSA (38%) and MSSA (40%) isolates.

## Pathogenic genes detection

Out of the 140 isolates, 50 were randomly selected and tested for the presence of pathogenic genes (staphylococcal enterotoxin genes (SE)). From the 50 isolates tested for the presence of staphylococcal enterotoxin genes, *Staphylococcus* enterotoxin A (SEA) was detected in 12(24%), of all tested isolates, while *Staphylococcus* enterotoxin D (SED) was detected in 3(6%) of the isolates. Among the 12 and 3 isolates harbouring SEA and SED, 2 (4%) harboured both SEA and SED together. SEB, SEC, SEE were not detected among the isolates tested (Figure 3).

Among the 50 isolates selected, thirteen 13 isolates were from males and 26 isolates were from females respectively. The gene distribution were 6 (46%) from males and 3 (11.5%) in females ($\chi^2$ = 3.938; p = 0.047). Out of the 23 isolates from urine, 8 (35%) were positive for SEA alone, while 1(5%) isolate was positive for both SEA and SED together (OR = 2240; CI: 0.61 – 8.11; p = 0.18). Among the 20 isolates from stools 1(5%) was positive for SEA alone, 1(5%) positive for SED alone, while 1 was positive for both SEA and SED together. From the 7 sputum isolates 1(14%) was positive for SEA, while none was positive for SED (Table 5).

Resistance to most of the antibiotics tested was similar among the isolates positive for pathogenic gene and those negative for pathogenic genes. While 92% of the positive isolates were resistant to amoxicillin, 92% of the negative isolates were also resistant. However, Isolates harbouring pathogenic genes were more resistant to meropenem (31%) and streptomycin 31% compared to the negative isolates (22 and 24% to the same antibiotics respectively).

Isolates positive for pathogenic genes showed higher rate of beta lactamase production (46.2%) compared to the negative isolates (22.2%); ($\chi^2$ = 2.680; p =0.102). Out of the 13 isolates that carried pathogenic genes, 15% were strong biofilm producers, compared to 11% among the isolates negative for pathogenic genes (P = 0.51).

## Impact of biofilm production on beta-lactamase and methicillin resistance

Out of the 14 strong producers 9(64.3%) were beta-lactamase positive compared to 40 and 34.2% of the moderate and non-producers respectively ($\chi^2$ = 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 ($\chi^2$ = 6.964; p = 0.021).

**Table 5.** Distribution of pathogenic genes from each sample source.

| Genes | Sputum (n=7) | Stool (n=20) | Urine (n=23) | Total (n=50) |
|---|---|---|---|---|
| SEA | 1(14) | 1(5) | 8(35) | 10(20) |
| SED | 0 | 1(5) | 0 | 1(2) |
| SEA& SED | 0 | 1(5) | 1(5) | 2(4) |
| Total with genes | 1 | 3 | 9 | 13(26) |

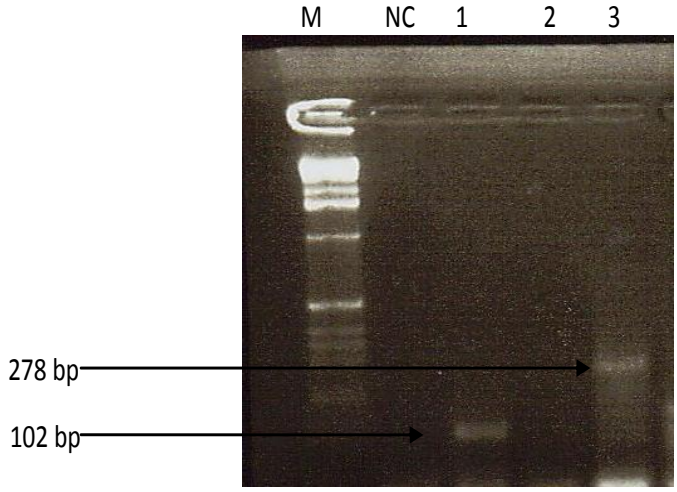


**Figure 3.** Agarose gel electrophoresis pattern showing 102 bp of the amplification product for *S. aureus* Enterotoxin genes. Lanes: M, DNA Molecular marker (Marker X); lanes NC, negative control; lane 1, 102 bp SEA amplicon product; lane 2, negative sample; 3, both 102 and 278 SEA and SED amplicon products.

## 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 patients with reduced immunity such as those undergoing chemotherapy or surgery, children, elders and patients with HIV and AIDS. *S. aureus* is an important cause of both community and hospital acquired infections resulting in high morbidity and mortality in tropical Africa (Awolude et al., 2010; van Rensburg et al., 2011). Apparently there is little data available in South Africa about this pathogen particularly among HIV and AIDS patients. Understanding the epidemiology of this organism as well as the potential pathogenicity of the infecting strains is important for the design control strategies for the concerned patients.

*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). However, laboratories often use PCR detection of the *mec A* gene, and an oxacillin MIC of ≥4 µg/ml, as alternative criteria for identifying MRSA. Our study could identify using the oxacillin agar methods 18 (14%) MRSA, of which 4 were negative for *mec* A gene. These findings are similar to those obtained by Essa et al (2009), in Kwazulu-Natal, where phenotypic methods could detect some MRSA for which the PCR was negative. Strains that were MRSA positive by the oxacillin agar screen test but negative by PCR for the *mec*A gene could have other mechanisms of methicillin resistance such as hyper production of β lactamase or modified penicillin-binding proteins (PBP) reactivities (MOD strains). Also the possibility of *mecA* mutation that could have prevented primer binding cannot be ruled out.

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). Factors such as overcrowding in big cities might play an important role in the transmission of MRSA as compared to our site which is more rural. Hundred (100%) susceptibility of MRSA isolates from South Africa to Vancomycin have been reported from other studies (Marais et al., 2009). However, in our study it was not the case, we identified vancomycin resistance rate of 14% among the MRSA strains, which is very high compared to those found by Klugman et al. (1998) and Sadaka et al. (2009). From this finding it is clear that serious attention should be given to the use of antibiotics by HIV/AIDS patients for the treatment of opportunistic pathogens. The difference between our findings and those of Klugman et al. (1998) and Sadak et al. (2009) might also be due to the type and characteristics of patients from whom the samples have been collected. Although no study has previously described vancomycin resistance in South Africa, several studies around the world have indicated a steady increase of vancomycin intermediate as well as vancomycin resistant *S. aureus* (Tiwari and Sen, 2006). A study in India by Poonam et al. (2010); indicated a steady increase of vancomycin resistant *S. aureus* among hospitalized patients which agrees with our study. Although we did not investigate the possible origin of such resistance, it is necessary to control the use of antibiotics in the hospitals.

The results of multiplex PCR showed that the production of enterotoxins genes, which play a major role in the pathogenecity of *S. aureus,* was low except for SEA which was harboured by 20% of the isolates. These results are similar to those reported by (Baba-Moussa et al., 2008; Naffa et al., 2006). Most of the urine sample

harboured the SEA which suggests the importance of *S. aureus* in the pathogenesis of urinary tract infections in HIV and AIDS patients in the Limpopo Province. There was a significant difference between sample origin and production of staphylococcal enterotoxin A with a p value (p = 0.042), and no significant different with SED p value (p = 0.743). In study by Asha et al. (2006), out of 10 *S. aureus* isolated from antibiotic associated diarrheal cases, diarrheal stool samples, five *S. aureus* isolates were enterotoxin C producers, one produced enterotoxins C and D while two produced enterotoxin A and toxic shock syndrome toxin 1, and two were nonentero-toxigenic. None of the *S. aureus* isolates produced detectable Panton-Valentine leucocidin, LukE-LukD, LukM-LukF′-PV-like, or exfoliative toxins A and B. We did not investigate the presence of the Panton–Valentine leucocidin gene in this study; however, the distribution of the different genes tested was different from that of Asha and colleagues. Further studies with larger numbers of isolates testing more genes are therefore recommended.

Fifty-four percent biofilm producers harboured pathogenic genes as compared to 46% of non producers. Only SEA and SED could be detected in all isolates, with high predominance of SEA. These might suggest a good correlation between biofilm and the presence of SEA. The high dominance of *mec* A gene in the strong producer (28.6%) as compared to 5.5% of non producers also suggests a strong link between biofilm and antibiotic resistance. There was also a good correlation between biofilm production and MRSA (p = 0.039). All these findings are in agreement with those found by Kwon et al. (2008) who indicated that biofilm producers are more likely to be MRSA as compared to non producers (p = 0.05). However, other authors have described opposite findings (Smith et al., 2008), where there was no correlation between methicillin resistance and the ability to form biofilm (P = 0.77). In a study by Mertz et al. (2007), a higher than expected percentage of *S. aureus* capable of producing the exfoliative toxins A and or B (ETA and or ETB) and Panton-valentine leukocidin (PVL) was seen in all skin lesions infected with *S aureus.* This study showed that it is possible that toxin producing isolates are pathogenic and able to cause diseases in the infected patients.

## Conclusions

Most *S. aureus* strains infecting HIV and AIDS patients in the Limpopo Province carry the *Staphylococcus* enterotoxin A. Males might be more susceptible to pathogenic *S. Aureus* compared to females. *S. Aureus* seems to be more involved in the urinary tract infections among HIV and AIDS patients or not involved in pneumonia or diarrhea. Also, water does not seem to be a transmission factor for pathogenic *S. Aureus* since no pathogenic strain was found in the water samples. 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 South Africa.

## ACKNOWLEDGEMENTS

The authors are grateful to the different following hospitals Thsilidzini, Donald Fraser and ELIM staff and patients. Authors are also grateful to the Department of Health in Polokwane. The present study was partially funded by a grant from the National Research Foundation. Furthermore authors are grateful to Dr Samie and other funding came from the University of Venda Research and Publication committee.

### REFERENCES

Almer LS, Shortridgea VD, Niliusa AM, Beyera JM, Sonia N, Buia HM, Stoneb GG, Flamma RK (2002). Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis., 43 (3):225-232.

Araki M, Kariyama R, Monden K, Tsugawa M, Kumon M (2002). Molecular epidemiological studies of *Staphylococcus aureus* in urinary tract infection. J. Infect. Chemother., 8 (2):168–174.

Asha NJ, Tompkins D, Wilcox MH (2006). Comparative Analysis of Prevalence, Risk Factors, and Molecular Epidemiology of Antibiotic-Associated Diarrhea Due to *Clostridium difficile*, *Clostridium perfringens*, and *Staphylococcus aureus.* J. Clin. Microbiol., 44 (8): 2785–2791.

Awolude OA, Adesina OA, Oladokun A, Mutiu WB, Adewole IF (2010). Asymptomatic bacteriuria among HIV positive pregnant women. Virulence, 1(3):130-3.

Baba-Moussa L, Werner S, Colin DA, Mourey L, Pédelacq JD, Samama JP, Sanni A, Monteil H, Prévost G (1999). Discoupling the Ca (2+)-activation from the pore-forming function of the bi-component Panton-Valentine leucocidin in human PMNs. FEBS Lett., 461(3):280-6

Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Riegel P, Haï€ıkou N, Hounsou F, Monteil H, Sanni A, Prée´vost G (2008). Virulence factors produced by strains of *Staphylococcus aureus* isolated from urinary tract infections. J. Hosp. Infect., 68 (1):32-38.

Baba-Moussa L, Ahissou H, Azokpota P, Assogba B, Atindéhou M, Anagonou S, Keller D, Sanni A Prévost G (2010). Toxins and adhesion factors associated with *Staphylococcus aureus* strains isolated from diarrheal patients in Benin. Afr. J. Biotech., 9 (5): 604-611.

Bremer PJ, Fletcher GC, Osborne C (2004). *Staphylococcus aureus*. New Zealand institute for crop and Food research Limited. http://www.crop.crinz/home/research/marine/Staphylococcus.pdf Accessed: 06 June 2009.

Chacko J, Kuruvila M, Bhat AK (2009). Factors affecting the nasal carriage of MRSA in human immunodeficiency Virus- infected patients. Indian J. Med. Microbiol., 2525 (2):146-148.

Dinges MM, Orwin PM, Schlievert PM (2000). Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev., 13 (1):16-34.

Essa ZI, Connolly C, Essack SY (2009). *Staphylococcus aureus* from public hospitals in KwaZulu-Natal, South Africa – infection detection and strain-typing. S. Afr. J. Epidemiol. Infect., 24(1):04-07.

Flemming K, Ackermann G (2007). Prevalence of Enterotoxin Producing *Staphylococcus aureus* in Stools of Patients with Nosocomial Diarrhea. Infection, 35 (5): 356–358.

Gravet A, Rondeau M, Harf- Monteil C, Grunenberger F, Monteil H, Scheftel JM, Prevost G (1999),. Predominant *Staphylococcus aureus* isolated from antibiotic-associated diarrhea is clinically relevant and produces enterotoxin A and the bicomponent toxin LukE-LukD. J. Clin. Microbiol., 37 (12): 4012-4019

Huggan PJ, Murdoch DR, Gallagher K, Chambers ST (2008). Concomitant *Staphylococcus aureus* bacteriuria is associated with poor clinical outcome in adults with *S. aureus* bacteraemia. J. Hosp. Infect., 69: 345-349.

Jimenez E, Delgado S, Fernandez L, Garcia N, Albujar M, Gomez A, Rodriguez JM (2008). Assessment of the bacterial diversity of human colostrums and screening of *Staphylococcal* and *enterococcal* population for potential virulence factors. Res. Microbiol., 159 (9-10):595-601.

Klugman KP (1998). Emerging Infectious Diseases—South Africa. *The* South African Institute for Medical Research Johannesburg, South Africa; 4 (4). URL:http://www.cdc.gov/ncidod/eid/vol4no4/klugman.htm. Accessed: 08 November 2009.

Kumar R, Yadav BR, Dev K Singh RS (2008). A Simple Protocol for DNA Extraction from *Staphylococcus aureus.* http://www.protocol Online .org. Accessed 30 June 2009.

Kwon AS, Park GC, Yeon Ryu S, Hoon Lim D, Yoon Lim D, Hee Choi C, Yong Lim (2008). Higher biofilm formation in multidrug-resistant clinical isolates of *Staphylococcus aureus*. Int. J. Antimicrob. Agents, 32(1):68-72.

Lechevaller MW, Seidler R (1980). *Staphylococcus aureus* in Rural Drinking water. Appl. Environ. Microbiol., 3039(4):*739*-742.

Lowy FD (1998). *Staphylococcus aureus* infections. N. Engl. J. Med., 339(8): 520-532.

Marais E, Aithma N, Perovic O, Osthuysen WF, Musenge E, DuséA G (2009). Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from South Africa. S. Afr. Med. J., 99(3):170-173.

Mehrotra M, Wang G, Johnson WM (2000). Multiplex PCR for detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic shock syndrome Toxin 1, and Methicillin Resistance. J. Clin. Microbiol., 38(3):1032-1035.

Mertz PA, Cardenas TCP, Snyder RV, Kinney MA, Davis SC, Plano LRW (2007). *Staphylococcus aureus* Virulence Factors Associated with Infected Skin Lesions Influence on the Local Immune Response. Arch. Dermatol., 143(10): 1259 – 1263.

Moussa IM, Al-Qahtani AA, Gassem MA, Ashgan MH, Dalia KI, Ghazy AI, Shibl AM (2011). Pulsed-field gel electrophoresis (PFGE) as an epidemiological marker for typing of methicillin resistant *Staphylococcus aureus* recovered from King Saudi Arabia KSA. Afr. J. Microbiol. Res., 5(12): 1492-1499.

Muder RR, Brennen C, Rihs JD, Wagener MM, Obman A, Stout JE, Yu VL (2002). Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. Clin. Infect. Dis., 15: 1504-150542(1): 46 – 50..

Mueller JH, Whitman L (1931). An Improved Method for the Detection of Hemolytic *Streptococcus* Carriers. J. Bacteriol., 21(3): 219–223.

Naffa RG, Bdour SM, MIgdadi HM, Shehabi AA (2006). Enterotoxicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan. J. Med. Microbiol., 55(Pt2):183-187.

Poonam SL, Taneja J, Bibhabati M (2010). Methicillin and vancomycin resistant *S. aureus* among hospitalized patients. J. Global Infect. Dis., 2 (3): 275 – 283.

Sadaka SM, El-Ghazzawy EF, Harfoush RA, Meheissen MA (2009). Evaluation of different methods for the rapid diagnosis of methicillin-resistance in *Staphylococcus aureus*. Afr. J. Microbiol. Res., 3(2): 049-055.

Shigemura K, Tanaka K, Okada H, Nakano Y, Kinoshita S, Gotoh A, Arakawa S, Fujisawa M (2005). Pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during a 20-year period (1983-2002) at a single institution in Japan. Japan Jpn. J. Infect. Dis., 58(5):303-308.

Shittu AO, Lin J (2006). Antimicrobial susceptibility pattern and characterization of clinical isolate of *S. aureus* in Kwazulu-Natal, South Africa, BMC Infect. Dis., 6: doi:10.1186/1471-2334-6-125.

Smith K, Perez A, Ramage G, Lappin D, Gemmell G, Lan GS (2008). Biofilm formation by Scottish clinical isolates of *Staphylococcus aureus*. J. Med. Microbiol., 57(Pt8): 1018–1023.

Tiwari HK, Sen MR (2006). Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis., 6:156 doi:10.1186/1471-2334-6-156.

van Rensburg MJ, Eliya Madikane V, Whitelaw A, Chachage M, Haffejee S, Gay Elisha B (2011). The dominant methicillin-resistant *Staphylococcus aureus* clone from hospitals in Cape Town has an unusual genotype: ST612. Clin. Microbiol. Infect., 17(5):785-92.

Zetola N, Francis JS, Nuermberger EL, Bishai WR (2005). Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect. Dis., 5(5): 275–286.