# Vancomycin Resistance in *Staphylococcus aureus* and Enterococcus Species Isolated at the University Teaching Hospital, Lusaka, Zambia: Should We Be Worried?

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## Abstract

**Background**: *Staphylococcus aureus* and Enterococcus species cause invasive infections such as bacteremia and infective endocarditis. Despite vancomycin being the drug of choice for drug-resistant infections caused by these species, few studies have been conducted to ascertain vancomycin resistance in the African setting. This is crucial given the rising resistance in these organisms. This study aimed at isolating *S. aureus* and Enterococcus species and determine their susceptibility to vancomycin and other antibiotics at the University Teaching Hospital in Lusaka, Zambia.

**Methods**: This was a cross-sectional study in which *S. aureus* and Enterococcus spp isolates from the skin, soft tissue and bloodstream infections were included. Standard microbiological and Kirby-Baur (disc diffusion and E-strips) methods were used to identify and determine the susceptibility of the organisms, respectively.

**Results**: From fifty-nine *S. aureus* isolates, thirty-seven were from the skin and soft tissue and twenty-two from blood culture. Twenty-six (44.1%) of these were Methicillin-resistant *S. aureus*. Thirty-nine Enterococcus were isolated from blood cultures only. Of the *S. aureus* [16] and Enterococcus [14] isolates tested with vancomycin E-strips, none were vancomycin-resistant. However, 12.5 per cent *S. aureus* and 14.3 per cent Enterococcus showed intermediate vancomycin susceptibility. *S. aureus* were resistant to penicillin (93.2%), erythromycin (52.5%) and tetracycline (50.8%). Enterococcus showed resistance to penicillin (83%) and tetracycline (84.6%).

**Conclusions**: There was no vancomycin resistance among *S. aureus* and Enterococcus, implying vancomycin is still a viable treatment option for invasive infections.

Given the intermediate vancomycin susceptibility, treatment guided by minimum inhibitory concentration results, continued surveillance and prudent use are key.

**Keywords:** *Enterococcus; Staphylococcus aureus; vancomycin resistance; Zambia*

## Introduction

Vancomycin, a glycopeptide antibiotic, has been the drug of choice for treatment of infections caused by resistant Gram-positive bacteria namely Enterococcus species (spp) and Methicillin-resistant *Staphylococcus aureus* (MRSA) including bacteremia and infective endocarditis [1].

However, resistance to vancomycin by these two organisms has been recorded in the recent past across the globe [2]. Enterococcus with a minimum inhibition concentration [3] of 8-16μg/ml are considered as intermediate and MIC of ≥32μg/ml are classified as Vancomycin Resistant Enterococci [4], while *S. aureus* isolates with MICs of 4-8μg/ml are termed as Vancomycin-Intermediate *S. aureus* (VISA), and those with an MIC of ≥16μg/ml are termed as Vancomycin-Resistant (VRSA) [1]. First encountered in about 1986, VRE have since emerged as important nosocomial pathogens in the last two decades throughout the world, leading to clinical treatment failures [5]. In Europe, the rise of VRE was principally in the community settings, due to transmission from animal food products to humans. It was thought to arise from using a glycopeptide antibiotic avoparcin as a growth promoter in livestock [6]. Conversely, in the US, the predominance of VRE was in the hospital setting, probably due to the increased use of the glycopeptide antibiotic vancomycin [7]. Extensive use of vancomycin to treat infections with MRSA has led to decreased susceptibility to vancomycin among *S. aureus* [8]. An MRSA isolate with decreased susceptibility to vancomycin was first reported in Japan in 1997 [9]. The isolate had only a modestly increased MIC value for vancomycin, in the range of 3–8μg/ml, and became known as vancomycin intermediate-resistant *S. aureus* (VISA). VISA isolates do not carry imported foreign genetic elements; rather, the increased vancomycin MIC values are related to mutations that appear in the invading of the pathogen during vancomycin use in vivo. Very limited options are currently available for treating severe infections caused by VRE and VRSA [1].

According to the World Health Organisation [10] African region, information concerning Antimicrobial Resistance (AMR) in Africa is limited with a scarcity of accurate and reliable data because surveillance of AMR is carried out in only few countries [11]. In Zambia, published data on AMR is rare despite antimicrobial usage and resistance in hospitals being widely spread. The recent studies conducted on *S. aureus* and MRSA isolates the at University Teaching Hospitals showed *S. aureus* isolation rates of 17.1 per cent among health care workers (hand and nasal carriage), [12] 17.8 per cent from lab coats worn by health care workers, [13] 30 per cent and 40 per cent from burns and bloodstream infections respectively [14]. At the same time, MRSA rates ranged from 43 per cent and 68 per cent [13] among the *S. aureus* isolates. One of these studies revealed a high prevalence of antimicrobial resistance, including multidrug resistance in the MRSA isolates [15]. There are relatively even fewer published studies on Enterococcus spp in Zambia. Until this study, there was no published study

on clinical Enterococcus spp. However, one study conducted in Kafue district, on cattle faecal samples, showed the presence of Enterococcus spp with high antimicrobial resistance to gentamycin, amoxycillin, ampicillin and tetracycline but all were susceptible to vancomycin. Regrettably, there are no comprehensive studies on vancomycin [3] resistance in clinical *S. aureus* and Enterococcus spp isolates in Zambia. Therefore, this study aimed at determining the susceptibility to vancomycin and other antibiotics including β-lactams, tetracyclines, macrolides and fluoroquinolones of *S. aureus* and Enterococcus spp isolates from blood, and skin and soft tissue infections at the University Teaching Hospital in Lusaka, Zambia.

## Materials and Methods

### Study Design and Site

The study was a cross-sectional study from April 2018 to July 2019 at the University Teaching Hospitals, in Lusaka, Zambia. The University Teaching Hospitals is the largest tertiary care and teaching hospital located in Lusaka, the capital city of Zambia. The hospital has a bed capacity of approximately 2000 and offers specialised care to millions of residents from the different provinces of the country. It also hosts the largest microbiological diagnostic centre in the country.

### Study Frame and Sample Size

*S. aureus* from the skin, soft tissue specimens and blood culture specimen and Enterococcus spp isolated from blood culture specimen routinely submitted to the Microbiology Laboratory of the University Teaching Hospital were used in this study. On average, the laboratory received 261 pus and 299 blood samples per month during the study. Convenient sampling was used, and a total of 384 samples were included in the study. Clinical and socio-demographic data of patients were not obtained.

### Detection of *S. aureus* and Enterococcus Species

The clinical samples were first inoculated on MacConkey, blood and chocolate agar (Oxoid, Basingstoke, UK). MacConkey agar plates were incubated aerobically at 35-37°C for eighteen to twenty-four hours while blood and chocolate agar plates were incubated in a 5 per cent $CO_2$ incubator to the same time. The resultant colonies were identified using standard microbiological methods including colony morphology, gram stain and biochemical tests. The suspect colonies were gram stained, and all gram-positive cocci were then subjected to a catalase test. For catalase-positive bacteria, a tube coagulase test was set and incubated aerobically for four hours; formation of a clot was recorded as a positive test. Further, the catalase-positive gram-positive cocci were also inoculated on mannitol salt agar (Oxoid, Basingstoke, UK) and incubated aerobically at 35-37°C for eighteen to twenty-four hours. Mannitol fermentation was observed and recorded. Coagulase positive isolates that fermented mannitol were reported as *S. aureus* and included in the study. All gram-positive cocci that were catalase-negative were considered as suspected Enterococcus. All suspected Enterococcus spp were inoculated on Bile esculin and incubated aerobically at 35-37°C for eighteen to twenty-four hours. Bile esculin positive isolates were reported as Enterococcus spp and were included in the study. Isolates were stored and analysed further depending on the availability of reagents.

**Determination of Antimicrobial Susceptibility Profiles by Kirby-Baur Disc Diffusion**

The antimicrobial susceptibility of the *S. aureus* and Enterococcus isolates was determined using the Kirby-Baur disc diffusion method and interpreted according to 2019 Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. Using a swab, one to two pure colonies of the organism grown overnight on Mueller-Hinton were suspended into 2mls of physiological normal saline to make a 0.5 McFarland density. These bacteria were then spread evenly on a Mueller Hinton agar plate using a sterile swab. Antimicrobial discs (Oxoid, Basingstoke, UK) were then gently placed on the Mueller Hinton agar plate ensuring that discs were not closer than 24mm from centre to centre after allowing the plate to air dry for a few minutes. The following antibiotic discs (Oxoid, Basingstoke, UK) were tested for *S. aureus* 10µg gentamycin, 5µg ciprofloxacin, 15µg erythromycin, 10µg clindamycin, 30µg amikacin, 30µg cefoxitin, 10 units penicillin G, 25µg co-trimoxazole, 30µg chloramphenicol and 30µg tetracycline. Methicillin resistance in *S. aureus* was detected using 30µg cefoxitin. For Enterococcus *species,* 10µg ampicillin, 10 units penicillin G, 5µg ciprofloxacin, 30µg tetracycline, 15µg erythromycin, 30µg chloramphenicol and 30µg vancomycin discs (Oxoid, Basingstoke, UK) were tested. The D-test using erythromycin and clindamycin discs, was also used to detect inducible resistance to macrolides, lincosamides, and group B streptogramins (MLSBi) in the *S. aureus* isolates.

**Determination of MICs**

MICs were determined for Enterococcus isolates resistant to vancomycin discs and the MRSA isolates using vancomycin E-test strips (Oxoid, Basingstoke, UK). Briefly, one to two pure colonies grown on Mueller-Hinton were suspended into 2mls of physiological normal saline to make a 0.5 McFarland density. The suspension was then spread evenly on a Mueller-Hinton agar plate using a sterile swab, sterile forceps were used to place the E-strip on the agar plate after allowing it to air dry for a few minutes. Interpretation of the antimicrobial susceptibility was done using the 2019 CLSI guidelines [16].

**Data Analysis**

Data obtained from this study was stored and analysed using WHONET version 5.6 software. The proportion resistant (R), intermediate (I) and susceptible (S) isolates among those tested were estimated using the RIS per cent command in WHONET. Graphs were also generated to visualise the distribution of resistance profiles of the tested bacteria.

**Ethics Considerations**

Ethics approval was obtained from the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC), the ethics approval number being 20190217066. A waiver of consent was obtained from UNZABREC to use archival clinical specimens that were collected routinely from the laboratory. Furthermore, permission to use the samples received in the laboratory for the study was sought from the hospital management.

**Results**

**Detection and Isolation of *S. aureus* and Enterococcus Species**

A total of 117 of *S. aureus* were detected and isolated. However, only fifty-nine of these were included in the study. Of the fifty-nine isolates, twenty-two isolates were from blood culture and thirty-seven from the skin and soft tissues specimen. Twenty-six isolates of the fifty-nine

(44.1%) were MRSA, as shown in Table 1. Forty-five Enterococcus spp were isolated, all from blood culture specimen, however, only 39 were included in the study.

**Antimicrobial Susceptibility Patterns of *S. aureus* and Enterococcus Species**

The antimicrobial susceptibility pattern results of *S. aureus* are shown in Figure 1. Most of the isolates were resistant to penicillin (93.2%), erythromycin (52.5%) and tetracycline (50.8%). The isolates were mostly sensitive to amikacin (96.6%) and clindamycin (72.9%). There was no clindamycin inducible resistance observed in all isolates (negative D test).

The Enterococcus isolates showed high resistance to many antibiotics, 97.4 per cent to erythromycin, 84.6 per cent resistance to tetracycline, 79.5 per cent to ampicillin and penicillin (Figure 2). The least resistance was 7.7 per cent to vancomycin discs. In line with the CLSI guidelines, vancomycin discs were used on Enterococcus isolates only while E-strips were used on *S. aureus* isolates. The Enterococcus isolates that showed vancomycin resistant and intermediate results using discs, were further subjected to E-strips.

**MICs of MRSA and Enterococcus Species**

Among the 16 MRSA isolates tested, most of them were vancomycin susceptible with ten (62%) having an MIC of 1.5µg/ml, and four (25%) had an MIC of 2µg/ml. Only two isolates (12.5%) were VISA with an MIC of 4µg/ml. No vancomycin resistance was observed in the *S. aureus* isolates. Among the fourteen Enterococcus isolates tested, 85.7 per cent were vancomycin susceptible while 14.3 per cent were vancomycin Intermediate Enterococcus with MICs ranging from 1µg/ml to 8µg/ml. No vancomycin resistant Enterococcus were detected.

**Discussion**

The present study reports the susceptibility patterns of MRSA and Enterococcus spp from the skin and soft tissue and blood culture specimens to vancomycin and other antibiotics at a large tertiary hospital in Lusaka, Zambia. A total of twenty-six (44%) MRSA were isolated during the study. This finding was very similar to the findings of a study that was carried out in the year 2015 at the same hospital which showed a prevalence of 43 per cent MRSA [15], indicating that occurrence of MRSA among patients seeking medical attention at UTH has remained relatively stable over time.

These findings were also similar to those of a study carried out in India by Rengaraj and colleagues who reported an MRSA prevalence of 49 per cent. However, a study in Kenya showed a slightly higher prevalence of 53.4 per cent than our findings [17]. The difference in the results could be attributed to the type of specimen, sample size, methods used, and the study's geographical locations. This present study also included thirty-nine Enterococcus out of forty-five isolated during the study period. Elsewhere, higher numbers of Enterococcus have been isolated from various clinical samples including urine, pus, blood, catheter tip, and tracheal aspirates that is 186 in Iran over one year (18) and 250 in South India over unknown study duration [19]. Lower numbers of Enterococcus were detected from urine and surgical site samples, but none were isolated from blood samples in a study conducted over six months in Nigeria [20]. Notably, all the Enterococcus in our study were from blood culture specimens and were confirmed using the Bile-esculin. However, we could not speciate

the isolates due to the unavailability of reagents at the study time. Future studies should include speciation of the Enterococcus isolates.

Antimicrobial susceptibility patterns of *S. aureus* showed that most of the isolates were moderately resistant to many antibiotics. Notably, the highest resistance was observed to penicillin, with about 93.2 per cent of the isolates being resistant. This was congruent with the findings of the study that was carried out earlier in Zambia, in which 95 per cent of *S. aureus* isolates were resistant to penicillin [15]. Again, it is noted that *S. aureus* resistance against penicillin had been maintained.

These findings were also similar to those a study carried out in Namibia in which there was 92.4 per cent resistance to penicillin in *S. aureus* [21]. However, a study carried out in Nigeria on *S. aureus* showed 87.5 per cent resistance to penicillin, which was slightly lower than our findings [22]. Penicillin is a beta-lactam antibiotic that inhibits the formation of peptidoglycan cross-linkages that provide the rigidity and strength in a bacterial cell wall, thereby killing the bacteria. Resistance to penicillin is usually mediated by the production of beta-lactamases whose production is usually induced by prolonged antibiotics use. Notably, methicillin resistance is conferred by the *mecA* gene, which encodes for an altered penicillin-binding protein that has a lower affinity of binding beta-lactams, including penicillins in MRSA isolates. The moderate resistance to erythromycin and clindamycin observed among the *S. aureus* isolates in our study (including the negative D test results) implies that none of the isolates had erythromycin inducible clindamycin. The resistance suggests that clindamycin can still be used in treating infections. However, since Samutela and others (2015) [15] detected erythromycin inducible clindamycin resistance in 68.3 per cent (28/41) of the isolates studied, the researchers recommend the routine use of the D test to enable microbiologist guide clinicians regarding judicious use of clindamycin. Notably, clindamycin is not a suitable drug for positive D test isolates because such strains may appear erythromycin-resistant and clindamycin sensitive in vitro, but when given in vivo, they have constitutive *erm* mutations that render clindamycin ineffective [23]. Notably, most of the *S. aureus* isolates were susceptible to amikacin in the present study, this agrees with the findings of our previous study [15] and thus, amikacin remains a viable treatment option for *S. aureus* infections in Zambia.

Among the MRSA isolates tested by the vancomycin E-strips, 85.5 per cent were vancomycin susceptible *S. aureus,* and 12.5 per cent were Vancomycin Intermediate *S. aureus* (VISA). The researchers' findings are similar to those of the study that was done by Ramakrishna and colleagues in which no resistance to vancomycin was observed [24]. Furthermore, the study conducted by Samutela *et al*., at UTH showed no resistance to vancomycin in the *S. aureus* isolates studied [15]. However, our results are different from those of a study carried out in Nigeria which showed 73.5 per cent vancomycin susceptible *S. aureus,* while 15 per cent were VISA and 44.5 per cent VRSA. According to these findings, vancomycin could still be used as a viable option in the treatment of MRSA in our setting. This could be attributed to the prudent use of vancomycin in treating infections by the clinicians. There has been an on-going campaign for the

prudent use of antibiotics at the hospital.

The Enterococcus isolates' susceptibility results showed that most of them were highly resistant to the seven drugs tested. The highest resistances were to erythromycin (97.4%), tetracycline (84.6%), ampicillin and penicillin (79.5%), and ciprofloxacin (71.8%) per cent. This was similar to a study carried out in India, which had high resistance rates of 79.0 per cent and 76.1 per cent to ciprofloxacin and erythromycin, respectively [2]. These findings of penicillin and ampicillin resistances were higher than those reported by a study in Ethiopia, which is 63.6 per cent and 54.5 per cent of penicillin and ampicillin resistance, respectively [25]. This difference in the findings can be attributed to the variable availability of drugs without prescriptions, which has made resistant bugs to spread in the different study settings. With the observed high levels of resistance to the commonly used drugs, routine susceptibility tests should be performed before treatment is started to prevent misuse of the antibiotics. Future studies should also include detection of the possible mechanisms of resistance using molecular techniques in these organisms.

About 7.7 per cent of the Enterococcus showed resistance to vancomycin when tested with vancomycin discs. However, when tested with the vancomycin E-strips to determine the MIC, no VRE was recorded. Interestingly, 85.7 per cent of these isolates were vancomycin susceptible with the E-strips while 14.3 per cent were vancomycin-intermediate. These findings were similar to a study conducted in Kenya, which did not record VRE [26]. Conversely, our study contradicted a study in Malaysia that reported VRE to be 20.8 [27] per cent and 15.3 per cent VRE in Tanzania [28]. Elsewhere, various vancomycin susceptibility rates have been detected; in Iran, 23.1 per cent VRE (18), in South India 17.2 per cent were VRE, 73.6 per cent with reduced susceptibility and only 9.2 per cent were susceptible [19]. Additionally, in Nigeria, 42.9 per cent were VRE [20] and a study carried out in Zambia on cattle showed no resistance of the Enterococcus to vancomycin [3]. Notably, there is no data published on clinical Enterococcus isolates in Zambia. The differences observed in our study can be attributed to geographical locations, isolation methods used, the antibiotic panel used in the treatment of Enterococcus infections, and selective pressure of the antibiotics on Enterococcus. Given these findings, it is recommended that all Enterococcus isolates resistant to vancomycin discs should be confirmed with an E-strips more so because discs do not differentiate between susceptible isolates and intermediate isolates [16].

It is worth noting that vancomycin-intermediate results were recorded for both *S. aureus* and Enterococcus in the present study. Therefore, there is a need for continued surveillance to rule out the development of vancomycin MIC creep in these species. Vancomycin MIC creep in MRSA and Enterococcus is a cause of concern in intermediate isolates because these isolates may be shifting to become resistant in long term therapy which in turn, leads to poor clinical outcomes such as morbidity and high mortality among patients. In addition, patients with such infections serve as sources of healthcare-associated infections which may cause opportunistic infections to individuals with weak immune systems.

One limitation of the study was the lack of clinical and socio-demographic data for patients due to insufficient records of such at the time the study was conducted.

Clinical and socio-demographic would give further insight into the epidemiology of the infections caused by *S. aureus* and Enterococcus in our setting.

## Conclusion

Resistance to vancomycin was not detected among the *S. aureus* and Enterococcus isolates. Therefore, vancomycin remains a viable option in the treatment of these two organisms in our setting. However, since vancomycin-intermediate isolates were detected, there is a need for continued surveillance for the emergence of resistance among these organisms. Additionally, prudent use of antibiotics with good infection control practices will help retain the susceptibility of these microbes to vancomycin.

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## Authors' Contributions

MM, MTS and KY conceived the idea, MM, FM, MB and CK collected the data, MM, MTS and KY analysed the data, MM drafted the manuscript, JBM, BMH, KY, LH, FNB and GK critically reviewed the manuscript. All authors read and approved the final draft of the manuscript. KY and MTS supervised the research.

## Competing Interests

The authors declare that there are no conflicts of interest associated with this study.

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**Figures**

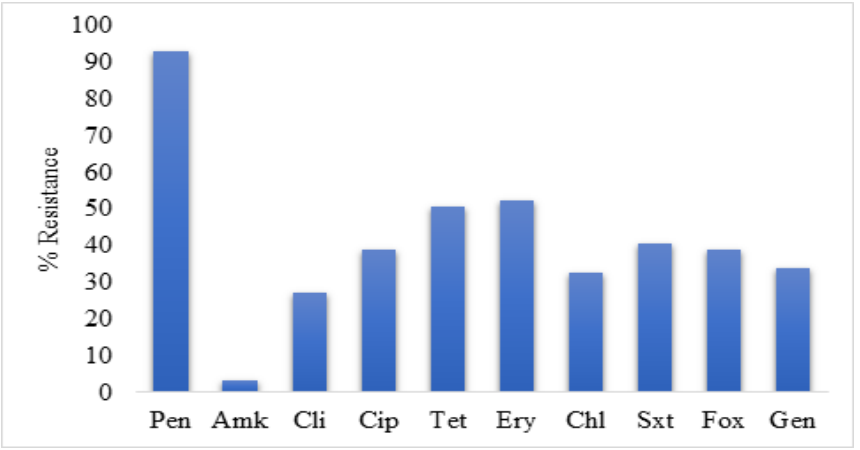


**Figure 1:** Resistance patterns of the S. aureus isolates (N=59)
Abbreviations: Pen, penicillin; Amk, amikacin; Cli, clindamycin; Cip, ciprofloxacin; Tet, tetracycline; Ery, erythromycin; Chl, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole; Fox, cefoxitin; Gen, gentamicin.

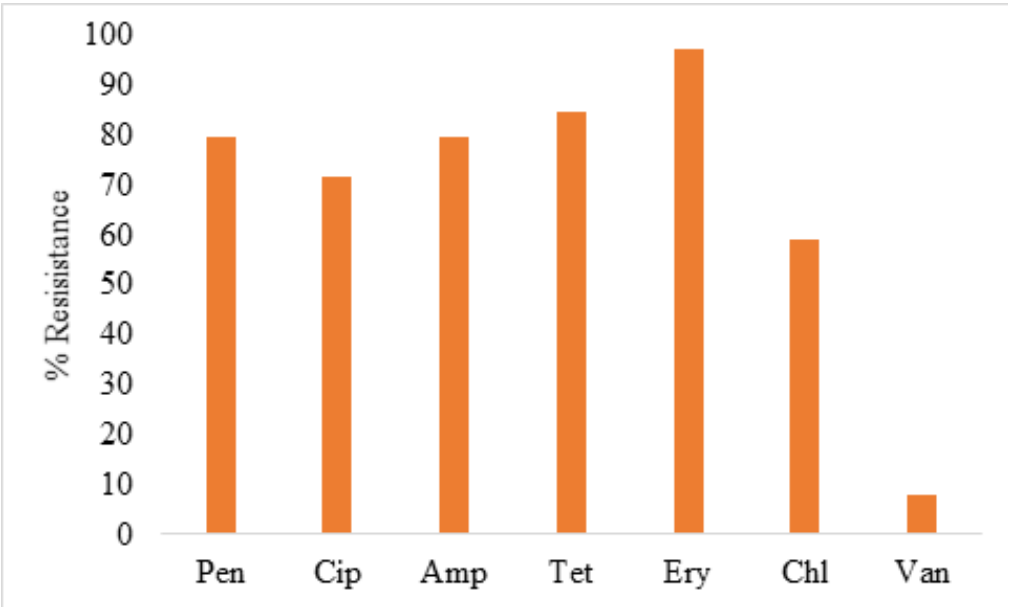


**Figure 2:** Resistance patterns of the Enterococcus isolates (N=39)
Abbreviations: Pen, penicillin; Cip, ciprofloxacin; Amp, ampicillin; Tet, tetracycline; Ery, erythromycin; Chl, chloramphenicol; Van, vancomycin.

## Tables

**Table 1: Detection of S. aureus and MRSA in the clinical specimens**

| Specimen Type | Number of Specimen tested | *S. aureus* % (N) | MRSA % (N) |
|---|---|---|---|
| Blood Culture | **4429** | **2.7 (117)**<br>**37.3 (22)*** | **18.6 (11)*** |
| Pus (Skin and Soft tissue) | **4279** | **6.5 (287)**<br>**62.7 (37)*** | **25.4 (15)*** |

*Calculated from the actual number of isolates included in the study.