Original article

Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococci* isolated from blood culture in Limpopo Province, South Africa

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Abstract

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) are the important nosocomial infectious agents. There is a growing concern about the rapid rise in the resistance of *Staphylococcus* to presently available antimicrobial agents.

Objective: The objective of this study was to evaluate the prevalence rate of MRSA and MR CoNS and their rate of resistance to different anti-staphylococcal antibiotics used broadly for treatment.

Methods: This study was carried out between November 2008 and December 2011 in Limpopo Province of South Africa. A total of nine hundred (900) *Staphylococcus* isolates were isolated from 5980 blood culture collected. The antibiotic susceptibility pattern of all the confirmed strains was determined by Kirby Bauer disc diffusion method. Screening Test for MRSA was performed following NCCLS guidelines using Oxacillin agar. Vancomycin resistance was tested by vancomycin agar screening test. ß-lactamase production was determined by iodometric strip method.

Results: MRSA was responsible for 9.89% (89 /900) of the infections while MRCoNS was responsible for 90.1 % (811/900). MRSA was resistant to cephalosporins, gentamycin, fluoroquinolones and even imipinem, so these are less effective in the treatment of MRSA infections. MRCoNS was resistant to augmentin, gentamycin and chloramphenicol.

Conclusions: The percentage of drug resistant isolates of both *Staphylococcus aureus* and coagulase negative *Staphylococcus* were seen to be high. Most of the clinical isolates of MRSA were resistant to cephalosporins, gentamicin, fluoroquinolones and even to imipenem, so these are less effective in the treatment of MRSA infections. Vancomycin use should be limited to those cases where they are clearly needed. *Ethiop. J. Health Dev.* 2015;29(1):37-42]

Introduction

Bloodstream infections (BSI) have high morbidity and mortality rates throughout the world (1, 2). The antimicrobial resistance of the pathogens isolated from BSI has been on the increase (1-3). *Staphylococcus aureus* was reported as one of the two most common causes of BSI in the United States and Europe (1, 4-6). Members of the genus *Staphylococcus* are non-motile, non-spore forming, usually un-encapsulated gram positive cocci; most species are facultative anaerobes. Besides their role as commensals on mucosal surfaces and the skin, staphylococci are often involved in a wide variety of diseases. These diseases include amongst others chronic blepharitis, conjunctivitis, and keratitis, skin and soft tissue infections, and pneumonia (7-10).

Staphylococcal infections are frequently treated with antibiotics and, consequently, antibiotic resistance or acquired resistance has developed (11-13). The high resistance being documented about these organisms in the new millennium is perhaps believed to have commenced at least a decade earlier, but with minimal attention or probably no recognition by the health personnel of the time (14). With the emergence of more disturbing strains of the organisms; being resistant to all the available common antibiotics plus the ones reserved as a last resort, the patient may apparently be at the mercy of these organisms (12-14). With the possibility of these highly resistant staphylococcal strains spreading from hospital settings to the community, the clinical relevance of these organisms become amplified (14, 15).

As the challenges in the management of staphylococcal infections worldwide deepens due to the varying but increasing resistance pattern of the organisms; the regional and geographic variations in their antimicrobial susceptibility patterns need to be established and probably institutionalized (16). The presence or absence of coagulase, an enzyme that clots plasma, divides the *Staphylococcus* species into two broad groups: the coagulase positive staphylococci (CoPS) and coagulase negative staphylococci (CoNS) (16, 17). In the microbiology laboratory, identification of staphylococci is often limited to a rapid screening test for *S.aureus*, while non-*S aureus* isolates are simply reported as CoNS species. Apart from being a common component of the

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normal flora, these bacteria have also been reported to cause serious infections in both hospitals and the community (18, 19). In the present study, we determined the prevalence and distribution of *Staphylococcal* blood infections and their antibiotic susceptibility profiles.

Methods

Study Sites and Sample Collection:

This study was carried out between November 2008 and December 2011 in Limpopo Province of South Africa. A total of nine hundred (900) *Staphylococcus* isolates were isolated from 5980 blood culture collected in the Province. Isolation of the bacteria was carried out by culturing the specimens on appropriate bacteriological media, including blood agar, chocolate agar, thioglycollate, and MacConkey agar media. Cultures were incubated at 37°C for 24 - 48 h. Blood samples were inoculated in trypticase soy broth bottles and incubated for at least 7 days at 37°C. Identification of organisms was performed by routine microbiological methods using Gram staining, catalase, coagulase tests and also using micro scan walk away. *In vitro* susceptibility of the isolates to 18 antibiotics was determined by standard disc diffusion method on Mueller-Hintonagar. Briefly, cell suspension inoculates were prepared from 18 - 24 h old pure cultures, in 0.85% sterile saline and adjusted to match a 0.5 McFarland standard tubes.

Antibiotic Susceptibility Testing:

The antibiotic susceptibility pattern of all the confirmed strains was determined by Kirby Bauer disc diffusion method (1966) against the following antibiotics. Penicillin (10µg) (PG), ampicillin (10µg) (AP), cloxacillin (10µg) (CLX), amoxicillin (AMX) (30µg), tetracycline (30µg) (TE), co-trimoxazole (25µg) (TS), augmentin (30µg) (AUG), colistin (10µg) (COL), streptomycin (STR), ciprofloxacin (5µg) (CIP), ofloxacin (5µg) (OFX), chloramphenicol (30µg) (C), cefttriaxone (30µg) (CTX), amikacin (30 µg) (AK), gentamicin (10 µg) (GN), erythromycin (15µg) (E), and rifampin (5µg) (R), cefuroxime (30µg) (CF) all of which were purchased from MAST.

Screening Test for MRSA:

Screening was performed following NCCLS guidelines using oxacillin agar. Briefly, a suspension equivalent to Mac Farland 0.5 was prepared from each strain. Then a swab was dipped and streaked on the surface of Muller-Hinton agar (Oxoid-UK) supplemented with6µg/ml oxacillin and 4% NaCl, growth was observed after incubation for 24 h at 35ºC (20). If any growth was detected, the isolate was considered oxacillin or methicillin resistant.

MRSA screening for decreased vancomycin susceptibility: Vancomyc in resistance was tested by vancomycin agar screening test whereby MRSA isolates were spot inoculated into the Muller-Hinton agar (Oxoid-UK) supplemented with 6 µg/ml of vancomycin from 0.5 McFarland standard suspensions. The plates were incubated at 35ºC for 24 h as recommended by the (16, 21). Any isolate growing two or more similar colonies on this agar would be considered as positive.

Determination of Minimum Inhibitory Concentration (MIC):

Micro dilution broth method, using Muller Hinton broth (Oxoid-UK) was used to determine the lowest concentration of antimicrobial agents (MICs) required to inhibit the growth of microorganism against methicillin, vancomyc in, tetracycline, rifampicin and gentamicin.

Bacterium inoculations of 5×10^5 cfu and incubation at 35ºC for 24 hours were done according to Clinical and Laboratory Standards Institute (CLSI) guidelines (17).

Detection of ß-lactamase:

ß-lactamase Production was determined by iodometric strip method, benzyl penicillin was dissolved in 0.2% starch solution; the mixture was soaked in what man No. 1 filter paper.

When the filter papers were saturated, they were dried and cut into strips; the strips were stored at -20ºC until use. Prior to test, strips were put in desiccators and brought to room temperature. Strips were moisturized with iodine and 2-3 similar colonies of bacteria were smeared. If the color of the strip changed in 5 min, the bacteria were ß-lactamase positive (15).

Ethical Considerations:

An ethical clearance and permission was obtained from Limpopo Department of Health.

Results

From all the blood samples collected, 900 staphylococcal organisms were isolated. *Methicillin Staphylococcus aureus* (MRSA) was responsible for 9.9% (89/900) (figure 1) of the infections while coagulase negative Staphylococci (CoNS) was responsible for 90% (811/900).

The age interval for isolation of *S. aureus* was between6 months to 69 years, with 30% (18/89) females and 70% (71/89) males. Infection rates were found to be significantly higher infection rates among females (Table 1). Of the 811 isolates of coagulase negative. Staphylococci recovered, 54% (489/900) and 46% (411/900) were from males and females respectively. The age interval for the isolation ranged between four months to 59 years (Table 2).

Table 1: **Age and gender distribution of MRSA recovered from blood culture specimens**

Age range	Male (%)	Female (%)	Total (%)
0-9	5	9	14
10-19	7	4	11
20-29	13	18	31
30-39	11	16	27
40-49	3	2	5
50-59	4	7	11
60-69	0	1	1

Table 2: **Age and gender distribution of coagulase negative Staphylococci recovered from blood culture specimens**

Age range	Male (%)	Female (%)	Total (%)
0-9	9	15	24
10-19	13	2	15
20-29	9	11	20
30-39	20	11	31
40-49	1	6	7
50-59	2	1	3

All the isolates of *S. aureus* tested were 30% to 40% resistant to augmentin, gentamycin and chloramphenicol, 10% to 19% resistant to penicillin G, ampicillin, cloxacillin, amoxicillin, tetracycline, co-trimoxazole, Ciprofloxacin and rifampicin (Figure 1). All the isolates of coagulase negative Staphylococci were30% to 40% resistant to augmentin, gentamicin and chloramphenicol with cloxacillin, amoxicillin, tetracycline, co-trimoxazole, Ofloxacin, ciprofloxacin, cefuroxime, and erythromycin being 10% to 19% resistant (Figure 2). Both isolates of *S. aureus* and that of CoNS showed high resistance to augmentin, gentamicin and chloramphenicol (30% to 40%).

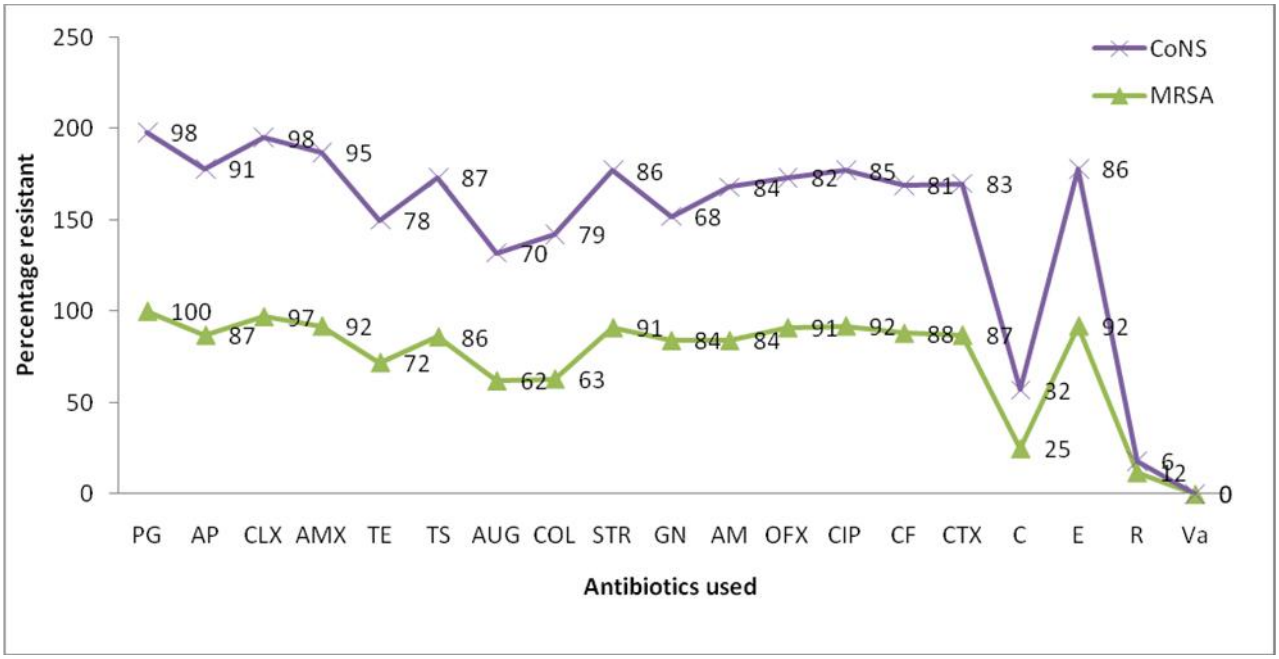

Figure 1: **Antibiotic resistance pattern of MRSA and MRCoNS**

Percentage resistance of MRSA and MRCoNS recovered from blood culture specimens. PG= Penicillin G, AP = Ampicillin, CLX = Cloxacillin, AMX = Amoxicillin, TE = Tetracycline,TS = Co-trimoxazole, AUG =Augmentin, COL = Colistin, STR = Streptomycin, GN = Gentamicin, AM = Amikacin,OFX = Ofloxacin, CIP = Ciprofloxacin, CF = Cefuroxime, CTX = Cefttriaxone, C= Chloramphenicol,E = Erythromycin, R = Rifampicin.

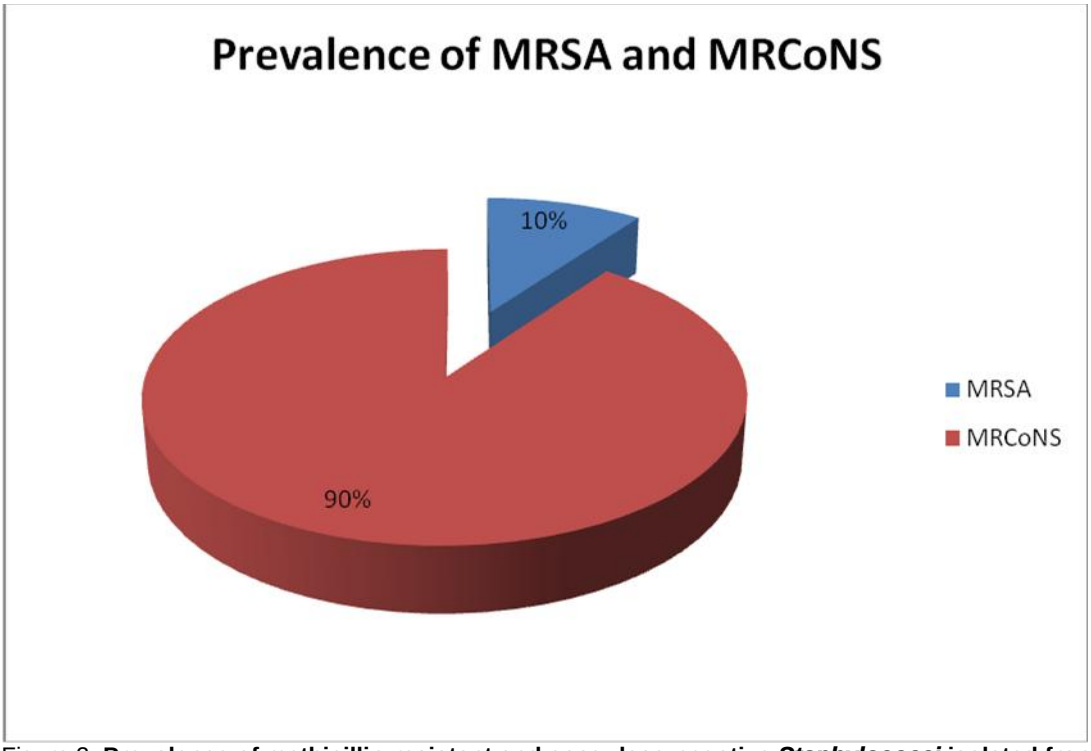

Figure 2: **Prevalence of methicillin-resistant and coagulase-negative *Staphylococci* isolated from blood culture**

Discussion

In general, bacterial isolates develop resistance to a number of commonly used antibiotics. This resistance has been attributed to indiscriminate use of broad-spectrum antibiotics, while infections due to hitherto common commensals have increased as a result of increased usage of immunosuppressive agent, intravenous catheters, and organ transplantation (22, 23). This has led to the intensive search for the alternative treatment. In developing countries, synthetic drugs are expensive and are very much prohibitive to the poor.

Most coagulase-negative staphylococcal clinical isolates were resistant to penicillin G (98%), gentamicin (68%), cloxacillin (98%) and amoxicillin (95%) and susceptible to Colistin (79%), ciprofloxacin (85%), and rifampicin (86%) (23-25). Also reported on the prevalence of vancomycin resistance CNS in a study asall the isolates in that study showed resistance to vancomycin and the commonly used antimicrobial agents that were tested against them; a finding that is quite alarming and different from other studies reported in other literatures. CoNS infections preferentially affect immunocompromised, long-term hospitalized and critically ill patients (24). Increasing antibiotic resistance of nosocomial isolates of CoNS aggravate the problem and pose a great challenge for the management of hospital acquired infections in general (25-27). The implications of this current finding is that little choice is available to the patients and this obviously will impact the general health care delivery in the region in the long run. As a consequence of this, the urgent need for an alternative therapy cannot be overemphasized.

This study further found all isolates of MRSA resistant to multiple antibiotics tested. Isolates exhibited resistance towards various antibiotics such as cephalosporins, tetracycline and gentamicin, which is almost similar to previous reports (20) where he reported 75% resistance. Another study (21) found MRSA strains resistant to first, second, third and fourth generation cephalosporins (22), reported 29% resistance of *S. aureus* against first generation cephalosporins which is similar to our findings. Gentamicin is an amioglycoside and is most often prescribed because of its low cost and synergistic activity with ß-lactam antibiotics. Rifampicin is a drug considered suitable for treatment of MRSA infection (15, 16, 18). In the present study MRSA resistance to rifampicin was found to be 12% while that of MRCoNS was 6%. Other studies (22-25) have reported MRSA resistance of 14% towards rifampicin. In the present study, we observed that 92% of MRSA were resistant to erythromycin while 86% CoNS were resistant, which is comparable to previous reports (19). Among fluoroquinolones, ciprofloxacin and ofloxacin were tested; the percentage resistance found in MRSA and MR CoNS was 91% and 82% for ofloxacin and 92% and 85%% for ciprofloxacin respectively. Previously reported resistance of ciprofloxacin shows a similar type of pattern (24). Since the emergence of methicillin resistant *S. aureus*, vancomycin a glycopeptides has been the only effective treatment for MRSA infections (23). In the present study, all the MRSA and MRCoNS isolates were

susceptible to vancomycin; ß-lactamase production rates found were 92% and 88% for MRSA and MRCoNS respectively. These results are similar to previous findings of (23-25).

The MIC values of MRSA were determined against antibiotics includes vancomycin, tetracycline, rifampicin and gentamicin. The MIC of vancomycin for MRSA isolates ranged from 1-5 µg/ml; 100% isolates were inhibited at concentration of 1µg/ml. There are studies (26) that reported 100% inhibition at concentration of 1 µg/ml, while another study (27) reported MRSA inhibition at concentration of 0.5-1 µg/ml.

For gentamicin the MIC value ranged between 0.5-61 µg/ml; 88% of isolates were inhibited at concentration 0.5 µg/ml. Similarly, there are reports (26) that showed 95% MRSA inhibition at 0.5 µg/ml and total 4% isolates inhibition at a concentration of ≥32 µg/ml.

There are few previous reports on antibiotic resistant patterns of *Staphylococcus* from patients in Limpopo Province in South Africa. Isolation of organisms with antimicrobial resistance patterns, as reported above, may impact the choice of medication for these patients.

Antibiotic sensitivity tests are warranted prior to prescription as most *Staphylococci* are likely to show some degree of resistance as a result of transfer of resistant plasmids among different genera. Glycopeptide susceptibility of CNS and other gram-positive pathogens can no longer be assumed and hence consistent routine susceptibility testing and elaborate monitoring are necessary imperatives for effective therapeutic strategies and interventions.

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