

Comparative Study of Antibiotic Resistance of *Staphylococcus* Species Isolated from Clinical and Environmental Samples

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Abstract

Comparative study of antibiotic resistance patterns of one hundred *Staphylococci* isolates comprising of fifty from clinical and fifty from environmental samples were evaluated. The isolates were identified to species level by use of both classical and API-Staph identification kit, screened for beta-lactamase production and their antibiograms determined using agar diffusion technique. Five species of *Staphylococcus aureus*, *S. haemolyticus*, *S. cohnii*, *S. xylosum* and *S. sciuri* were identified. The 50 isolates from the environment showed multiple antibiotic resistance to selected antibiotics tested, 13(26.0%) were resistant to augmentin, 19(38.0%) to amoxicillin, 30(60.0%) to both cloxacillin and cotrimoxazole, 38(76.0%) to chloramphenicol, 43(86.0%) to erythromycin, 44(88.0%) to tetracycline and 48(96.0%) to gentamicin. The multiple antibiotic patterns of the clinical samples showed that 29(58.0 %) were resistant to augmentin, 39(78.0%) to chloramphenicol, and erythromycin while 42(84.0%) were resistant to cloxacillin, 45(90.0%) to amoxicillin, 48(96.0%) to gentamicin, and tetracycline, while all the isolates were resistant to cotrimaxazole, 50(100.0%). Only 8.0% of the environmental strains and 24.0% of the clinical strains had detectable beta-lactam enzyme activity. The results showed that *S. xylosum* had a wider range of antibiotic resistance activities when compared to other coagulase negative *Staphylococci*. This result showed the consequences of antibiotic resistance patterns in the environment and the need for urgent management.

Keywords: Antibiotics, Coagulase negative, Resistance, *Staphylococcus aureus*.

Introduction

Infections caused by *Staphylococcus aureus* poses serious threat in health care institutions. (Panlilio *et al.* 1992; and NNIS 2001, 2004). It is one of the most widely spread and virulent nosocomial pathogen and is usually resistant to multiple antibiotics making infections difficult to treat (Cooper *et al.* 2004). It appears to add to the total burden of *Staphylococcus* infections in the hospitals, rather than replacing sensitive *S. aureus*, and is associated with sharp risk in mortality attributable to *Staphylococcal* infection (Crowcroft & Catchpole 2002). *Staphylococcus aureus* strains continue to be a major problem in many healthcare institutions especially with

emergence of Methicillin resistant *Staphylococcus aureus* (MRSA) and now account for more than 50% of *S. aureus* recovered from patients in intensive care units and about 40% of *S. aureus* isolated from non intensive care unit (Boyce 2003).

Although, the clinical significance of methicillin resistance has been questioned in the past, there is now widespread acknowledgement of the pathogenicity of MRSA. It has emerged as a significant cause of both nosocomial and community-acquired infections. Recent report of strains of MRSA isolated from children in the community has led to speculation that the epidemiology of *S. aureus* is changing (CDC 1999; and Boyce 1998). Traditionally, MRSA infections have

been acquired almost exclusively in hospitals, long-term care facilities or similar institutional settings (Thompson *et al.* 1982). Health-care associated infection commonly caused by MRSA include surgical site infections, bacteremia and endocarditis, pneumonia, soft-tissue infections and urinary tract infections.

However, the emergence of community-associated MRSA (CA-MRSA) infections is of major concern to both public health officials and clinicians. The first report of CA-MRSA infection occurred among Australian aboriginals and Native Americans in Canada in the early 1990s (Boyce 2003). The earliest reported cases of CA-MRSA infection in the United States occurred in children with little or no recognized contact with the hospitals or other health care institutions (Herold *et al.* 1998). Coagulase negative *Staphylococci* (CNS) belong to the group of opportunistic pathogens since they are found as normal flora of the skin and mucus membranes in different part of the body (Eisenstein and Schaechter 1994). For this reason, CNS are often reported without further specification, assuming that they are contaminating clinical samples but are not involved in the primary infection.

However, there is mounting evidence that these bacteria may be responsible for primary infections as a result of increased use of medical in dwelling plastic devices and compromised or immunodepressed patients (Jarvis and Martone 1994; and Kloos and Bannerman 1994). Methicillin resistance among CNS is particularly important due to cross resistance to virtually all B-lactam agents and other antimicrobial classes. As a result, therapeutic approaches are restricted to glycopeptide and new antimicrobial agents as Linezolid (Woods *et al.* 2002). Therefore, an accurate analysis of resistance between clinical and community strains may allow the provision of better antimicrobial therapy. Besides, the importance for patient care the detection also has implications for the validity of antibiotic resistance surveillance. Hence the purpose of this study is to isolate *Staphylococcus* species from both clinical and community based samples and to determine the antibiogram of the isolates against some selected commercial antibiotics.

Materials and Methods

Bacterial Isolates

Clinical samples were isolated between February 2007 and June 2007 from patients admitted to University College Hospital, Ibadan. Of the 50 clinical samples, 8(16.0%) were from ear, 15(30.0%) wound, and 27(54.0%) eye swabs.

Whereas in the 50 community based samples, 9(18.0%), 7(14.0%), 3(6.0%) were isolated from ear, skin and nose respectively of apparently healthy individuals while 31(62.0%) were isolated from polluted water.

The various isolates were identified to species level according to standard microbiological methods (Kloos and Lambe 1991; and Holt *et al.* 1994). Also, API-Staph identification 25E system (BioMerieux, France) was employed.

Detection of Beta-lactamase

The cell-suspension iodometric method was employed, as described by Adeleke and Odelola (2007). Bacterial cell suspension equivalent to 10^9 cells/ml was prepared for each strain from overnight nutrient agar plate culture, in 0.5ml of freshly prepared phosphate buffer solution containing penicillin G (0.05mg/ml). The suspension was shaken briefly in a vortex mixer. Ordinary penicillin G phosphate buffer served as control. All test tubes were left on the bench at room temperature for minimum of 1h. Thereafter, two drops of freshly prepared 1% aqueous starch solution was added to suspension, without shaking. The mixtures were allowed to stand for 10minutes, for a possible colour change from blue/blue-black to colourless.

Susceptibility Test

Disc diffusion testing was performed according Kirby-Bauer method, as described in the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS 2000, 2002), using discs (Abterk) containing 10µg Gentamicin (GEN), 25 µg Cotrimaxazole (COT), 30 µg Chloramphenicol (CHL), 30 µg

Augumentin (AUG), 25 µg Amoxicillin (AMX), 5 µg Erythromycin (ERY), 10 µg Tetracycline (TET) and 5 µg Cloxacillin (CXC).

Results

The 50 isolates from the environment showed multiple antibiotic resistance activities to selected antibiotics tested, 13(26.0%) were resistant to augumentin, 19(38.0%) to amoxicillin, 30(60.0%) to both cloxacillin and cotrimoxazole, 38(76.0%) to chloramphenicol 43(86.0%) to erythromycin, 44(88.0%) to tetracycline and 48(96.0%) to gentamicin. The multiple antibiotic patterns of the clinical samples were augumentin 29(58.0 %), 39(78.0%) chloramphenicol, and erythromycin,

42(84.0%) cloxacillin, 45(90.0%), amoxicillin 48(96.0%), gentamicin, and tetracycline, while all the isolates were resistant to cotrimaxazole, 50(100.0%) as shown in Table 1. The difference in resistance patterns between the clinical and community strains were evaluated using Chi-square analysis and statistical significance was set at $\alpha = 0.05$.

There was no significant difference in antibiotic resistance pattern amongst isolates from clinical and community strain to gentamicin, chloramphenicol, erythromycin, and tetracycline. ($p > 0.05$). However, there are significant difference in antibiotic resistance pattern amongst isolates from clinical and community strain to gentamicin, chloramphenicol, erythromycin, and tetracycline ($p < 0.05$).

Table: 1 Comparison of antibiotic sensitivity and resistance of Community and Clinical strains of *Staphylococcus* species.

| Drugs | Community (N=50) | | | | Clinical (N=50) | | | | χ^2 | df | P |
|-----------------|------------------|------|-----------|------|-----------------|-------|-----------|------|----------|----|--------|
| | Resistant | | Sensitive | | Resistant | | Sensitive | | | | |
| | N | % | n | % | N | % | N | % | | | |
| Gentamicin | 48 | 96.0 | 2 | 4.0 | 48 | 96.0 | 2 | 4.0 | 0.000 | 1 | 1.000 |
| Cotrimaxazole | 30 | 60.0 | 20 | 40.0 | 50 | 100.0 | 0 | 0.0 | 25.000 | 1 | 0.000* |
| Chloramphenicol | 38 | 76.0 | 12 | 24.0 | 39 | 78.0 | 11 | 22.0 | 0.056 | 1 | 0.812 |
| Augumentin | 13 | 26.0 | 37 | 74.0 | 29 | 58.0 | 21 | 42.0 | 10.500 | 1 | 0.001* |
| Amoxicillin | 19 | 38.0 | 31 | 62.0 | 45 | 90.0 | 5 | 10.0 | 29.340 | 1 | 0.000* |
| Cloxacillin | 30 | 60.0 | 20 | 40.0 | 42 | 84.0 | 8 | 16.0 | 7.143 | 1 | 0.008* |
| Erythromycin | 43 | 86.0 | 7 | 14.0 | 39 | 78.0 | 11 | 22.0 | 1.084 | 1 | 0.298 |
| Tetracycline | 44 | 88.0 | 6 | 12.0 | 48 | 96.0 | 2 | 4.0 | 2.174 | 1 | 0.140 |

Key: n = number of isolates; level of significance = 0.05; * = significant.

Discussion

Coagulase negative *Staphylococci* were mostly encountered in this study. This is in conformity with previous work of Bannerman (2003) and Kwok and Chow (2003). *Staphylococcus xylosus*, *S. scuri*, and *S. haemolyticus* were isolated from eye swab and wound swab while *S. aureus* was only isolated from eye swab. Although, the most reported CNS of clinical importance are *S. saprophyticus* and *S. haemolyticus* (Kloos and Wolfshohl 1982; and Sewell *et al.* 1982). The isolation of *S. xylosus* and *S. scuri* were in accord with the more recently reports which implicated a much wider range of species of

Staphylococcus as etiologic agents of infections (Schnitzler *et al.* 1997; and Bannerman 2003) especially with infections associated with medical devices and in immunocompromised patients. Boagdo *et al.* (2001) reported that the incidence of resistant CNS to antimicrobial agents are high and this is similar to the findings of other authors (Hedin 1996; and Martínez *et al.* 1997) especially with respect to nosocomial infection, highlighting the therapeutic and economic problems raised by these strains.

The occurrence of *S. xylosus* from wound and eye infections and its predominance over any other CNS encountered in this study raises the question to whether this is the most virulent

species or simply the most predominant on the skin or in the environment of patients who become infected with these organisms.

In the determination of the susceptibility of these strains on eight selected antibiotics by agar diffusion technique showed that *S. xylosus* tend to be resistant to a wider spectrum of antibiotics than other CNS. This finding is in agreement with the work of Herold *et al.* (1998), Adcock *et al.* (1998) and CDC (1999) who reported that clinical Staphylococci are resistant to multiple antibiotics. The reason for the predominance of CNS in clinical samples in this study is unknown. Coagulase negative *Staphylococci* also have a variety of multiple resistance genes on their plasmid which can be exchanged and spread among different species including *S. aureus*. (Neihart *et al.* 1988).

S. cohnii which were all isolated from community based samples were found to be resistant to most of the antibiotics used in this study. The reason for this is unclear, but may also be attributed to genetic exchange of materials occurring among the closely related strains or different strains by any of the vehicles of genetic transfer. This is the first time is been reported that *S. cohnii* is exhibiting multiple resistance to antibiotics.

It is interesting to note that 92.0% and 76.0% of the strains from community and clinical environments were non-producer of beta-lactamase, yet were resistant to beta-lactam and other classes of antibiotics. This suggests that beta-lactamase production is just an integral part of factors accounting for resistance in these bacteria.

These results suggest that CNS strains isolated from patients with infection should be considered as a possible etiological agent of the infection. Antibiotic susceptibility should be carried out on the isolates considered being the cause of the infection due to the resistance of these organisms to a wide spectrum of antibiotics.

This work advocates for a cost-effective and fast-reliable Staph-identification system in Africa which will reduce the level of assumption that *S. aureus* is the major cause of Staphylococcus-associated infections in our community.

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