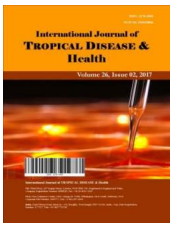

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Cefoxitin Resistant Profile of *Staphylococcus aureus* Isolated from the Environment of a Tertiary Health Institution

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors CIM, CFU, ENM, SA and UOE designed the study. Authors UOE and UEG performed the statistical analysis. All the authors were involved in writing the protocol and the first draft of the manuscript. All the authors managed the analyses of the study. Authors SA and ENM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Staphylococcus aureus has a notable ability to acquire resistance to methicillin and other antibiotics, and represents a growing public health challenge globally. This study was aimed at evaluating the cefoxitin resistance profile of *Staphylococcus aureus* isolated from the University of Calabar Medical Centre, Calabar. A total of 50 swab specimens were collected from the hospital environment of the University of Calabar Medical Centre and analyzed following standard microbiological techniques. Isolates were subjected to antimicrobial susceptibility testing using commonly used antibiotics. A total of 20 (40%) *S. aureus* strains were isolated and exhibited 80%

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resistance against cefoxitin, 75% against ampiclox and 65% against amoxyl, confirming their ability to secrete β -lactamases against β- lactam agents. Furthermore, *S. aureus* strains also exhibited varying degrees of resistance to non β-lactam antibiotics including streptomycin (50%) and ciprofloxacin (60%). Considerable susceptibility was however observed with other antibiotics including levofloxacin (75%) and gentamycin (70%), suggesting that these drugs could be employed as combination therapy in the management of CRSA- related infections. This study revealed a high level of resistance of *S. aureus* to cefoxitin. In addition, isolates also exhibited resistance to routinely used antibiotics and makes need for urgent review of antibiotics, hospital sanitation and disinfection policies.

Keywords: Cefoxitin; Staphylococcus aureus; resistant profile; susceptibility.

1. INTRODUCTION

Staphylococcus aureus is a common bacterium carried on the skin and/or in the nose of approximately 20 - 40% of otherwise healthy individuals [1]. It has been reported to cause a wide array of infections including minor skin conditions such as furuncles or boils as well as life-threatening conditions usually involving the lungs, blood, other organs and tissues in the body under certain conditions [2]. However, under normal conditions, this organism has been reported to be a normal flora of the skin and surfaces [1-2].

Before the emergence of resistance to penicillin *S. aureus,* penicillin was initially very effective against *S. aureus* [3]. Its introduction into clinical practice and wide spread use and abuse has made this organism to became penicillin-resistant [3-4]. Resistant strain of *S. aureus* was first reported in the hospital setting and eventually moved into the community setting [2-5]. *Staphylococci species* have been reported to possess penicillin-binding proteins (PBPs) used in the synthesis of peptidoglycan, a cell wall component in the absence of β-lactam antibiotics [2,4.6].

Furthermore, Garvin et al. [4] revealed that this mechanism of resistance may be brought about by the secretion of β-lactamase enzymes which has the ability of deactivating the active sites of penicillin thus, rendering it inactive. The rapid emergence and spread of β-lactamase producing *S. aureus* led to the production and introduction into clinical practice semi-synthetic penicillins including methicillin, nafcillin, oxacillin and cloxacillin which these β-lactamase enzymes could not destroy. These drugs became known as β-lactamase stable penicillins [7]. However, in the early 60s, this organism developed resistance to these synthetic drugs by acquiring a gene known as *mecA* [8].

Methicillin-resistant *Staphylococcus aureus* (MRSA) also known as Cefoxitin resistant *Staphylococcus aureus* (CRSA) has been reported as a significant nosocomial pathogen which emerged immediately after the introduction of semi-synthetic penicillins and was reported first in 1961 in the United Kingdom [3-4]. This species has been reported as a significant public health challenge globally, being generally incriminated in infections ranging from minor skin infections, catheter-associated bacteraemia and ventilator-associated pneumonia to many other complicated infections among patients [2]. Similar to other major infections, MRSA-related infections are of major public health concerns because they are known to cause increased cost of treatment and prolonged hospital stay [8].

MRSA-related infections have been significantly associated with high rates of mortality compared to those caused by methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin resistance has been reported to be independently associated with death [9]. In a prospective study of ventilator-associated pneumonia patients, caused by MSSA and MRSA, Francois [9] reported a higher incidence of extremely difficult to treat bacteraemia that was associated with MRSA. Many researchers have reported the prevalence of cefoxitin-resistant *Staphylococcus aureus* in developed countries but information in the study area is scarce. The study was aimed at determining cefoxitin resistance profile of *Staphylococcus aureus* isolated from the University of Calabar medical centre.

2. METHODOLOGY

2.1 Study Site

This study was carried out at the University of Calabar (UNICAL) located in Calabar Municipality Local Government area of Cross

River State, Southern Nigeria [10-11]. The University of Calabar Medical centre is health service department of the University saddled with the responsibility of providing appropriate healthcare for staff, students and others who engage in downstream activities within the University of Calabar community and environs.

2.2 Materials

A total of 50 swab specimens were collected from the hospital environment of University of Calabar medical centre. The antimicrobial agents employed for this study included: cefoxitin (FOX 30 μg) (Oxoid, England), streptomycin (30 μ), chloramphenicol (30 μg), erythromycin (30 μg), Amoxil (20 μg), ampiclox (20μg), levofloxacin (20 μg), rifampicin (20 μg), ciprofloxacin (10 μg), norfloxacin (10 μg) and gentamycin (10 μg) (Optun. Lab. Nig. Ltd.).

2.3 Sample Collection and Preparation

All samples were obtained from the hospital environment (table tops, hands and laboratory coats) of the University of Calabar medical centre using an environmental sample transport swab stick and transported within 30 minutes of collection to the Microbiology laboratory where they were analyzed following standard microbiological procedures contained in Holt et al. [12] and Murray et al. [13]. Briefly, samples were inoculated unto already prepared nutrient and blood agar plates and incubated at 37℃ for 24 hours. Following incubation, discrete colonies were sub-cultured onto Mannitol salt agar plates and incubated at 37℃ for 24 hours. A series of biochemical tests including Gram's reaction, catalase and coagulase tests were carried out to confirm the isolates.

2.4 Sensitivity Testing

This test was carried out following Kirby-Bauer modified disc diffusion technique described by NCCL [14] and CLSI, [15]. Briefly, using a sterile cotton swab, standardized inoculums were inoculated unto plates containing freshly prepared Muller Hinton agar after which standard antimicrobial discs were placed firmly on the surfaces of the inoculated agar plates using sterile forceps. The plates were then incubated at 35^0C overnight after which zones of inhibition were measured following CLSI interpretive chat.

2.5 Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC)

This was performed following procedures described by CLSI [14]. Briefly, 2-3 colonies of the test isolate was inoculated into 5ml of sterile peptone broth and incubated for 30 minutes. Antibiotics of various concentrations were dissolved in sterile test tubes containing 5ml of diluents (distilled water) to make stock solutions. Doubling dilutions of the antibiotics in the order of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024 were carried out, respectively. Standardized *S. aureus* inoculums were added to each of the tubes and incubated overnight. The MIC was then reported as the lowest concentration of antimicrobial required to prevent visible growth. The MBC was determined by sub-culturing tubes which showed no growth (turbidity) during the MIC test into plates containing freshly prepared nutrient agar and incubated over night at 37℃.

2.6 Data Analysis

All data obtained in this study were analyzed using descriptive statistics such as simple percentages with SPSS version 17.0.

3. RESULTS

3.1 Sensitivity Test of Isolates to Cefoxitin and Other Commonly Used Antibiotics

Out of a total of 50 samples analyzed, 20 (40%) of organisms recovered were *Staphylococcus aureus* species. Of the 20(40%) of the *S. aureus* strains isolates that were subjected to testing, 16(80%) were resistant while 4(20%) were sensitive to cefoxitin as presented in Table 1. The test isolates when subjected to sensitivity testing using antibiotics other than cefoxitin exhibited varied resistance as shown in Table 1. Out of 20 isolates tested, 60% (12/20) were resistant to ciprofloxacin while 25% (5/20) and 15% (3/20) were intermediate and susceptible, respectively. Similarly, isolates were 45% (9/20) norfloxacin resistant while 20% (4/20) and 35% (7/20) were intermediate and susceptible, respectively. Furthermore, isolates were 40% (8/20) resistant to chloramphenicol while 20% (4/20) and 40% (8/20) were intermediate and susceptible, respectively. Isolates were further resistant to amoxyl 65% (13/20) and 35% (7/20)

Table 1. Susceptibility pattern to cefoxitin and other antibiotics

Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
CPX	12 (60.0)	5 (25.0)	3 (15.0)
NB	9 (45.0)	4 (20.0)	7 (35.0)
CH	8 (40.0)	4 (20.0)	8 (40.0)
AML	13 (65.0)	3 (15.0)	4 (20.0)
S	10 (50.0)	5 (25.0)	5 (25.0)
E	7 (35.0)	7 (35.0)	6 (30.0)
CN	6 (30.0)	7 (35.0)	7 (35.0)
APX	15 (75.0)	3 (15.0)	2 (10.0)
LEV	5 (25.0)	8 (40.0)	7 (35.0)
RD	9 (45.0)	6 (30.0)	5 (25.0)
FOX	16(80.0)	-	4(20.0)

Key: E = Erythromycin, CPX = Ciprofloxacin, CH = Chloramphenicol, APX = Ampiclox, CN = Gentamycin, NB = Norfloxacin, LEV = Levofloxacin, S = Streptomycin, AML = Amoxyl, FOX= Cefoxitin and RD = Rifampicin

were susceptible. Isolates were 50% (10/20) resistant to streptomycin while 25% (5/20) were each intermediate and susceptible, respectively. Consistently, isolates were 35% (7/20) resistant to erythromycin while 35% (7/20) and 30% (6/20) were intermediate and susceptible, respectively. Resistance of 30% (6/20) was exhibited against gentamycin while 35% (7/20) each were intermediate and susceptible, respectively. Isolates were 75% (15/20) resistant to ampiclox while 25% (5/20) were susceptible. A resistance of 25% (5/20) was exhibited against levofloxacin while 40% (8/20) and 35% (7/20) were intermediate and susceptible, respectively. The minimum inhibitory concentration (MIC) of the test isolates was 1:32 while the minimum bactericidal concentration (MBC) was 1:16.

4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The hospital setting according to Anupurba, [14] serves as a reservoir of infections with modes of transmission ranging from cross contamination between healthcare workers, surfaces, patients, water as well as air [16]. Some studies have reported the resistance of *S. aureus* to cefoxitin. This study reports the resistance of *Staphylococcus aureus* strains isolated to cefoxitin. Out of the 20 (40%) *S. aureus* strains that were isolated, 16 (80%) were resistant to cefoxitin. The 80% resistance observed in this study is somewhat higher than the 43.6% and 52.8% reported by Rongpharpi et al. [17] and Kakhandki and Peerapur, [18], respectively.

The high resistance exhibited by *S. aureus* isolates to cefoxitin shows the all-roundness of this organism; making it the most common pathogen in the hospital setting. This resistance as revealed by Hackbarth, [19] and Byarugaba, [20] could be due in part to the ability of this organism to secrete extracellular enzymes that deactivate the β-lactam ring of this drug, rendering it ineffective. Furthermore, the organism have the ability acquire extra chromosomal elements including plasmid and transposons; resulting in the acquisition and spread of *mecA* gene by these organisms [2]. Generally, cefoxitin resistance in *S. aureus* has been reported to be due to PBP2a; a penicillin-binding protein found in the bacterial cell wall with a low binding affinity to β-lactam enzyme encoded by *mecA* gene. This may be suggestive that *mecA* gene not only mediates cefoxitin resistance but also influence resistance of *S. aureus* strains to other antibiotics. However, the mechanism of resistance of these isolates was not investigated in this study. This considerable resistance was further confirmed by the high MIC and MBC of 1:32 and 1:16, respectively. This is in line with the 1:32 reported by Obajuluwa, [21].

Staphylococcus aureus strains employed in this study also exhibited resistance to other β-lactam drugs such as ampiclox (75%) and amoxil (65%). The high level of resistance to these β-lactam agents further confirms the ability of these organisms to excrete extracellular enzymes against the β-lactam drugs. This observation is consistent with that of Chambers, [22] who stated that resistance to the β-lactam antibiotic cefoxitin implies resistance to other members of the class.

Cefoxitin resistance is a good indicator of MRSA and this is usually confirmed by the presence of *Mec A* genes [2,23-26]. Thus, the existence of cefoxitin-resistant *S. aureus* which are susceptible to non β-lactam antibiotics including

those mentioned above could present a possibility of these drugs being employed for management of CRSA-related infections.

4.1 Conclusion and Recommendations

This study revealed a high level of resistance of *S. aureus* to cefoxitin. In addition, isolates also exhibited resistance to other routinely used antibiotics employed in this study. The isolation and treatment of CRSA positive patients, high risk patients, screening of an index case is advocated. Furthermore, implementing control measures including hand hygiene, proper sanitation, wearing of disposable aprons and gloves among others could help prevent and control the spread of cefoxitin-resistant *Staphylococcus aureus* (CRSA) in the study area.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was sought for and obtained from the management of the University of Calabar Medical Center.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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