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Research Article

Antibiogram of Staphylococci Isolates from Orthopaedic Patients

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Abstract

Background: Staphylococci has been implicated on bone infections and other infections worldwide. The increasing rate of these bacterial species to antibiotics is also alarming.

Objective: The aim of this study was to isolate Staphylococci from orthopaedic patients in a tertiary institution in Zaria, Kaduna State, Nigeria, and to compare the antibiotics susceptibility pattern of both the coagulase positive and coagulase negative Staphylococci.

Method: A total number of 104 Staphylococci isolates were collected from orthopaedic patients, biochemical tests were carried out to characterize these isolates into coagulase positive (Staphylococcus aureus) and coagulase negative Staphylococci. Disc agar diffusion method was used to determine the antibiotics susceptibility while the resistance pattern was classified as either multidrug resistance (MDR), extended drug resistance (XDR) or pandrug resistance (PDR).

Results: Out of the 104 Staphylococci isolates, 40 (38.5%) were coagulase positive, out of which 25 (62.5%) were methicillin-resistant *Staphylococcus aureus* (MRSA). Of the 64 (61.5%) coagulase negative Staphylococci, 49 (76.6%) were methicillin-resistant coagulase negative Staphylococci (MRCoNS).The coagulase positive isolates were more susceptible to antibiotics than the coagulase negative Staphylococci. Resistance pattern showed 11 (44%), 3 (12%) and 1 (4%) of the MRSA isolates were MDR, XDR, and PDR respectively while 14 (28.6%), 3 (6.1%) and 2 (4.1%) of MRCoNS were MDR, XDR, and PDR respectively.

Conclusion: High prevalence of MRSA and MRCoNS was observed and the isolates were generally resistant to beta lactam antibiotics used in this study.

Keywords: Staphylococci; Methicillin-Resistant Staphylococcus aureus; Methicillin–Resistant Coagulase Negative Staphylococci, Multi-Drug Resistance

Introduction

Staphylococci are Gram positive cocci, non-motile, non-spore forming and aerobic or facultative anaerobic except *Staphylococcus saccharolyticus* which is an obligate anaerobe. Staphylococci are members of the newly formed family, Staphylococcaceae [1]. They can be differentiated by coagulase production. An example of coagulase producing Staphylococci is *Staphylococcus aureus*, examples coagulase negative Staphylococci (CoNS) are *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* [2]. *Staphylococcus epidermidis* has been reported to cause various health care acquired or nosocomial infections while *Staphylococcus saprophyticus* is associated with urinary tract infections especially in adolescent girls and young women, Staphylococcus haemolyticus is occasionally recovered in wounds, septicaemia and urinary tract infections [3-5].

Staphylococcus aureus causes diseases ranging from mild skin infections (including boils, impetigo, carbuncles) to serious systemic diseases such as pneumonia, bacteremia, wounds, osteomyelitis [6]. *Staph aureus* especially MRSA is known for its ability to develop resistance to several antibiotics, this is a major health care problem worldwide. Current estimate in the USA indicates that MRSA causes approximately 95000 invasive infections and 19000 mortality cases per year [7,8]. Multi-drug resistant MRSA are also prevalent in some African countries e.g. Nigeria, Morocco, Kenya and Cameroon [9].

Methicillin-resistance is also frequently observed with coagulase negative Staphylococci especially in surgical site infections and device associated infections [10,11].

In orthopaedics the genus *Staphylococcus* are the principal causative agents of septic arthritis and osteomyelitis which involve inflammatory destruction of joint and bone, they are often difficult to manage and may cause serious morbidity [12].

Aim of the Study

The aim of this study was to isolate Staphylococci from skin and beddings orthopaedic patients in Ahmadu Bello University Teaching Hospital, a tertiary institution in Zaria, Kaduna State, Nigeria, and to compare the antibiotics susceptibility pattern of both the coagulase positive and coagulase negative Staphylococci.

Materials and Methods

Isolation and Classification of Staphylococci strains

After obtaining patients' consent, 179 clinical samples were collected from the wound, skin and beddings of orthopaedic patients in Ahmadu Bello University Teaching hospital, Zaria, Nigeria. Gram staining and other biochemical tests including catalase, coagulase and deoxyribonuclease tests were carried out using the methods described by Cheesbrough [13]. Isolates that were Gram positive cocci, catalase positive, coagulase positive and deoxyribonuclease positive were classified as coagulase positive Staphylococci while isolates that were Gram positive cocci, catalase positive, deoxyribonuclease negative and coagulase negative were classified as coagulase negative Staphylococci.

Detection of Methicillin Resistance

Cefoxitin disc diffusion method was used according to Clinical Laboratory Standards Institute (CLSI) recommendation [14].

Antibiotics Susceptibility Test

Antibiotics susceptibility test of all the Staphylococci isolates were carried out using agar disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke) according to the Clinical and Laboratory Standards Institute (CLSI) [14]. The following antibiotics was used: Cefoxitin 30 µg, Ceftriaxone 30 µg, Vancomycin 30 µg, Ampicillin 10 µg, Gentamicin 10 µg, Pefloxacin 5 µg, Ciprofloxacin 5 µg, Amoxicillin-clavulanic acid 30 µg, Erythromycin 15 µg and Clindamycin 2 µg (Oxoid Ltd. Basingstoke, London).

Determination of Resistance Pattern of MRSA and MRCoNS Isolates.

This was done in accordance with the standard given by Joint initiative by the European Centre for Disease Prevention and Control (ECDC), and the Centre for Disease Prevention and Control (CDC) for proper description of multidrug resistant (MDR), extensively drug-resistant (XDR) and pandrug resistant (PDR) profiles, in their conclusion, acquired, non-susceptibility to at least one agent in three or more antimicrobial categories were considered MDR, while non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two antimicrobial categories) were considered XDR. PDR was considered as non-susceptibility to all agents in all antimicrobial categories [15,16].

Results

Distribution of Staphylococci isolates and Methicillin resistance

Total number of Staphylococci isolates were 104, 40 (38.5%) were coagulase positive (*Staphylococcus aureus*) while 64 (61.5%) were coagulase negative Staphylococci. Methicillin resistance prevalence was high, out of the *Staphylococcus aureus* isolates, 25 (62.5%) were methicillin-resistant while 49 (76.6%) were methicillin- resistant coagulase negative Staphylococci.

Antibiotics Susceptibility test

The result of the antibiotics susceptibility testing of all the Staphylococci isolates showed the highest activity with gentamicin (95.2%), ciprofloxacin (94.2%) and vancomycin (88.5%) as presented in table 1.

	No of Staphylococci isolates (%) n = 104		
Antibiotics	**Sensitive**	**Intermediate**	**Resistant**
Ampicillin 10 µg	2 (1.92)	-	102 (98.1)
Ceftriaxone 30 µg	26 (25.0)	23 (22.1)	55 (52.9)
Vancomycin 30 µg	92 (88.5)	-	12 (11.5)
Cefoxitn 30 µg	30 (28.8)	-	74 (71.2)
Amoxicillin-clavulanic acid 30 µg	38 (36.5)	-	66 (63.5)
Erythromycin 15 µg	38 (36.5)	32 (30.8)	34 (32.7)
Clindamycin 2 µg	26 (25.0)	27 (26.0)	51 (49.0)
Ciprofloxacin 5 µg	98 (94.2)	5 (4.8)	1(1.0)
Pefloxacin 5 µg	88 (84.6)	2 (1.9)	14 (13.5)
Gentamicin 10 µg	99 (95.2)	2 (1.9)	3 (2.9)

Table 1: Antibiotics susceptibility of all the Staphyloccoci isolates.

The antibiotics susceptibility of Staphylococcus aureus and coagulase negative Staphylococci isolates showed high level of resistance to ampicillin (95:96.9%), ceftriaxone (77.5:73.4%), clindamycin (67.5:79.7%), cefoxitin (62.5%:76.6%), erythromycin (50:70.3%). The detail is as shown in figure 1. However, higher level of resistance was observed with MRSA and MRCoNS isolates (Figure 2).

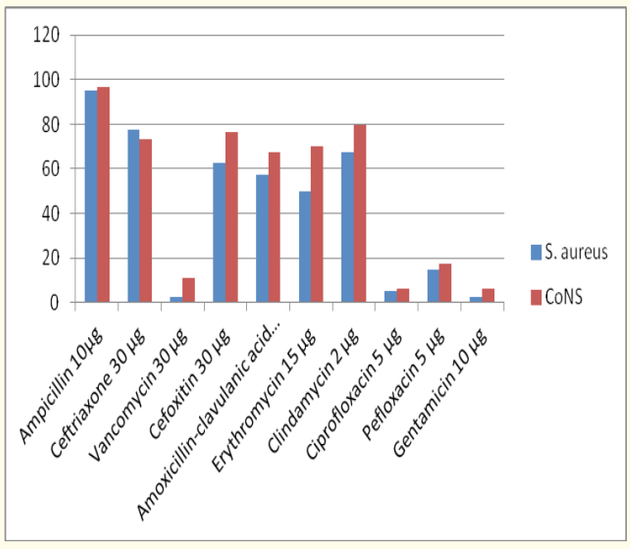

Figure 1: Comparison of percentage antibiotic resistant pattern of *S. aureus* and CoNS isolates.

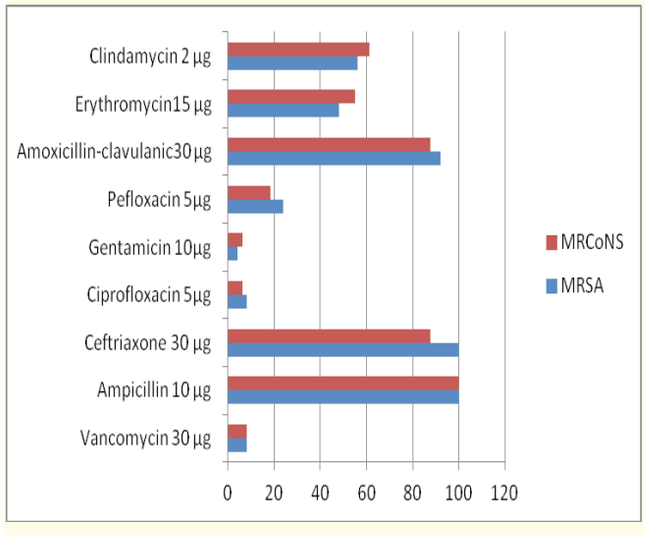

Figure 2: Comparison of antibiotic resistance percentage in MRSA and MRCoNS isolates.

Further analysis of the resistance pattern showed that 11 (44%), 3 (12%) and 1 (4%) of the MRSA isolates were MDR, XDR, and PDR respectively while 14 (28.6%), 3 (6.1%) and 2 (4.1%) of MRCoNS were MDR, XDR, and PDR respectively (Table 2).

Resistance Level	MRSA	MRCoNS
Multi-drug resistant (MDR)	11 (44%)	14 (28.6%)
Extended –drug resistant XDR)	3 (12%)	3 (6.1%)
Pan-drug resistant (PDR)	1 (4%)	2 (4.1%)

Table 2: Resistance level of MRSA and MRCoNS isolates.

Discussion

Staphylococci species especially *Staphylococcus aureus* has over the decades emerged as a very important human pathogen, a leading cause of nosocomial infections [17]. Both the Staphylococcus aureus and coagulase negative staphylococci species were isolated from the skin and beddings of the orthopaedic patients; this is a clear indication that staphylococci species are possible cause of nosocomial infections. Several previous studies are also in support of this [18-20].

In this study, the staphylococci isolates were generally susceptible to gentamicin (aminoglycosides) ciprofloxacin (fluoroquinolones) and vancomycin (glycopeptides) and highly resistant to beta lactam antibiotics used that is ampicillin, amoxycillin-clavulanic acid and ceftriaxone. The difference in the mechanism of action of these various classes of antibiotics might be responsible for the various activities observed. Beta lactams inhibit bacteria cell wall synthesis while aminoglycosides and fluoroquinolones inhibit protein synthesis and DNA synthesis respectively [21,22]. *Staphylococcus aureus* produces beta lactamase enzymes which hydrolyses the beta lactam ring rendering the antibiotics ineffective [23]. This might be responsible for high level of resistance to beta lactam antibiotics observed in this study which is in correlation with a previous study [19]. In Nigeria, beta lactam antibiotics are generally prescribed and may even be obtained over the counter without prescription and this gives room for its being abused. Also inability to follow a complete dosage regimen can lead to antibiotic resistance all these may have also contributed to the high level of resistance to beta lactams and other antibiotics observed. High prevalence of MRSA and MRCoNS was observed in this study this is in accordance with previous in some parts of Nigeria [24-27]. Methicillin-resistant *Staphylococcus* are now major problem in hospitals and the rate of their incidence is rising considerably in recent years [28]. Comparing the resistance pattern of MRSA and MRCoNS, a higher percentage MDR was observed in MRSA (44%) this shows a possibility that MRSA isolates may be more virulent than MRCoNS even though this is beyond the scope of our study; however a previous study reported higher mortality rate in MRSA than MRCoNS infection [29]. The increasing rate of prevalence of MRSA and MRCoNs isolates is alarming especially in developing countries like Nigeria where this study was carried out. Increase rate of drug (antibiotics) abuse and misuse in our society might have also contributed to high level of multidrug resistance observed.

Gentamicin was highly active against the MRSA and MRCoNS isolates followed by ciprofloxacin and vancomycin; the dosage form for gentamicin is injection this may make its abuse difficult.

Conclusion

There was high prevalence of MRSA and MRCoNS isolates in this study; the isolates were generally resistant to beta lactam antibiotics. There is a great need for intensive campaign against misuse and

abuse of antibiotics in our society, in like manner the government should enforce restricting the prescribing and dispensing of antibiotics to professionals alone. Infection control measures is highly recommended in our hospitals and community in order to reduce the incidence of methicillin resistance.

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