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## Prevalence and resistance pattern of methicillin-resistant coagulase negative Staphylococci among orthopaedic patients in a tertiary institution in North western Nigeria

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### Abstract

**Background:** Increasing rate of antibiotic resistance in coagulase negative Staphylococci is of great concern especially methicillin – resistant coagulase negative Staphylococci (MRCoNS). It causes increased mortality, morbidity and increased health care costs

**Objective:** The aim of this study was to determine the prevalence of methicillin resistance among the coagulase negative Staphylococci isolates from orthopaedic patients in Ahmadu Bello University Teaching Hospital in North-west Nigeria and their resistance pattern to antibiotics.

**Method:** In this study, 114 clinical samples were collected from orthopaedic patients in Ahmadu Bello University Teaching Hospital, located in North West Nigeria. Biochemical tests were carried out to isolate the coagulase negative Staphylococci while Cefoxitin disc diffusion test was used to determine their methicillin resistance. Antibiotic susceptibility test was carried out using disc agar diffusion method.

**Results:** Using biochemical test 74 Staphylococci isolates were confirmed to be coagulase negative, 48 out of CoNS isolates were found to be methicillin resistant. The antibiotics susceptibility test carried out on the MRCoNS isolates showed the resistance pattern to be in this order: ampicillin (100%), amoxicillin-clavulanic acid (87.5%), ceftriaxone (87.5%), clindamycin (60.5%) and erythromycin (54.2), 39.6% were multi-drug resistant.

**Conclusion:** High prevalence of methicillin-resistant coagulase negative staphylococci was observed in this study. The MRCoNS were generally resistant to beta lactam antibiotics.

**Keywords:** Coagulase, Staphylococci, orthopaedics, methicillin resistance.

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### Introduction

Coagulase negative Staphylococci (CoNS) belong to the family Micrococcaceae and several species has been identified. Since the 1970s, it has been suggested that CoNS are of great importance as true pathogens, and they have become a common cause of a wide

variety of infections (Dubois *et al.*, 2010; Otto, 2009). CoNS colonize different parts of human skin and mucous membrane, this is the major source of endogenous infection by CoNS (Roth and James, 1988, Becker *et al.*, 2014). They are associated with

the use of implant, once inserted, foreign bodies can become colonized by CoNS and the success of the respective medical procedure is significantly impaired, resulting in enormous medical and economic burdens. They have also been recognised as the cause of prosthetic valve endocarditis, neurosurgical shunt infection and infection of prosthetic orthopaedic devices (Huebner & Goldmann, 1999; Zong *et al.*, 2011)

Increasing rates of antibiotic resistance in CoNS is a great problem and this is limiting our therapeutic options. Methicillin resistance in Staphylococci emerged from the acquisition of the *mecA* gene encoded by penicillin-binding protein 2a (PBP2a), a transpeptidase conferring broad-spectrum beta-lactam resistance (IWG-SCC, 2009). Over the last decade, methicillin resistant Staphylococci strains have become endemic in hospitals worldwide. Also, they are now an endemic community pathogen in many geographical regions (Tiwari and Sen, 2006; Lowy, 1998), the strains have also been reported to be implicated in causing progressively increased mortality, morbidity and increased health care costs (Cosgrove *et al.*, 2003; Tiwari *et al.*, 2008).

This study was aimed at determining the prevalence of methicillin-resistant coagulase negative Staphylococci among the orthopaedic patients in Ahmadu Bello University and their antibiotics resistance pattern.

## Materials and Methods

### Isolation and classification of Staphylococci

After obtaining consent clinical samples were collected aseptically from the skin, beddings and wounds of orthopaedic patients in Ahmadu Bello University Teaching Hospital, North-west Nigeria. Biochemical tests were performed on the purified isolates to classify the Staphylococci isolates based on their coagulase properties. API STAPH identification kit (bioMerieux, Inc., Durham, NC) was used to further characterize the coagulase negative Staphylococci into species.

### Detection of methicillin resistance

Clinical Laboratory Standards Institute (CLSI) has recommended cefoxitin disc diffusion method for the detection of Methicillin-resistant *Staphylococcus aureus* due to its ability to enhance induction of PBP2a. A 0.5 Mc Farland standard suspension of the isolate was made and a lawn culture was done on Mueller Hinton Agar plate. Cefoxitin 30 µg discs were

placed on the plates and incubated at 37 °C for 18 hours after which zone of inhibition diameter was measured in reflected light. An inhibition zone diameter 21 mm was reported as methicillin resistant and 22 mm was considered as methicillin susceptible (CLSI, 2013).

### Antibiotics susceptibility test

Kirby Bauer disk diffusion method was used to determine the antibiotics susceptibility test of the methicillin - resistant CoNS isolates according to the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute., 2012). The following antibiotics was used: Cefoxitin 30µg, Ceftriaxone 30µg, Vancomycin 30µg, Ampicillin 10µg, Gentamicin 10µg, Pefloxacin 5µg, Ciprofloxacin 5µg, Amoxicillin-clavulanic acid 30µg, Erythromycin 15µg and Clindamycin 2µg (Oxoid Ltd. Basingstoke, London).

### Determination of resistance pattern of MRCoNS isolates

Joint initiative by the European Centre for Disease Prevention and Control (ECDC), and the Centre for Disease Prevention and Control (CDC) gave a standard method 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 (Magiorakos *et al*, 2012, Silpi *et al*, 2016). This was used as the standard for the characterization of resistance profile of MRCoNS isolates in this study.

## Results

### Isolation and classification of Staphylococci

A total number of 114 samples were collected from the orthopaedic patients, with the use of conventional methods 74 (64.1%) were confirmed to be coagulase negative Staphylococci. The characterization of the CoNS isolates is presented in Table 1, 10(13.5%) were undetermined.

**Table 1: Identification of Staphylococci species**

Staphylococci species	Number of isolates (%)
<i>S. xylosus</i>	23 (31.1)
<i>S. lentus</i>	8 (10.8)
<i>S. hominis</i>	8 (10.8)
<i>S. cohnii cohnii</i>	4 (5.4)
<i>S. epidermidis</i>	3 (4.1)
<i>S. cohnii ureal.</i>	2 (2.7)
<i>S. lugdunensis</i>	2 (2.7)
<i>S. hyicus</i>	2 (2.7)
<i>S. caprae</i>	1 (1.4)
<i>S. warneri</i>	1 (1.4)
<i>S. sciuri</i>	1 (1.4)
<i>S. chromogens</i>	1 (1.4)
<i>S. capitis</i>	1 (1.4)
<i>S. haemolyticus</i>	1 (1.4)

### Antibiotics susceptibility

Gentamicin and ciprofloxacin were the most active (95.8%) (Table 2)

**Table 2: Antibiotic susceptibility of MRCoNS**

Antibiotics	No of CoNS isolates (%) n= 48		
	Resistant	Intermediate	Sensitive
Vancomycin	3 (6.3)	-	45 (93.8)
Ampicillin	48 (100)	-	-
Ceftriaxone	34 (70.8)	8 (16.7)	6 (12.5)
Amoxicillin-clavulanic	42 (87.5)	-	6 (12.5)
Pefloxacin	7 (14.6)	1 (2.1)	40 (83.3)
Gentamicin	1 (2.1)	1 (2.1)	46 (95.8)
Erythromycin	12(25.0)	14 (29.2)	22 (45.8)
Clindamycin	14 (29.2)	15 (31.3)	19 (39.6)
Ciprofloxacin	1 (2.1)	1 (2.1)	46 (95.8)

### Detection of methicillin resistance

Methicillin resistance was detected in 48 (64.9%) out of the 74 CoNS isolates

### Resistance pattern of MRCoNS isolates

Out of the 48 MRCoNS, 14 (29.2%) were multi-drug resistant (Fig. 1). The MRCoNS isolates were generally resistant to beta-lactam antibiotics.

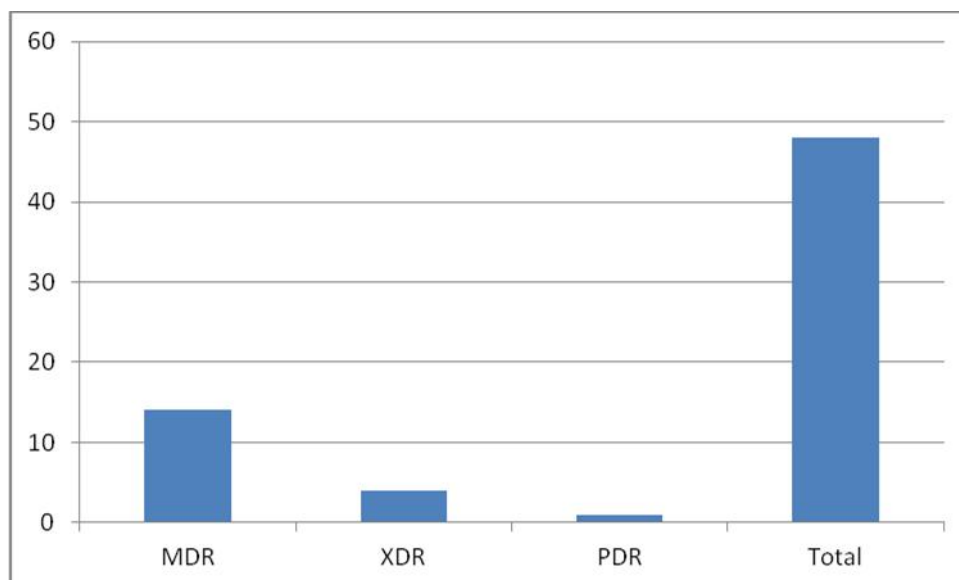


Fig.1: Incidence of MDR, XDR and PDR among the MRCoNS isolates

## Discussion

Prevalence of methicillin resistance observed in this study was high (64.9%), in Nigeria where this research was carried out there were reports on methicillin-resistant *Staphylococcus aureus* but not much has been reported about methicillin-resistant coagulase negative Staphylococci in clinical setting. However the prevalence observed in this study was consistent with the reports from other parts of the world: 67.5% prevalence of MRCoNS was reported from blood cultures of septicaemia patients in Turkey (Ko Ksai et al, 2009) and 45% prevalence from surgical wounds (Rahman et al, 2013), 52% from a tertiary care hospital in Iran (Sharma et al, 2010). Iyamba et al, 2014 reported 60% prevalence of MRCoNS from surgical site infections from Kinshasa. The high prevalence of MRCoNS should be of great concern to health institutions and the community at large due to its antibiotics multi-resistance challenge and the financial burden on the in-patients especially here in Nigeria, a developing country.

Antibiotic resistant coagulase negative Staphylococci has emerged as a major cause of morbidity and mortality in hospital setting. The level of antibiotics resistance in MRCoNS isolates observed in this study is high, this is an indication of possible misuse of antibiotics by the patients before hospital admission or possible acquiring of resistant strains through nosocomial infections since coagulase negative staphylococci has been reported to be an agent of nosocomial infections (Washington et al, 2001). There is a great need for a campaign against misuse and abuse of antibiotics in our society.

The isolates were generally resistant to beta lactam antibiotics, lincosamine and macrolides which are cheap readily available antibiotics. In a report by Diekema et al, 2001, resistance to penicillin among the coagulase-negative staphylococci (CoNS) approaches 90 to 95 percent, resistance to methicillin and semisynthetic penicillins has been observed in more than 80 percent of CoNS isolates; these isolates are often resistant to multiple classes of antibiotics in addition to beta-lactams, therefore limiting traditional treatment options, like those with semi-synthetic penicillins. Iyamba et al, 2014 reported high resistant of MRCoNS isolates to ampicillin (beta lactam), erythromycin (macrolide), clindamycin (lincosamine) and ciprofloxacin.

The multidrug resistance observed may also be probably due to the ability of MRCoNS strains to produce biofilms. Staphylococci are known as especially good biofilm formers, which is due primarily to the production of a series of surface molecules that promote extracellular matrix formation (Otto, 2008). However, high antibiotics susceptibility was observed with gentamicin (95.8%), ciprofloxacin (95.8%) and vancomycin (93.8%). Even though vancomycin is a drug of choice in the treatment of methicillin-resistant infections (Whitener et al, 2004) 6.2% resistance was observed in this study, this correspond with previous reports from Nigeria (Alo et al, 2013, Obajuluwa et al, 2016) even though vancomycin is not a readily available drug in the country.

## Conclusion

In this study high prevalence of methicillin-resistant coagulase negative staphylococci was observed therefore importance should be accorded to the isolation of coagulase negative and their antibiotics susceptibility studies. Also there is the need for proper surveillance studies and preventive measures in our hospitals to control the emergence and spread of MRCoNS. It suggested from the findings in this study that gentamicin and ciprofloxacin be used in the treatment of methicilli-resistant coagulase negative staphylococcal infections.

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