Prevalence and antimicrobial susceptibility pattern of coagulase-negative staphylococci obtained from nares of adult patients admitted to Ahmadu Bello University Teaching Hospital (ABUTH), Zaria

Cecilia T. Atolagbe, Babajide A. Tytler, Olanrewaju Jimoh, Adebola T. Olayinka and Busayo O. Olayinka

ABSTRACT

Coagulase-negative staphylococci (CoNS) are part of the normal microbial flora of the skin and mucous membranes. Nasal colonization with antibiotic-resistant CoNS represents both a risk factor for the colonized individual and their immediate contacts. This study determines the antibiotics susceptibility pattern and resistance phenotypes to a specific group of antimicrobial agents in CoNS isolate from the nares of adult patients from Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria. One hundred and twenty-three presumptive staphylococci isolate from nasal colonization surveillance cultures of adult patients were collected from the diagnostic medical microbiology laboratory of ABUTH, Zaria and were characterized using standard microbiological procedures and their susceptibility to commonly used antimicrobial agents determined using the guideline of European committee on antimicrobial susceptibility testing (EUCAST). A total of 60 of the 123 (49%) staphylococcal isolate were CoNS. Characterization of the sixty CoNS isolate showed that the most predominant species were S. chromogenes (30.0%), S. xylosus (15.0%) and S. schleiferi subsp. coagulans (13.3%). The highest level of resistance was observed to Cefoxitin (95.0%) and the lowest to Tigecycline (1.7%). S. epidermidis isolates were observed to show ≥60% resistance to all tested antibiotics with the exception of Tigecycline (0%) and Gentamicin (20%). Analysis of the multiple antibiotic resistance index (MARI) showed that majority (96.7%) of the isolates were resistant to 3 or more of the antimicrobial agents. No isolate was resistant to all the tested antimicrobial agents. A very high proportion of the CoNS isolates were resistant to cefoxitin, penicillin, tetracycline, amoxicillin-clavulanate, and vancomycin. In conclusion, the high proportion of isolates with MARI of 0.3 and above indicates a high level of antibiotic use or exposure in the study area. These findings revealed the need for continued surveillance for resistant phenotype to inform clinical therapy decisions.

Keywords: Coagulase-negative staphylococci (CoNS), methicillin-resistant – coagulase-negative Staphylococci (MRCoNS), multiple antibiotic resistance index (MARI), antimicrobial agents

1.0 Introduction

Antimicrobial resistance in most human pathogens is increasing worldwide and if the trend continues, the repercussion for public health and for the general community could be disastrous [1]. Coagulase-negative staphylococci (CoNS), a prominent part of the normal microbial flora of the skin and mucous membranes, can colonize the nasal mucosa, the lower airways and invasive devices making colonization a risk factor for infection [2]. CoNS are increasing resistant nosocomial pathogens and methicillin-resistant coagulase-negative staphylococci (MRCoNS) have emerged as the most important cause of hospital-acquired infections (HAI) especially in the intensive care unit (ICU), in
the elderly and immunocompromised patients [3]. Long period of stay or admission in the hospital and the use of medical devices also increase the chances of infection by these opportunistic pathogens [3, 4]. CoNS have a greater tendency to develop multidrug resistance, this characteristic is important in their ability to cause infection [5-7].

The number of CoNS strains isolated from the hospitals is constantly increasing and so is the medical importance due to the presence of several antimicrobial resistance genes making treatment very challenging [8]. This is why there is need for continued surveillance for resistance phenotype.

The purpose of this study was to determine the pattern of resistance in the nasal isolate from the surveillance culture obtained from Ahmadu Bello University Teaching Hospital (ABUTH) to antimicrobial agents that should be used in the treatment of infection and also determine the prevalence of their multidrug resistance.

2.0 Methodology

2.1 Ethical Clearance

Ethical clearance/approval for this work was obtained from the ethical committee of the Ahmadu Bello University committee for Research in human subjects.

2.2 Isolate Collections

A total of 123 stored suspected staphylococci isolate were collected into sterile nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) from the diagnostic medical microbiology laboratory of the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State and processed at the Pharmaceutical Microbiology laboratory of Ahmadu Bello University (ABU), Zaria, Kaduna State.

2.3 Isolation and Characterization of the isolate

Overnight growths (37°C for 18 hours) from the nutrient broth were streaked onto sterile blood agar (Oxoid Ltd., Basingstoke, Hampshire, England) and mannitol salt agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates and incubated at 37°C for 24 hours. Colonies with hemolysis and distinct features that appeared round, raised and opaque with 1-2mm in diameter on the respective media were subcultured on nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, England) in order to obtain pure colonies. Isolates were characterized conventionally based on the method by Cheesebrough [9].

Preliminary identification of the isolates was based on their cultural characteristics and confirmed using standard biochemical tests provided by the Microgen Staph ID kit (Microgen Bioproducts, UK) [10].

2.4 Antimicrobial Susceptibility testing

The antimicrobial susceptibility pattern of the CoNS isolate was investigated using Modified Kirby Bauer disk diffusion method on Mueller Hinton agar (Oxoid Ltd. Basingstoke, London) and interpreted according to the European committee on antimicrobial susceptibility testing [11] breakpoints. The CoNS strains were tested for their sensitivity to a panel of antimicrobial agents; Benzylpenicillin (P, 1 unit), Ciprofloxacin (CIP, 5μg), Amoxicillin-Clavulenate (AMC, 30μg), Gentamicin (GEN, 10μg), Vancomycin (VAN, 1gm), Clindamycin (DA, 2μg), Erythromycin (ERY, 15μg), Quinupristin-dalfopristin(QD, 15μg), Tigecycline (TGC, 15μg), Tetracycline (TET, 30 μg), Linezolid (LZD, 10μg) (all from Oxoid Ltd. Basingstoke, London). The minimum inhibitory concentrations (MIC) value for Vancomycin was determined using broth microdilution method as described by Balouiri et al [12] and interpreted according to EUCAST, 2019.

2.5 Determination of MAR Index

The multiple antibiotics resistance (MAR) index was determined for each isolates by dividing the numbers of antibiotics to which the isolate is resistant to by the total number of antibiotic tested Paul et al [13].

3.0 Results

3.1 Identification of the isolates

Of the One hundred and twenty-three (123) presumptive staphylococci isolate, 66 (53.7%) were confirmed coagulase-negative staphylococci (CoNS) and 39 (31.7%) as coagulase-positive staphylococci (CoPS) and 12 showed no growth on blood agar. 6 out of the 66 that were confirmed CoNS were positive rods during Gram reaction. The 60 CoNS were further characterized. Species differentiation is shown in Table 1; Staphylococcus chromogenes (30%) were the most prevalent organisms, followed by S. xylosus (15%).
Table 1: Identification of staphylococci species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. chromogenes</td>
<td>18(30.0)</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>09(15.0)</td>
</tr>
<tr>
<td>S. schleiferi subsp.coagulans</td>
<td>08(13.3)</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>06(10.0)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>05(8.3)</td>
</tr>
<tr>
<td>S. caprae</td>
<td>04(6.7)</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>04(6.7)</td>
</tr>
<tr>
<td>S. simulans</td>
<td>04(6.7)</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>01(1.6)</td>
</tr>
<tr>
<td>S. hominis subsp. Hominis</td>
<td>01(1.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>60</strong></td>
</tr>
</tbody>
</table>

Figure 1: Antibiotic resistance profile of the isolates

3.2 Resistance pattern of the isolates

The resistance profile of CoNS isolate to test antibiotics is as shown in Figure 1, the highest level of resistance was observed against Cefoxitin (95.0%) and the lowest was recorded against Tigecycline (1.7%).

3.3 Antibiotic resistance classification

The CoNS isolate were categorized based on the International Expert Proposal on Interim Standard Definitions for acquired resistance [14] as Multi-drug resistance MDR (55.0%), Extensively-drug resistance (XDR) (33.3%) and Pan-drug (PDR) (8.3%) (Table 2).
3.4 Multiple antibiotic resistance index (MARI)

Analysis of the MAR index showed that 96.7% of the isolates were resistance to 3 or more antimicrobial agents (MARI ≥0.3) which is an indication of high level of antibiotic use or exposure in the study (Table 3).

3.5 Occurrence of methicillin-resistance coagulase-negative staphylococci (MRCONS)

In this study, resistance to cefoxitin was used in classifying isolates as methicillin-resistance coagulase-negative staphylococci (MRCONS) and methicillin-sensitive coagulase-negative staphylococci (MSCoNS). Table 4 shows that a total of 57 (95.0%) of CoNS isolate tested were MRCONS and 3 (5%) were MSCoNS.

Table 2: Antibiotic resistance classification of coagulase-negative staphylococci isolate

<table>
<thead>
<tr>
<th>S/N</th>
<th>Resistance Classification</th>
<th>Number of Isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MDR</td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>XDR</td>
<td>20</td>
<td>33.3</td>
</tr>
<tr>
<td>3</td>
<td>PDR</td>
<td>05</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
<td>Non-MDR</td>
<td>02</td>
<td>3.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

Keys: MDR = multidrug-resistant; XDR = extensively drug-resistant, PDR= Pandrug-resistant

Table 3: Multiple antibiotic resistance index (MARI) of coagulase-negative staphylococci isolate from nasals of adult patients in Zaria, Nigeria

<table>
<thead>
<tr>
<th>MARI</th>
<th>Distribution</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>01</td>
<td>1.7</td>
</tr>
<tr>
<td>0.2</td>
<td>01</td>
<td>1.7</td>
</tr>
<tr>
<td>0.3</td>
<td>11</td>
<td>18.3</td>
</tr>
<tr>
<td>0.4</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td>0.5</td>
<td>12</td>
<td>20.0</td>
</tr>
<tr>
<td>0.6</td>
<td>04</td>
<td>6.7</td>
</tr>
<tr>
<td>0.7</td>
<td>11</td>
<td>18.3</td>
</tr>
<tr>
<td>0.8</td>
<td>09</td>
<td>15.0</td>
</tr>
<tr>
<td>0.9</td>
<td>01</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100%</td>
</tr>
</tbody>
</table>

MARI: Multiple antibiotic resistance index

96.7%
Table 4: Occurrence of methicillin-resistance coagulase-negative staphylococci (MRCoNS)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Methicillin resistance classification</th>
<th>Number of Isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methicillin-resistance coagulase-negative staphylococci</td>
<td>57</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Methicillin-sensitive coagulase-negative staphylococci</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>60</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

3.6 macrolide, lincosamide and streptogramin B (MLSB) resistance phenotypes of the isolates

Erythromycin and clindamycin were used to determine the macrolide-lincosamide-streptogramin B resistance (MLSB) of the isolates. Among the 60 isolate, 6(10%) showed inducible resistance (iMLSB), 21(35%) showed constitutive clindamycin resistance (cMLSB), 6(10%) showed erythromycin resistance (MS) and 3(5%) showed true sensitivity to clindamycin. The percentage resistance is shown in Figure 2. The CoNS isolate susceptible to both clindamycin and erythromycin were 24(40%).

Figure 2: Macrolide, lincosamide and streptogramin B (MLSB) resistance phenotypes of the isolates

4.0 Discussion

Coagulase-negative staphylococci (CoNS) were considered as non-pathogenic bacteria but are now known to be opportunistic organism, responsible for various nosocomial infections [15]. Colonized individuals are potential reservoirs for transmission of CoNS infections, which could be detrimental to public health especially when antibiotic resistant strains are present [16].

In this study, among the 123 presumptive staphylococci isolate collected, the prevalence of CoNS nasal colonization is 53.7%. The reported isolation of CoNS varies with different authors. Percentages reported range from 14.8% in a study in Jordan (17), 49% in Iran (18), 52.14% in India (19), 57.4% in China (20), 60.6% in Brazil (21) and 72% in Switzerland (22). The findings in this study fall within the range reported. Prevalence of nasal carriage of CoNS varies by country, demographic group, and medical condition.
The most prevalent CoNS in the nares of adult patients in this study is *Staphylococcus chromogenes* (30%), followed by *S. xylosus* (15.0%), *S. schleiferi* subsp. *coagulans* (13.3%), *S. hyicus* (10.0%), while *S. epidermidis* made up 8.3% of the organisms. Findings differed from the report by Al-Tamimi et al. [17] who reported *S. haemolyticus* (8.4%), *S. sciuri* (3%), *S. epidermidis* (1%), *S. warneri* (1%), *S. hominis* (1%) as the most prevalent CoNS isolated from nostrils of hospitalized patients in Jordan. Sabzehali et al. [23] reported *Staphylococcus haemolyticus* (51.9%), *S. epidermidis* (21.7%), *S. lugdunensis* (9.8%) as the most prevalent CoNS isolated from hospitalized patients in Iran. Obajuluwa et al. [24] reported *S. xylosus* (31.1%), *S. lentus* (10.8%), *S. hominis* (10.8%), *S. cohnii cohnii* (5.4%), *S. epidermidis* (4.1%) as the most prevalent CoNS isolated from orthopaedic patients in Kaduna, Nigeria. The difference in the prevalent species may be due to variability in colonization by CoNS in diverse hospital settings owing to differences in antimicrobial pressure. Microbial communities are known to vary across various body sites, geographical locations, and sometimes between seasons.

Resistance rates above 50% were found to a number of antibiotics among the isolates from the nares of adult patients. Although, none of the tested isolate was 100% resistant to all the antibiotics tested. The least resistance was to Tigecycline (1.7%), the findings in this study is similar to the report by Shariati et al. [25] who reported 1.6% resistance. Tigecycline may be the drug of choice for the treatment of severe infections [26].

Resistance to penicillin in CoNS has been very high; resistance of CoNS to penicillin in this study is 81.7%. Resistance rates ranging from 81 – 100% has been reported. The percentage resistance in this study of 81.7% can be considered to be on the lower range. Morgenstern et al. [22] reported a similar percentage of 81.4% in a prospective point prevalence study in an international cohort of surgeons. Goudarzi et al. [18] reported 96.1% in the nasal cavity of hospital employees in Khorramabad, Iran. Al-Tamimi et al. [17] and Alharbi [27] both reported 100% resistance from the nostrils of hospitalized patients in Jordan and in the nasal cavity of patients in Saudi Arabia respectively. Resistance in this study could be due to production of the beta-lactamase enzymes.

Compared to the findings of this study, lower percentage resistance to gentamicin ranging from 6 - 25% have been reported (6.3%) [22], (11.1%) [28], (17.6%) [27] and (25%) [17].

The percentage resistance to ciprofloxacin in this study (51.7%) is higher than other reported studies which ranged from 10 - 41% (10.7%) [22], (22%) [29] and (37.5%) [17] and (41.2%) [27], although report of resistance rate as high as 96% have also been reported [18].

Resistance to Linezolod in this study is higher than that reported by Morgenstern et al. [22] (0.1%). Linezolid is currently used for treating glycopeptides and methicillin-resistance staphylococci. Linezolid resistance is uncommon among staphylococci but Tewhey et al. [30] and Mittal et al. [31] reported emerging resistance through mutation.

In this study, high percentage of vancomycin resistance was observed, with majority of CoNS isolate having MIC values above 4ug/ml [11]. Vancomycin is considered a drug of last resort for multidrug resistant staphylococci; hence observed resistance is worrisome and portends danger for treatment efficacy. Susceptibility of vancomycin were reported (0%) [22] and (0%) [27]. There have also been reports of reduced susceptibility of CoNS to vancomycin from studies in Nigeria (4mgL) and Egypt (2μg/mL) [32, 33].

Methicillin resistant Staphylococci spp pose a major health problem, as they are known to be generally resistant to many other antimicrobial agents [34]. The percentage of methicillin- resistant coagulase-negative staphylococci (MRCoNS) in this study is higher than that reported in Brazil (44.4%), Iran (42.8%) and Saudi Arabia (91.4%) [21, 18, 27]. The abundance of mobile genetic elements in staphylococci spp increases the risk of transfer of resistance determinants within the hospital setting [22]. Phenotypically, 57 (95%) of the 60 isolates tested showed methicillin resistance. Methicillin resistance has been associated with multiple resistances to other antibiotics [29]. The link between methicillin resistance and multiple drug resistance (MDR) was observed in this study.

Multiple antibiotic resistance index (MARI) helps in analyzing health risk, and is used to check the extent of antibiotic resistance [35]. In this study, 96.7% of the isolates had MARI of ≥0.3 when tested for susceptibility against 12 antibiotics. The MARI analysis also revealed that 15.0% of the isolates
had a very high MAR index value of 0.8, this indicates previous exposure to antibiotics and development of resistance to commonly prescribed antibiotics. This will make selection of antibiotics more difficult.

It was reported that a MAR index value greater than 0.2 indicates high risk source of contamination where antibiotics are often used [36]. This suggests that the CoNS isolates from the nares of adult patients would have spread from a niche of high antibiotic use. The classification of resistance profile as MDR (55%), XDR (33%) and PDR (8%) showed that resistance observed in the study could possibly be due to adaptation and gene transfer, as a result of antimicrobial pressure associated with antibiotic misuse or overuse. Michalik et al. [37] reported that CoNS in the nares are severe pathogens responsible for infections and associated with multiple antibiotics resistance. The high prevalence of methicillin resistance and multidrug-resistance in CoNS isolate necessitates surveillance of this emerging pathogen.

The percentage of resistance to Macrolides and Lincosamides (ML) antibiotics in this study is lower compared to that reported in Iran (erythromycin 87.7%, clindamycin 75.5%) [18]. However, it is higher than that reported in Switzerland (erythromycin 39.3%, clindamycin 23.6%) [22] and comparable to the report in Saudi Arabia (erythromycin 41.2%, clindamycin 35.3%) [27]. A higher prevalence of constitutive Macrolide, Lincosamide and Streptogramin B (MLSb) resistance (35%) was observed in this study compared to the inducible resistance (iMLSb) phenotype (10%). This could be due to high exposure over time to the antibiotics within the environment, leading to antimicrobial pressure and consequent inclusion of the _erm_ gene (which mediates constitutive MLS resistance) into the core genome. Similar reports were made in Brazil, where constitutive MLS resistance was 65.2% [38]. Macrolide, Lincosamide and Streptogramin B (MLSb) are one of the few therapeutic alternatives treatment of staphylococcal infections but the development of resistance especially inducible resistance with in-vitro testing and in-vivo during clindamycin therapy leading to therapeutic failure is a major concern.

It was also observed that all the isolates with inducible clindamycin resistance were MRCoNS. Debnath and Sande [39] reported similar findings that inducible clindamycin resistance was significantly higher in MRCoNS isolates as compared to methicillin-susceptible coagulase-negative staphylococci (MSCoNS).

5.0 Conclusions

There is need for more clinical data to access the clinical significance of the antimicrobial resistance encountered in this study. There is also need for consistent and continuous antimicrobial surveillance for important and commonly isolated clinically significant pathogens like MRCoNS to guide the development of antimicrobial therapy, establishing their role as significant pathogens and developing and implementing measures that can reduce antimicrobial resistance burden.

Conflict of Interest:

We have no conflict of interests.

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Author 5: BO Olayinka – Conceived and designed the analysis, contributed data or analysis tools, data analysis and interpretation, correction of draft, final approval of the version to be published.
Reference


