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Antimicrobial resistance pattern of Coagulase-negative staphylococci isolates from medical students of some Nigerian teaching hospitals

Etim Marylene E.[1], Eze Chinwe C.[1], Ikeanumba Michael O.[2] and Adieze Ifechukwu E[1*]
[1]Department of Microbiology, Federal University of Technology, P. M. B. 1526, Owerri, Imo State, Nigeria
[2]Department of Biology, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria
ifechukwu.adieze@futo.edu.ng

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Abstract

The antimicrobial resistance or otherwise of Coagulase-negative Staphylococci (CoNS) isolates from medical students of some South Eastern Nigerian Universities were determined. Swab samples were obtained from the students and were cultured for CoNS using standard microbiological methods. Isolates obtained were assayed for antibiotic resistance using disc diffusion method. MecA and blaZ genes on 533bp and 510bp respectively, of the isolates were amplify using the Polymerase Chain Reaction (PCR). Fingerprinting of isolates' genomes was also conducted by Randomly Amplified Polymorphic DNA. Results obtained showed that of the 200 students screened, 26 (13.0%) harbour CoNS. Observed resistance against the tested antimicrobials were as follows; trimethoprim (100%), oxacillin, penicillin, ampicillin (88.5%), clindamycin (61.5%), ciprofloxacin (57.7%), erythromycin and vancomycin (15.4%), and nitrofurantoin (0%). All the isolates showed multi-drug resistance to at least four antibiotics assessed. PCR amplification yielded no antibiotic resistant gene and fingerprinting by RAPD revealed that the isolates were not genetically related. The elevated level of resistance among isolated CoNS to the antimicrobials studied is of great concern. The isolates can serve as reservoirs of resistance determinants that can be transmitted to pathogens. The spread of CoNS can however be prevented by effective infection control measures. This study underlines the necessity for policies on the use of antibiotics in healthcare and agriculture, and the development and enforcement of measures that will prevent the spread of CoNS in Nigerian hospitals. It also calls for periodic studies for the evaluation of current resistance patterns of CoNS.

Keywords: Antimicrobial resistance, Coagulase-Negative Staphylococci, Resistance determinant reservoirs.

Introduction

There has been a progressive increase in knowledge regarding the virulence of Coagulase-negative Staphylococci, hitherto seen as commensals with minimal pathogenicity. Coagulase negative *Staphylococcus* spp. ranks among the most important pathogens accounting for nosocomial infections of the blood stream, in patients using intravenous catheters and implants. They function also as drug resistance determinant genes' reservoirs for *Staphylococcus aureus*[1]. Coagulase-negative Staphylococci is characteristically less virulent than *Staphylococcus aureus*, it also has a different array of disease spectrum. Thus, host susceptibility is very important. Because of the large proportions of CoNS strains resistant to methicillin and their growing numbers showing reduced susceptibility towards glycopeptides, Coagulase-negative Staphylococci are challenging therapeutically. Given that they are commensals on the skin, it is difficult to differentiate infection resulting from it and, colonization and contamination, making the epidemiological studies of Coagulase-negative Staphylococci infection to be difficult and complicated[2]. *Staphylococcus epidermidis* and *S. saprophyticus* are among the Coagulase-negative Staphylococci species most frequently encountered in human infections. While *S. epidermidis* is usually associated with the use of intravascular catheters, *S. saprophyticus* are linked to a range of infections which include urinary tract infections, mostly in young women[3].

Since antibiotics were introduced in the 1940s, the emergence of antibiotic-resistance in bacteria has been increasing at a disturbing pace. This increasing spread of resistance even to relatively new antimicrobial agents could be a consequence of the combination of several factors which include; incorrect diagnoses, wrong prescriptions, patients' non-conformity with prescribed antibiotics regime, antibiotics overuse or misuse. The *mec* A gene which encodes for penicillin-binding protein (PBP) 2A, resulting in the reduction of affinity for the β-lactam antibiotics, usually facilitates methicillin-resistance in Staphylococci. The gene is extensively spread amongst Staphylococcus species, probably as a result of horizontal transmissions among *Staphylococcus aureus* and other Staphylococcus species. The important role played by antibiotics in the control of infections, it's high cost and the damage being done by increasing resistance to useful antibiotics justifies the need for identification, prevention and control of Coagulase-negative Staphylococci infection.

Although, CoNS were historically considered harmless with relatively low virulence[3,4], they are increasingly acknowledged

as agents of infections of clinically significance especially of the bloodstream and other sites. Data from a previous United States National Nosocomial Infections Surveillance System report showed that between *Staphylococcus aureus* and Coagulase-negative Staphylococci, CoNS were more commonly reported as pathogens of bloodstream, having been isolated from 37.3% bloodstream infections as against 12.6% for *S. aureus* in patients of intensive care units[5]. Of particular note, is the propensity of some Coagulase-negative Staphylococci to form a protective biofilm which interferes with phagocytosis and efficacy of antimicrobial peptides[6,2]. More importantly, Coagulase-negative Staphylococci often function as repository of antimicrobial resistance genes; with resistance to penicillin approaching 90 to 95%, while resistance to methicillin and semisynthetic penicillins has been found in greater than 80% of CoNS isolates[7], in addition to β-lactams, CoNS are also commonly resistant to several other groups of antibiotics. Patients at particular risk for CoNS infections include those with prosthetic devices, pacemakers, intravascular catheters, and immunocompromised status[8]. However, despite the increasing recognition of Coagulase-negative Staphylococci as agents of clinically important infection and their resistance to multiple groups of antibiotics including beta-lactams, very little is known in Nigeria regarding Coagulase-negative Staphylococci strains obtained from healthy persons in the community and hospital environment.

In this study, the pattern of antimicrobial resistance and the clonal affinity of CoNS isolates of palms, noses and throats of clinical medical students from some University Teaching Hospitals of South Eastern Nigeria was assayed.

The antimicrobial resistance pattern of the Staphylococcal isolates may give information on the possible sources from which antimicrobial resistance in the community may have arisen. The sensitivities of the isolates to selected antimicrobials may also guide clinical treatment decisions. A surveillance study of this nature in the hospital environment may highlight the need for reassessment of policies on antibiotics use within and outside the Teaching Hospital environments, and development of measures to prevent the spread of Coagulase-negative Staphylococci infections.

Materials and methods

Study area: This study covered three (3) universities within the South Eastern part of Nigeria (Imo State University, Abia State University and University of Uyo).

Subjects: A total of 200 clinical medical students, 89 males and 111 females who gave their informed consent were screened.

Ethical Consideration: The essence of the study was explained to the prospective candidates after which their informed consent was sought to be enrolled for the study. Candidates who met the inclusion criteria and who freely granted their informed consent were enrolled for the investigation.

Exclusion criteria: Relevant information on antibiotic usage, presence of any skin abrasion or open wound and hospitalization history in the previous one month was collected of each enrolled candidate using a well-structured questionnaire. Candidates that used antibiotics, had skin abrasion or open wound and/or hospitalized within the preceding one month prior to sampling were excluded from the study.

Sample collection: A total of 205 swab samples (103 palm swabs, 23 throat swabs and 79 nasal swabs) were collected from consenting clinical medical students from the University Teaching Hospitals. All samples were collected using swab sticks soaked in sterile saline. Collected samples were immediately labeled, packaged and transported to the laboratory for microbiological studies. The media used for this study included the following: Mueller-Hinton agar, mannitol salt agar and nutrient agar (Oxoid Ltd).

Identification and characterization of bacterial isolates: The collected swab specimens were inoculated into freshly prepared nutrient broth in bijour bottles, and incubated for 4 - 6 hrs after which culture from the broth was transferred onto Mannitol salt agar plates and then incubated at 37°C for 24-48 hrs. Following the incubation, plates were checked for colony development, and mannitol fermentation shown by a colour change from red to yellow around isolated colonies. Isolates that fermented mannitol were purified by sub-culturing on nutrient agar. Obtained pure isolates were transferred onto nutrient agar slants in test tubes by streaking, incubated at 37°C for 24 hrs, and then stored for further studies at 4°C in a refrigerator. Further confirmation of their identities was by standard microbiological methods which include Gram staining to determine the cellular morphologies and biochemical reactions including catalase, citrate utilization, coagulase, Vogues-Proskauer, methyl red, gelatin hydrolysis, indole and fermentation of carbohydrates (sucrose, maltose, lactose and glucose).

Determination of antimicrobial susceptibility pattern: The isolated Staphylococci were tested for susceptibility or otherwise to nine (9) antimicrobial agents using the disc-diffusion method[9]. Antimicrobial agents tested were: Oxacillin (1µg), Penicillin (10units), Ampicillin (10µg), Vancomycin (30 µg), Erythromycin (15µg), Clindamycin (2µg), Ciprofloxacin (5 µg), Trimethoprim (5µg), Nitrofurantoin (300µg). The disc of each antibiotic was placed on Mueller Hinton agar plates earlier inoculated with Staphylococci isolates. The plates were then incubated at 37°C for 24hrs, after which diameter of observed zones of inhibition were measured using meter rule and recorded. Susceptibility of isolates to antibiotics was deduced following the guidelines of the Clinical and Laboratory Standards Institute[10]. All isolates showing resistance to more than two antimicrobial agents were also recorded as Multiple antibiotic resistant.

Molecular studies on isolated Staphylococci: Plasmid and Genomic DNA extraction: The extraction of isolates' DNA

was by modified alkaline lysis method Sambrook *et al.*[11]. To extract DNA, the broth (1.5ml) containing the isolates was centrifuged for 5mins at 10,000 rpm. Supernatant was discarded after centrifugation, and obtained pellets washed in sterile distilled water, and then resuspended in sterile water (200µl). The suspension was vortexed to homogenize, boiled (100°C) for 10mins in dry bath, then vortexed and centrifuged at 12,000rpm for 5mins. Obtained supernatant contained the DNA and were stored at -20°C. A nano drop spectrophotometer was used to assess the purity and concentration of the extracted DNA.

Polymerase Chain Reaction (PCR): The PCR amplifications were carried out with a mixture of the genomic DNA, PCR H_2O, primers and Red Taq Mastermix (Sigma-Aldrich, USA) containing Taq DNA polymerase, antibodies to Taq DNA polymerase, Tris HCl (130mM), $(NH_4)_2SO_4$ (32mM), $MgCl_2$ (2 mM), Tween 20 (0.02%) and dNTPs (dGTP, dCTP, dTTP, dATP) (25µl). The protocol for amplification was set in the PCR thermocycler (Bio-Rad, Germany) depending on the primers used. After the amplification, the PCR products were separated by agarose gel electrophoresis.

Agarose Gel Electrophoresis: To separate the DNA products of PCR based on their sizes, an electric field was applied through the agarose matrix which moved the DNA based on their sizes. Agarose powder (Segenetic, Germany) was used in preparing the agarose gels used in this study at different concentrations. The agarose was dissolved in 0.5xTBE: Tris-Borate-EDTA and microwaved to dissolve the agarose. The molten agarose was placed on a stirrer and allowed to cool down. Casting of the gel was done by placing a comb into the cast and then pouring the agarose gently into the cast. The comb was removed after the gel has solidified. The electrophoresis chamber was then filled with the buffer (0.5xTBE) and casting tray was placed in the chamber and totally submerging it in the buffer. A dye was added to the colourless PCR products to make them visible for tracking. The amplicon (10µl) was loaded into each well. A 1 kb molecular weight marker- *Hind* III Lambda DNA ladder (New England Biolabs, Germany) was used as standard for estimating molecular weight of the resulting DNA fragments by adding it to the first well and last well. Electricity (160V) was supplied to the electrophoresis chamber and the DNA separation ran for 30-45 min. Separated DNA fragments were then visualized by staining the gel for 15min with ethidium bromide and then de-staining with water for 15min. DNA bands were then viewed following illumination by UV light. Images were recorded by photography.

Detection of antibiotic resistance genes: The antibiotic resistance genes present in the Staphylococci strains were detected on the PCR amplification products *mec A* and beta-lactamase genes using agarose gel electrophoresis.

Detection of mecA gene: Resistance to methicillin was determined through *mecA* PCR as described[12]. The *mecA* gene amplification was with primer set *mecA*1 (AAAATCGATGGTAAAGGTTGGC) and *mecA*2 (5' AGTTCTGCAGTACCGGATTTTGC 3') following the method of Del Vechio *et al.*[13]. PCR was conducted in a reaction mixture (25µl) composed of DNA (10-200ng), and 1X Master Mix (Thermo Scientific Dream Taq Green PCR Master mix). Additional Taq DNA polymerase was included to make the final concentration of Taq DNA polymerase 2.5U, the reaction mixture was made up with sterile distilled water. Thermal cycling was conducted in an Eppendorf (Nexus Series) cycler at 95°C for 5 mins for the initial denaturation, followed by 30 amplification cycles at 95ºC for 30sec; at 55ºC for 30sec and at 72ºC for 1 min. The amplification products were then separated using agarose gel electrophoresis and visualized by staining with ethidium bromide. Molecular weight of DNA was estimated using 100bp DNA ladders (Solis Biodyne). The *mec A* gene is signified by the presence of a band with a molecular weight of 533bp.

Detection of beta-lactamase gene (blaZ gene): The *blaZ* gene amplification was with primer set *blaZ*-F (AAG AGA TTT GCC TAT GCT TC) and blaZ-R (GCT TGA CCA CTT TTA TCA GC). PCR was conducted in a reaction mixture (25µl) composed of DNA (10-200ng), and 1X Master Mix (Thermo Scientific Dream Taq Green PCR Master mix). Additional Taq DNA polymerase was included to make the final concentration of Taq DNA polymerase 2.5U, the reaction mixture was made up with sterile distilled water. Thermal cycling was conducted in an Eppendorf (Nexus Series) cycler at 95°C for 5mins for the initial denaturation, followed by 30 amplification cycles at 95ºC for 30 sec; at 55ºC for 30sec and at 72ºC for 1min. Agarose gel electrophoresis was then used to separate the DNA amplification products which were then stained with ethidium bromide for visualization. DNA molecular weight was estimated using 100bp DNA ladders (Solis Biodyne). The *blaZ* gene is signified by the presence of a band with a molecular weight of 517bp.

Random Amplified Polymorphic DNA (RAPD-PCR) fingerprinting: This typing was conducted following the methods of Casey *et al.*[14] with few modifications. RAPD-PCR was used to assess the clonal relatedness of 18 tested Coagulase-negative Staphylococci. PCR was conducted in a reaction mixture (25µl) containing 1X PCR buffer (Solis Biodyne), $MgCl_2$ (2.5mM), each of the dNTP (dGTP, dCTP, dTTP, dATP) (0.2mM), 40pMol of primer, DNA (10-200ng) and 1U Taq DNA polymerase. The reaction mixture was made up by adding sterilized deionize water. A thermal cycler was used for the amplification with the following cycling parameters; denaturation for 5mins at 95ºC, followed by 40 cycles of amplification at 95ºC for 1min, 30ºC for another 1min and at 72ºC for 2 mins, and extension for 10mins at 72ºC.

Agarose gel (1.5%) was used to separate the products from the PCR. DNA molecular weight was estimated using 100bp DNA ladders (Solis Biodyne). The evaluation of RAPD fingerprints was with Sequentix - Digital DNA Processing (Germany).

Based on RAPD data sets, cluster analysis of the binary matrix distance were transferred into dendrogram using Pearson coefficient method. A dendrogram based the unweighted pair group method with arithmetic averages (UPGMA) analysis using RAPD data generated by primer set *blaZ*-R (GCT TGA CCA CTT TTA TCA GC) and *blaZ*-F (AAG AGA TTT GCC TAT GCT TC) was generated.

Results and discussion

Study: The medical students screened reported that they had not taken any antibiotics medication in the last one month, nor were they hospitalized or had any skin abrasion or open wound prior to and during the sampling period. From the questionnaire, 90% of the students were aged 21-30, were always in the hospital and were not smoking. The frequencies of the distribution of CoNS spp. isolates against palms, noses and throats of clinical medical students from the screened institutions is shown in Table-1.

Table-1: Spread of Coagulase-negative Staphylococci isolates against palms, noses and throats of clinical students

Swab Samples	Swab samples collected	Positive for CoNS (%)	Negative for CoNS (%)
Palm swabs	103	9 (8.7)	94 (91.3)
Throat swabs	23	0 (0)	23 (100.0)
Nasal swabs	79	17 (21.5)	62 (78.5)
Total	205	26 (12.7)	179 (87.3)

In this study, out of 205 swab samples screened, Coagulase-negative Staphylococci isolates were recovered from 26 (12.7%). Obtained swabs positive for Coagulase-negative Staphylococci comprises of 9(8.7%) palm swabs and 17 (21.5%) nasal swabs samples. Of the 23 throat swab samples screened, no Coagulase-negative Staphylococci was isolated.

Also, among the 26 CoNS, 15 (57.7%) strains were isolated from males and 11(42.3%) strains from females (Table-2).

Table-2: The occurrence of Coagulase-negative Staphylococci among male and female clinical students.

Sex	Number of Samples	Prevalence (%)
Female	11	42.3
Male	15	57.7
Total	26	100

Table-3: Resistance pattern of Coagulase-negative Staphylococci isolates to test antimicrobials.

Antibiotics	Susceptible (%) n=26	Resistance (%) n=26
Penicillin	3(11.5)	23(88.5)
Oxacillin	3(11.5)	23(88.5)
Ampicillin	3(11.5)	23(88.5)
Nitrofurantoin	26(100.0)	0(0.0)
Erythromycin	22(84.6)	4(15.4)
Trimethoprim	0(0.0)	26(100.0)
Ciprofloxacin	11(42.3)	15(57.7)
Vancomycin	22(84.6)	4(15.4)
Clindamycin	16(61.5)	10(38.5)

Results of the disc diffusion tests shows that the tested isolates were 100% susceptible to nitrofurantoin, 84.6% susceptible to erythromycin and vancomycin respectively, while resistance (100%) was recorded against trimethoprim, followed by (88.5%) against oxacillin, penicillin and ampicillin and (57.7%) for ciprofloxacin.

Table-4: Frequency of multi-drug resistance in coagulase-negative Staphylococci isolates of clinical medical students.

Number of antimicrobials isolates are resistant to	Number of isolates with multi-drug resistance	Frequency (%)	Multi-drug resistance indices
3	0	0	0.33
4	9	34.6	0.44
5	12	46.2	0.56
6	4	15.4	0.67
7	0	0	0.78
8	1	11.1	0.89
9	0	0	1.0

Multi-drug resistance indices = Number of antimicrobials isolated spp. are resistant to/Number of tested antimicrobials.

The frequency of Multi-antibiotic resistance (MAR) is as given in Table-4. Of the twenty-six (26) CoNS isolated from clinical medical students, all 26 (100%) are MAR. Twelve isolates (46.2%) are jointly resistant to five antimicrobial agents with a

MAR index of 0.56. Nine isolates (34.6%) were resistant to four antimicrobials. Four isolates (15.4%) were resistant to six antimicrobials while one (3.8%) was resistant to eight antimicrobials studied, with MAR indices of 0.89.

Table-5: Resistance and susceptibility patterns of multi-drug resistant coagulase-negative Staphylococci isolates from clinical students.

Isolates (n)	Resistant antimicrobials	Susceptible antimicrobials
Pm ABU 41, Ns UYC 35, Ns ABU 3 (3)	W, OX, PENI, AMP	F, VAN, ERY, CLIN, CIP
Ns IMU 36 (1)	W, OX, PENI, AMP, CLIN	F, VAN, ERY, CIP
Ns UYCL 5, Ns IMU 10, NS UYCL 2, Pm UYCL 6, Ns UYCL 83, Ns ABU 201, Pm ABU 17 (7)	W, OX, PENI, AMP, CIP	F, VAN, ERY, CLIN
Ns UYCL 81, Ns UYCL 82 (2)	W, PENI, AMP, CLIN	F, VAN, ERY, CIP, OX
Ns IMU 178, Ns UYCL 40, Ns IMU 6 (3)	W, OX, CIP, CLIN	F, VAN, ERY, PENI, AMP
Pm UYC 1 (1)	W, PENI, AMP, CLIN	F, VAN, ERY, CIP, OX
Pm ABU 200, Pm UYC 83, Pm UYCL 160 (3)	W, OX, PENI, AMP, CLIN, VAN	F, ERY, CIP
Pm ABU 20, Pm ABU 8, Ns IMU 12, Ns ABU 11 (4)	W, OX, PENI, AMP, CIP	F, VAN, ERY, CLIN
Ns IMU 10 (1)	W, OX, PENI, AMP, CIP, CLIN, ERY, VAN	F
Ns UYCL 40 (1)	W, OX, PENI, AMP, CLIN, ERY	F, VAN, CIP

Legend: OX-Oxacillin, PENI- Penicillin, AMP- Ampicillin, ERY-Erythromycin, CLIN-Clindamycin, CIP-Ciprofloxacin, W-Trimethoprim, F-Nitrofurantoin, VAN-Vancomycin

All the isolates showed resistance to at least four (4) antibiotics and were therefore designated multiple drug resistant. Polymerase Chain Reaction amplification yielded no antibiotic resistant gene and fingerprinting by Randonly Amplified Polymorphic DNA as revealed by Unweighted Pair Group Method with Arithmetric averages (UPGMA) dendrogram revealed that the isolates were widely disseminated and not genetically related.

Discussion: This study isolated 26 (12.6%) Coagulase-negative Staphylococci from 205 swab samples obtained from palms, noses and throats of clinical medical students. From 103 palm swabs, 9(8.7%) Coagulase-negative Staphylococci were obtained and from 79 nasal swabs, 17 Coagulase-negative Staphylococci isolates (21.5%) were obtained. From 23 throat swabs screened, zero (0%) Coagulase-negative Staphylococci isolates was obtained. From the 26 Coagulase-negative Staphylococci isolated, 9 (34.6%) were from palm swabs and 17 (65.4%) were from nasal swabs. It can be seen that nasal swabs yielded more Coagulase-negative Staphylococci than palm swabs. Nasal swab carriage of 65.4% Coagulase-negative Staphylococci observed here is greater than the 18% observed by Baragundi *et al*.[1]. Our Coagulase-negative Staphylococci nasal carriage rate corroborates with the 30% Coagulase-negative Staphylococci carriage rate recorded by Akinjogunla *et al*.[15] in their assessment of antibiotic resistance and virulence elements in *Staphylococcus* spp. isolates of the nose of seemingly healthy undergraduates in Uyo. The anterior nares of humans have been recognized as the primary habitat of opportunistic pathogens as well as commensals as *S. aureus*, Coagulase-negative Staphylococci and *Moraxella* spp.[16].

The occurrence of CoNS here corroborates those of Silva *et al*., [17]. The occurrence of CoNS in the nose observed in the current assessment also corroborates the findings of Nakamura *et al*.[18]. From the 26 Coagulase-negative Staphylococci isolated, 15 (57.7%) were from male clinical students and 11(42.3%) were from female clinical medical students. Male students' Coagulase-negative Staphylococci carriage rate is clearly higher than that of the females. This does not agree with reports of Akinjogunla *et al*.[15] who reported 66.7% Coagulase-negative Staphylococci in females and 33.3% in male undergraduate students. The differences in colonization of nose by *Staphylococcus* spp. observed in the two studies could be as a result of factors such as immunity of the host, gender, age and the environment[19].

All the Coagulase-negative Staphylococci in our study showed high resistance to the β–lactams (oxacilli, ampicillin and penicillin), trimethoprim and ciprofloxacin. The mechanism of resistance by Staphylococci spp. to β-lactam antibiotics as cephalosporins and penicillins, is the production of the enzyme β-lactamase[20]. The isolates were susceptible to nitrofurantoin and the glycopeptide- vancomycin. This corroborates the findings of many authors[21-23]. The sensitivity of Coagulase-negative Staphylococci to nitrofurantoin (100%), vancomycin (85.6%) and erythromycin (85.6%) may be an indication that these antibiotics have not been misused or over used in these areas studied. There are however reports from other studies, indicating reduced susceptibility to outright resistance to vancomycin and teicoplanin[24,25]. Resistance to frequently used antibiotics observed in this study and others, may have resulted from miss use of product and/or inefficient infection control exercises. Ogbulie *et al*.[26] reported resistance to extensively used biocides to be a result of adaptation to diverse environmental, physical and chemical conditions arising as a result of insufficient cleaning, improper product use and inefficient infection control practices.

Multi-antibiotics resistance to tested drugs was also recorded in all the isolated species in our study. A study on Coagulase-negative Staphylococci by Nahaei *et al*.[27] also reported multi-drug resistance in most of their isolates. MAR indices recorded were between 0.33 and 1.0. MAR indices greater than 0.2 indicates that isolated species are from an area where antimicrobial agents are easily accessible and available with high probability for misuse and overuse[28].

Thus, the multiple antibiotics resistance index obtained in this study, shows that the organisms are from environments where antibiotics are freely available and are abused and/or misused[28]. The data obtained here may be used to formulate regional antibiotic policies to compare regional with national data and over all to aid clinicians in the rational choice of antibiotics therapy. The results of the study points to an emerging resistance to the drugs tested. It is therefore important that this resistance be monitored to prevent the spread. The presence of multidrug resistance in CoNS has been reported by various authors from different parts of the globe[22]. Many studies from India have also reported multi-drug resistance in Coagulase-negative Staphylococci[21,29]. In their study, Patricia *et al*.[30] observed 61% of their isolates to be multi-drug resistant. This may be as a result of several prescriptions and types of antimicrobials used in different health care settings and maybe due also to clonal manifestation differences, and antimicrobial pressure in the studied community[23]. Susceptibility (84.6%) of Coagulase-negative Staphylococci isolated from this study to glycopeptides may suggest its prudent use and continuous monitoring is required to ensure that its efficacy is not lost.

PCR amplification yielded no antibiotic resistant gene. Thus, the resistance to antimicrobials observed here could be as a result of biofilm formation. Otto[6] reported that Staphylococci also have non-specific mechanisms of resistance in addition to the specific mechanisms resulting from uptake of genetic resistance determinants (chromosomally or plasmid-encoded). Of these non-specific mechanisms of resistance, the formation of biofilm is incontrovertibly the most significant. Fingerprinting by Randonly Amplified Polymorphic DNA as revealed by Unweighted Pair Group Method with Arithmetric averages (UPGMA) dendrogram indicated that all isolates were widely disseminated and may not be genetically related. However, bacterial clones are genetically duplicate cells resulting from one progeny. With time, members of a clone might start differentiating as a result of the deletion or gaining of a mobile genetic element, point mutations and recombination. This distinction provides increased chances for obtaining distinct characteristics, as pathogenicity and antibiotic resistance. Genetic distinction thus brings about an increase in broad array of phenotypic and genomic features[31].

Conclusion

Routine screenings among clinicians for Coagulase-negative Staphylococci colonization of the nose should be regularly carried out. Hospitals should adopt strategies for the creation of awareness on the implications of drug abuse and misuse, as well as on prevention of the spread of multi-drug resistant strains.

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