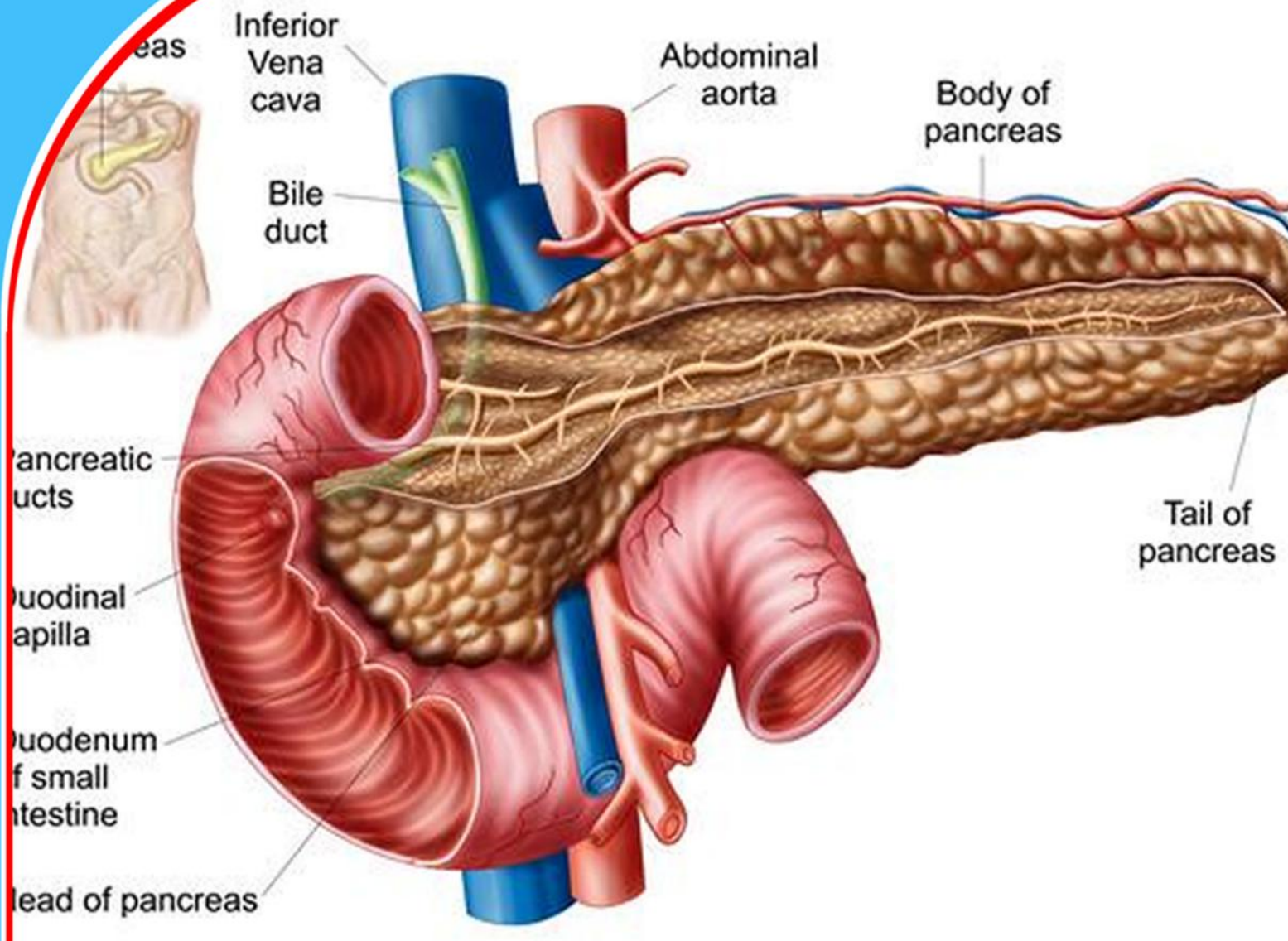
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Threat of Coagulase Negative Staphylococci (CoNS) Acquiring Methicillin Resistant Genes and Other Virulent Signatures within Irrua Specialist Teaching Hospital, Edo State

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ABSTRACT

Purpose: Coagulase-negative staphylococci (CoNS) form a group ofGram-positive cocci united by their lack of the virulence factor coagulase. Methicillin-resistant CoNS refers to strains of CoNS that has developed resistance to methicillin. The aim of this study was to determine the prevalence of MR-CoNS within Irrua Specialist Teaching Hospital (ISTH), antibiogram and molecular studies of the isolates to acertain the how CoNS which were believed to be non pathogenic acquired some pathogenic traits.

Materials and Methods: A total of 299 swabs were collected from different departments in ISTH and processsed using standard microbiological procedures. The 16S rRNA sequencing was conducted on six MR-CoNS isolates.

Findings: Results revealed a prevalence of 12% for MR CoNS. The distribution of the MR-CoNS isolates within ISTH revealed thus; fomites (38%), cleaners (24%), patients (16%), nurses (13%), and doctors (8%) while 5% of MR-CoNS possessed *nuc*, 49% possessed *mec*A, 41% had *blaZ,* while 21% had *pvl* genes respectively. Results of the antibiogram revealed 96% of the isolates were resistant to cloxacillin, while vancomycin and linezolid were the best drug of choice because all isolates were sensitive to these two antibiotics. The 6 MR-CoNS sequenced in this study were identified as *Staphylococcus chromogenes*, *Staphylococcus scuiri* and *Staphylococcus cohnii* strains.

Implications to Theory, Practice and Policy: In conclusion, the CoNS isolated from this study revealed that there is acquisition of methicillin resistant and some virulent genes, most likely from other bacteria, which pose a major threat to Hospitals and Communities.

Keywords: *Methicillin Resistant Coagulase-negative Staphyloccocci (MR CoNS), mecA and pvl Genes, Public Health*

INTRODUCTION

Coagulase-negative staphylococci (CoNS) form a large group of Gram-positive cocci united by their mutual lack of the virulence factor coagulase (Chenouf et al., 2021). These CoNS are a heterogeneous group comprising over 80 species. In comparism to coagulase-positive staphylococci, such as *Staphylococcus aureus*, CoNS have been considered to be less pathogenic or non-pathogenic for decades. Most CoNS species are confined to the human skin and mucosal membranes and rarely do they involve in clinically symptomatic infections (Medis et al., 2022). However, given new groups of immunocompromised patients and the growing complexity of medical procedures, CoNS have emerged as one of the leading group of nosocomial pathogens in this dispensation. CoNS are often associated with foreign body-associated infections and infections in preterm neonates (Grazul et al., 2023). While the infections are usually subacute and associated with only mild inflammation, they usually comprise a heavy clinical burden due to their widespread occurrence and multifactorial challanges with choosing an appropriate antibiotic therapy. Acquisition of resistant genes to antimicrobial agents is a common pattern and challenge with CoNS and which also spreads quickly between species (Bonvegna et al., 2021).

Among all the multiple mechanisms of acquired drug resistance displayed by CoNS, the resistance to beta-lactam antibiotics appears the most important from a clinical and epidemiological view. The two principal mechanisms of staphylococcal resistance to beta-lactams include the production of enzymes, beta-lactamases, and acquisition of a defuncted penicillin-binding proteins (PBP) (Shresta et al., 2020). Due to the generation of new PBP, with low affinity to beta-lactams, staphylococci has developed resistance to all beta-lactam antibiotics used in clinical practice nowadays. This type of resistance is now referred to as methicillin or oxacillin resistance, as it was first documented in the case of methicillin, which was the first semisynthetic anti-staphylococcal penicillin (Yang et al., 2024). Any CoNS that displayed this type of resistance are refered to as methicillin-resistant coagulase-negative staphylococci (MRCoNS). The presence of methicillin resistance is determined by the presence of acquired *mec*A, *mec*B, or *mec*C genes that encode additional penicillin-binding proteins, PBP2a (PBP2'), PBP2b, or PBP2c, respectively. Ordianrily, *Staphylococcus aureus* that are resistant to methicillin are found to harbor the mobile genetic element, Staphylococcal cassette chromosome *mec* (SCC*mec*) that carries the *mecA* gene which encodes low affinity penicillin binding proteins such as PBP2a, bypassing the bactericidal action of bata-lactam antimicrobials but recent studies have shown that some CoNS have acquired these genetic elements, which has endowed them with resistance to beta-lactam antibiotics (Wu et al., 2025).

Problem Statement

Over the past years, Ekpoma and Irrua community of Edo State, has been challenged with the burden of post hospital-acqured infections (HAIs), majorly targetting device-related sepsis, surgical site infections and the empirical failure of standard antibiotic regimen as drivers of this problem. Some analysis done by some researchers such as Isibor and Otabor (2014) pointed that some of these post HIAs could be attributed to CoNS and *Staphylococcus aureus*. The dilemma of how possible these infections can be caused or initiated by CoNS led to this research investigation to unravel the true status of CoNS isolates around this study environment.

Literature Review

Coagulase negative staphylococci (CoNS) are a heterogeneous group of Gram-positive bacteria that constitute part of the human normal microbiota of the skin and mucous membranes. Although they are regarded as harmless commensals, CoNS have increasingly been recognized

as important opportunistic pathogens, especially in healthcare settings. The pathogenicity of CoNS is largely associated with their ability to adhare to host tissues and medical devices, form biofilms and evade host immune responses (Shresta et al., 2020).

Methicillin resistance in CoNS represents a major therapeutic challenge because it limits the effectiveness of commonly used beta-lactam antibiotics. The principal mechanism involves the acquisition of the mecA gene usually carried on the staphylococcal cassette chromosome mec which encodes an altered penicillin binding protein, PBP2a. This protein has a low affinity foe most beta lactam antibiotics, allowing bacterial cell wall synthesisto continue despite exposure to these agents (Chenouf et al., 2021).

Consequently, the emergence of methicillin resistant CoNS highlights the need for routine antimicrobial susceptibility testing, molecular surveillance of resistant determinants and effective infection prevention measures.

MATERIALS AND METHODS

Study Design

This study was aimed at investigating the presence of methicillin resistant genes and other virulent genes from supposed non pathogenic Coagulase Negative Staphylococci (CoNS). Swab samples were collected from Doctors, Nurses, Ptients, Cleaners and Fomites from 14 departments within ISTH Hospital. These swabs were cultured on Mannitol agar for growth of Staphylococci and further screened for methicillin resistance. Islates were also subjected to different panel of antibiotics commonly used in this locality for the resistant pattern. Molecular identification using the 16S rRNA sequencing was done on some isolates with highest and lowest MAR index. These isolates were also assayed for prsesnce of some virulent factors.

Area of Study

The study was carried out in Irrua Specialist Teaching Hospital, Irrua, Edo State.

Irrua Specialist Teaching Hospital (ISTH), which is a Federal Government of Nigeria Teaching Hospital located in Irrua, Edo State Nigeria, is the administrative headquarters of Esan Central Local Government Area. It is situated at Latitude $5^0$15 48^0E. It has a population of about 40,000 people whose major occupation includes Farming, Teaching, Civil services and Trading (Isibor and Otabor 2014). The hospital is less than 2km from Ekpoma town and serves as the teaching hoapital to Ambrose Alli University Ekpoma medical programm.

Determination of Sample Size

According to study by Ruiz-Ripa et al (2020), sample size was determined using the formula;

$$N = \frac{P(100-P)2}{E2}$$

Where:

N= required sample size

P= Percentage occurrence of the event (27%; Igbinosa et al., 2026)

E= Percentage error required (5%)

Z= Statistical value corresponding to confidence level (95%) which is 1.96

Inputing these figures into Bartlet's formula,

$$N = \frac{27(100-27)\ 1.96^2}{5^2}$$

N= 299 samples for this study population.

Hence, a total number of 299 samples was needed for this study.

Sample Collection

In the sample collection, a total of 299 nasal and fomite samples were collected from Irrua Specialist Teaching Hospital (ISTH), Irrua for the detection of Methicilin Resistant Coagulase Negative Staphylococci (MR CoNS). These samples were collected from personnels and fomites of 14 major clinic departments/wards in the hospital. From each department/ ward, sterile swab sticks (oxoid) were aseptically used to collect nasal samples from Doctors, Nurses, Cleaners, Patients and Fomites.

Isolation of Staphylococci spp

These nasal and fomite swabs were aseptically streaked on Mannitol salt agar and incubated at 37ºC for 24 hours. Presumptive growths of coagulase negative Staphylococci (isolates that do not change color of the media) were then, further subjected to Gram staining and biochemical tests.

Isolation of Methicillin Resistant CoNS/Antibiogram

The CoNS isolated after biochemical identification were further cultured on the surface of Oxacillin Resistant Screening Agar Base (ORSAB) medium which is selective for the isolation of methicillin resistant isolates. The methicillin resistant isolates were confirmed on ORSAB by a change in the colour of the medium from pale blue to deep blue.

Presumptive Methicillin resistant coagulase negative Staphylococci (MRCoNS) isolates from the ORSAB media were further selected for methicillin resistance, using the Kirby Bauar disk diffusion method. Briefly on this, a 0.5 McFarland turbidity standard match of these isolates were flooded on the surface of Mueller Hinton agar, which was allowed to dry a little before 1µg of Oxacillin single disc was placed on the surface of the plates and incubated at 37ºC for 24 hours. After 24 hours' incubation, zones of clearance were measured according to the guidelines of Clinical and Laboratory Standard Institute (CLSI), 2014 interpretative chart to screen for MR CoNS.

The methicillin resisitant CoNS were further screened through some antibiotics which included single disk Vancomycin (1µg) and Linezolid (1µg), multiple disk of Cloxacillin (5µg), Ciprofloxacin(5µg), Gentamycin(5µg), Tetracycline(10µg) and Erythromycin(5µg). This was carried out by preparing a 0.5 McFarland standard match of the MR CoNS isolates which was used to flood the surface of Mueller Hinton agar. It was notched around and the excess fluid decanted into a disinfectant jar. After drying, the antibiotic disks were placed on the surface of the media and incubated at 37ºC for 24 hours. After 24 hours incubation, the plates that showed zones of inhibitions were read and measured according to guidelines of CLSI (2014) interpretative chart to screen for resistant isolates.

Determination of Multiple Antibiotics Resistant (MAR) Index

According to Igbinosa et al (2026), the MAR index which was calculated as the ratio of number of antibiotics resistant to the total number of antibiotics exposed to. MAR index greater than 0.2 reveals that there is a high-risk source of resistance where antibiotics are frequently used. Therefore, the formula for calculating MAR index which is $\frac{a}{\text{bx c}}$

Where "a" represents number of antibiotics to which an isolate was resistant to and "b" represents total number of antibiotics tested while "c" is the total number of isolates obtained from sample source.

Assay for Biofilm Formation

The MR CoNS isolates were assayed for biofilm formation. Biofilms depict the aggregates of microorganisms suspended in a matrix of extracellular polymeric substances attached to a surface. Possession of these biofilms by bacteria gives them a high survival chance by making them resistant to antibiotics and evading host cells. Adopting the technique used by Igbinosa et al (2026), the Congo red agar method was used in this study to detect biofilm formation.

Assay for Haemolysin Production

Haemolysins, which are lipids and proteins produced by some bacteria that causes lysis of the red blood cells by disrupting the cell membrane. Some pathogenic bacteria produce haemolysins to obtain nutrient from lysing red blood cells of the host and evade host destruction.

According to Okon et al. (2011), the blood agar test was performed to detect for haemolysin production in the MR CoNS isolates. Briefly, 5% sheep blood agar (Oxoid) plates were prepared according to the manufacturer's instructions. The isolates were then streaked on the surface of the blood agar plates, which were then incubated at 37ºC for 24 hours. After 24 hours' incubation, plates that showed a complete zone of clearance aeound the streaked bacterium, indicates positeve for haemolysin production.

Assay for Dnase Production

Deoxyribonuclease (Dnase), which are short group of glycoprotein endonucleases enzymes catalyzes the hemolytic cleavage of phosphodiester linkage in the DNA backbone, thus degrading DNA. According to methods performed by Igbinosa et al. (2016), the isolates were aseptically streakied on Dnase agar (prepared according to manufacturer's instruction) using a sterile wire loop. Thereafter, plates were incubated at 37ºC for 24 hours. After incubation, plates were observed for color change. Medium that appeared colorless around the test organism were reported positive to Dnase production while medium that remained green around the test organism were reported negative to Dnase production.

Molecular Identification of Isolates and Gene Detection by PCR

On the molecular analysis of the isolates from this study, all gene detection using the polymerase chain reaction (PCR) was carried out in Irrua Specialist Teaching Hospital, while the identification through 16S rRNA sequencing, was done in Lahor Research Institute, Benin City, Edo State, Nigeria.

Genomic Deoxyribose Nucleic Acid (DNA) Extraction Protocol

The genomic DNA extraction protocol was carried out as described by Igbinosa et al. (2016), where single colonies of Methicillin Resistant *Staphylococci* isolates grown on Brain Heart Infusion agar was transferred to 1.5 mL of Brain Heart Infusion broth and the cultures were grown on a shaker water bath for 48 hours at 28 ^{0}C. Thereafter, cultures were centrifuged at 4600 rpm for 5 minutes. The resulting pellets were each re-suspended in 520 µL of TE buffer, and 5 µL of 20% SDS and 3 µL of Proteinase K (20mg –ML^{-1}) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µL of 5M NaCL and 80 µL of a 10% cetyltrimethyl ammonium bromide (CTAB) solution in 0.7M NaCl were added and mixed. The suspension was incubated for 10 minutes at 65ºC and kept on ice for 15 minutes before centrifugation at 7200 rpm for 20 minutes. The aqueous phase was transferred to a new tube followed by addition of isopropyl (1:0:6) and DNA was precipitated at -20^{0}C for 16 hours. DNA was collected by centrifugation at 7200 rpm for 10 minutes, washed with 500 µL of 70 % ethanol, air dried at room temperature for approximately 3 hours and finally dissolved in 50 µL of TE

buffer. The genomic DNA was stored at -20ºC and for molecular identification and used for bacterial typing protocols.

Amplification of *mecA, blaZ, nuc,* and *pvl* Genes

For the detection of these genes, it was carried out as described by Igbinosa et al. (2016). The 25.0 µL volume of PCR reaction mixture contained a 1.0 µL of template genomic DNA, 12.5 µL of PCR master mix, 7.5 µL PCR H_2O and 2 µL each of the primers. DNA amplification was carried out for 40 cycles according to the following protocols: denaturation at 94 ºC for 30 seconds and annealing at 55 ^{0}C for 30 second; extension at 72 ^{0}C for 1 minutes with a final extension at 72 ^{0}C for 5 minutes and cooling at 4 ^{0}C. Electrophoresis of amplicons were performed with 1 % agarose gel containing ethidium bromide (Et Br) 0.5 mg L^{-1}, for 1 hour at 100 V in 0.5 x TAE buffer (40mM Tris-HCL, 20 mM Na-acetate, 1mM EDTA, pH 8.5) and visualized under a UV trans illuminator.

Amplification of Staphylococcal Cassette Chromosome (SCC) *mec* I, II, III, IV, and V Genes

The PCR detection of the SCC*mec* I, II, III, IV and V was performed as described by [11]. The 25.0µL volume of PCR reaction mixture contained a 1.0µL of genomic DNA, 12.5µL of PCR master mix, 7.5µL PCR H^2O and 2µL each of the SCCmec primers. DNA amplification was carried out for 45 cycles according to the following protocols; denaturation at 94℃ for 30s, extension at 72℃ for 1 mins with final extension at 72℃ for 5 mins and cooling to 4℃. Electrophoresis of amplicons was performed with 1% agarose gel containing ethidium bromide (EtBr) 0.5mgL^{-1} , for 1 hour at 100V in 0.5×TAE buffer (40Mm Tris-HCL, 20Mm Na-acetate, 1mm EDTA, PH8.5) and visualized under a UV transilluminator

Amplification of 16S rRNA Gene Sequence of Coagulase Negative Staphylococci Isolates

Molecular identification of CoNS isolates was carried out as described by Igbinosa et al. (2016). The PCR consisted of a final volume of 50 µL which included 8µL DNA and 42 µL reaction specific for~1500 bp conserved domain of the 16S rRNA gene of bacteria and 0.3 unit of Taq DNA polymerase. The primers 27F; 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R; 5'-GGTTACCTTGTTACGACTT-3' were used for the PCR thermal cycling regime. An initial denaturation at 94 ºC for 1 minutes, extension at 72 ^{0}C for 1.5 minutes, a final extension at 72 ºC for 15 minutes and cooling at 4 ^{O}C, for 40 cycles. Electrophoresis of amplicons was performed with 1% agarose gel containing ethidium bromide (Et Br) 0.5 mg L^{-1}, for 1 hour at 100 V in 0.5 x TAE buffer (40 mM Tris – HCL, 20 mm Na –acetate, 1mM EDTA, pH 8.5) and visualized under a UV trans illuminator.

Table 1: Primers Used for the Amplification of Relevant Genes

Gene Target	Sequence (5′→3′)	Product Size (bp)	Reference
nuc-F	TAATCCAAGAGCAATAAGGGC	227	Igbinosa et al. (2016)
nuc-R	GCCACACTATCATAACCACTA		
blaZ-F	ACTTCAACACCTGCTGCTTT	173	Igbinosa et al. (2016)
blaZ-R	TGACCACTTTTATCAGCAAC		
pvl-F	CTTGTTGATCACGATAATTTCC	167	Igbinosa et al. (2016)
pvl-R	ATCTTTTAGCAAACCCGTATTC		
mecA-F	GGCACAATAAGAGTGTTTAAAGG	940	Igbinosa et al. (2016)
mecA-R	AGTTATATCATGAATAGATTGTCCTGTT		

Table 2: Primers Used for the Amplification of SCCmecs

SSCmec Type	Orientation	Oligonucleotide Segments (5′-3′)	Size of Base Pair (bp)
Type I	Forward	GCTTTAAAGAGTGTCGTTACAGG	613
	Reverse	GTTCTCTCATATAGTATGACTCC	
Type II	Forward	GATTACTTICAGAACCAGGTCAT	287
	Reverse	TAACTGTGTCACACGATCCAT	
Type III	Forward	CATTTGTGAAACACAGTACG	243
	Reverse	GTTATTGAGACTCCTAAAGC	
Type IV	Forward	GCCTTATTCGAAGAAACCG	776
	Reverse	CTACTCTTCTGAAAAGCTCG	
Type V	Forward	GAACATTGTTACTTAAATGAGCG	325
	Reverse	TGAAAGTGTATAAATGAGCG	

FINDINGS

Prevalence of CoNS in the Studied Populations

An overall prevalence of 12% MR CoNS isolate was recorded in this study comprising 37 CoNS of the total 299 samples (Table 3).

Table 3: Prevalence of CoNS among the Studied Population

Sample obtained from: n = 299	No of Samples	No (%) positive for CoNS
CoNS	299	37 (12)

Key: CoNS: Coagulase Negative Staphylococci

Identification of Coagulase Negative Staphylococci Isolates

The results showed that all MR CoNS isolates grew as tiny pale whitish colonies on mannitol salt agar, were Gram positive cocci in clusters and were resistant to oxacillin. Biochemical characteristics of these isolates shows that all were catalase positive, coagulase negative, oxidase negative, citrate positive, indole variable, urease positive and were able to utilize the sugars glucose, mannitol and lactose (Table 4).

Table 4: Characterization of the Coagulase Negative Staphylococci Isolates

Identification Criteria	Result
Gram reaction	+
Catalase	+
Coagulase	−
Oxidase	−
Citrate	−
Indole	−
DNase	−
Urease	+
Mannitol	−
Glucose	+
Lactose	+
Growth on MSA	+
Resistance to Oxacillin	±

Key: + = positive; - = negative; ± = variable; MSA = Mannitol Salt Agar; Dnase: Deoxyribonuclease

Occurence of Virulent Factors and Genes in CoNS Isolates

Table 5 shows the occurence results of the MR CoNS isolates on haemolysin production 4 (11%), DNAse production 3 (8%), biofilm formation 21 (57%) as well as presence of *nuc* gene 2 (5%), *pvl* gene 8 (21%), *mecA* 18 (49%), *blaZ* 15 (41%) and *SCCmec* types I, II, III (Hospital lineage), 9 (24%) and *SCCmec* IV-V (Community lineage) 4 (11%) genes.

Table 5: Occurrence of virulent factors and genes in MR CoNS Isolates (n=37)

Characteristic Feature	Number of Positive Isolates, n (%)
Production of haemolysin	4 (11)
Production of DNase	3 (8)
Biofilm formation	21 (57)
Presence of mecA gene	18 (49)
Presence of blaZ gene	15 (41)
Presence of pvl gene	8 (21)
Presence of nuc gene	2 (5)
SCCmec I-III	9 (24)
SCCmec IV-V	4 (11)

Key: SCC*mec*: Staphlococcal Cassette Chromosome *mec*

Dnase: Deoxyribonuclease

Distribution of MR CoNS according to Subjects and Hospital Departments

A total of 37 (12%) of the 299 nasal and fomite samples yielded MR CoNS isolates ans of the 37 isolates, 5 (14%) was recovered from male surgical ward, 4 (11%) each from female surgical ward and laundry, while the least number of MRSA isolates 1(3%) were recovered each from Mother and Child Health (MCH) department and Gynecology (Table 6). In addition, the highest number of the MR CoNS isolates were recovered from fomites (38%), followed by cleaners (24%), patients (16%), nurses (13%), and the least from doctors (8%).

Table 6: Distributions and Prevalence of MR CoNS Isolated in ISTH

Department	Doctors	Nurses	Patients	Cleaners	Fomites	Total No. (%)
SCBU	0	1	0	0	1	2 (5)
Gynecology	0	0	0	0	1	1 (3)
Lassa	0	0	0	1	1	2 (5)
MCH	0	0	1	0	0	1 (3)
Ophthalmology	0	1	0	0	2	3 (8)
Female Surgical	0	1	1	1	1	4 (11)
ICU	1	0	0	0	0	1 (3)
Pediatrics	0	1	1	0	1	3 (8)
Labour Wards	0	0	0	1	1	2 (5)
Post-natal	1	0	1	0	1	3 (8)
A & E	0	1	1	1	1	4 (11)
Male Surgical	1	0	1	1	2	5 (14)
Laundry	–	–	–	3	1	4 (11)
Radiology	0	0	0	1	1	2 (5)
Total (%)	**3 (8)**	**5 (13)**	**6 (16)**	**9 (24)**	**14 (38)**	**37 (100)**

Key: SCBU =Special Care Baby Unit; MCH = Mother and Child Health; ICU = Intensive Care Unit; A&E= Accident and Emergency; MRSA: Methicillin Resistant *Staphylococcus aureu*

Antibiogram of the MR CoNS Isolates

In the incidence of the antibiogram on all 37 MR CoNS isolated from ISTH, it was revealed that vancomycin and linezolid represent the best drugs of choice in the treatment of infections caused by these isolates. All 37 isolates were sensitive to 1*ug* of vancomycin and linezolid, while for cloxacillin, 92% of the isolates were resistant. The results also showed that for ciprofloxacin, 41% of the isolates were resistant, while for Tetracycline only 9% of the isolates were resistant. For Gentamycin, a total of 45% of isolates were resistant to it and finally, Erythromycin had 26% of the isolates were resistant (Table 7).

Table 7: Antibiogram of MR CoNS Isolates

No of MR CoNS	Antibiotics						
n = 37	Van	Lin	Clox	Cipro	Tetra	Gent	Ery
S	37	35	2	20	33	19	28
I	0	2	1	1	1	0	1
R	0	0	34	16	3	18	8
Percentage of Resistant Isolates	0	0	96	41	10	45	21

Key:

S= Sensitive, I= Intermediate, R=Resistant, Van=Vancomycin, Lin= Linzolid, Clox= Cloxacillin, Cipro= Ciprofloxacin, Tetra=Tetracyclin, Gent=Gentamycin, Ery=Erythromycin, MRSA: Methicillin Resistant *Staphylococcus aureus*.

Minimum Inhibitory Concentration (MIC) Value and Multiple Antibiotic Resistsnce (MAR) Index of MR CoNS

It was shown from the results (Table 8) that the CoNS isolates recovered from SCBU, Lassa ward, pediatrics had the lowest MIC value 0.5-2ug/ml, while isolates from male surgical ward, had the highest MIC value ranging from 2-8ug/ml Female surgical ward and laundry isolates had a MAR index of 0.4, Lassa ward, A&E and male surgical ward isolates had MAR index of 0.3. I solates from other departments and wards from this study recorded a MAR index of 0.2.

Table 8: MIC Value and MAR index of MR CoNS Isolates

Department / Source of Isolates	No of MRSA	MIC Value (ug/ml)	No of Resistant Antibiotics	No of Antibiotics tested	MAR index
SCBU	2	1 - 2	6	7	0.2
Gynecology	1	2	12	7	0.2
Lassa	2	0.5 - 2	13	7	0.3
MCH	1	4	6	7	0.2
Ophthalmology	3	0.5 - 4	10	7	0.2
Female Surgical	4	2 - 8	13	7	0.4
ICU	1	2	5	7	0.1
Pediatrics	3	0.5 - 2	6	7	0.2
Labour Wards	2	1 - 4	14	7	0.2
Post natal	3	2 - 4	7	7	0.2
A&E	4	0.5 - 4	14	7	0.3
Male Surgical	5	2 - 4	26	7	0.3
Laundry	4	0.5 - 4	26	7	0.4
Radiology	1	2	7	7	0.2

Key: SCBU= Special care baby unit; A&E= Accident and Emergency; MCH= Mother and Child health; ICU= Intensive care unit; MIC: Minimum Inhibitory Concentration; MAR: Multiple Antibiotic Resistance

Identification of Methicillin Resistant Coagulase Negative Staphylococci (MR-CoNS) Strains using 16S rRNA Sequencing

The genomic (chromosomal) DNA extracts from these isolates were of high quality, as shown in plate 1. Amplification of the 16S rRNA gene fragment (1500 bp) of six selected strains of MR-CoNS were shown in plate 2 a, b and c.

Multiple nucleotide sequence aligments of the gene fragment of the isolates (Figures 4.1 and 4.5) revealed that the isolates have strong homology (99-100%) with members of *Staphylococcus* gemus sequences deposited in the National Center for Biotecnlogy Information (NCBI) data base. The sequences of isolated strains have been deposited in the NCBI data base with ths accession numbers as shown in Table 7.

The results of neighbourhood joining trees or phylogenic tree of these six MR-CoNS isolates constructed using Molecular Evolutionary Genetics Analysis version 7.0 (Mega 7.0) are depicted in figure 2. The results revealed that the isolates from this study clustered among related Staphylococci specios already deposited in the NCBI database. The isolates were identified as Staphylococcus chromogenes, Staphylococcus scuiri (three strains) and Staphylococcus cohnii (two strains).

Table 9: Molecular Identification of MR-CoNS Isolates Based on 16S rRNA Gene Sequencing Data.

Isolate Code	Identified Bacteria	% Similarity	Assigned Gene Bank Accession Number
Igbins 1	Staphylococcus chromogenes strain	99.77	PQ198534
Igbins 2	Staphylococcus cohnii strain	99.89	PQ198535
Igbins 3	Staphylococcus sciuri strain	100	PQ198536
Igbins 4	Staphylococcus sciuri strain	100	PQ198537
Igbins 5	Staphylococcus cohnii strain	99.86	PQ198538
Igbins 6	Staphylococcus sciuri strain	99.82	PQ198539

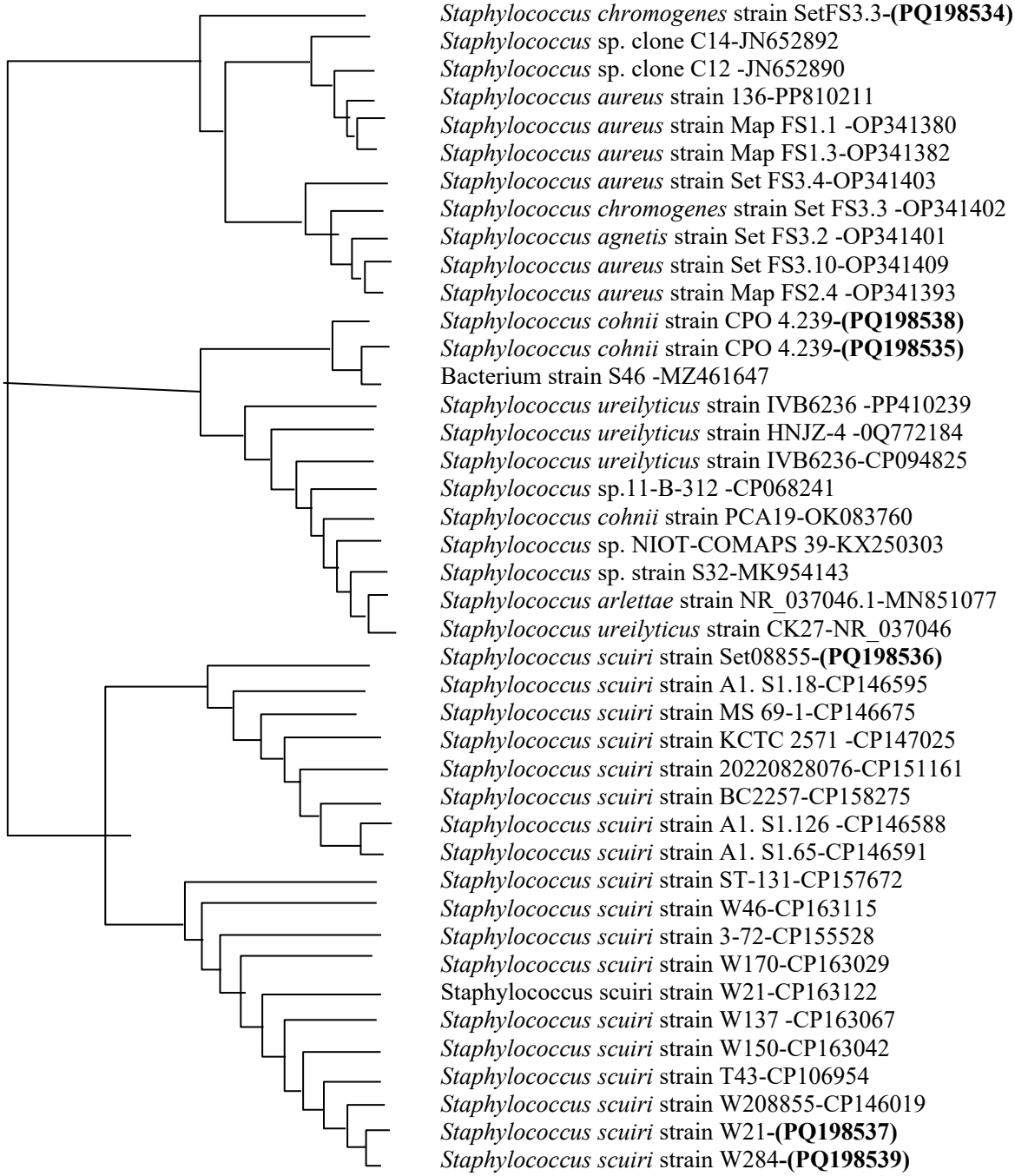


Figure 2. *Phylogenetic tree indicating the phylogenetic relationship of the 16S rRNA gene sequence from bacteria isolated in the study to those in the GenBank database. The accession numbers of the bacterial isolates with brackets are isolates from this study, while the accession numbers in the study without brackets are those in the GenBank database.*

Detection of Genes Coding for Methicillin Resistance

For the detection of the genes that codes for methicillin resistance in the MR CoNS isolates, the two genes targeted for amplification, were the *mecA* and the *blaZ* genes with an amplified product of 940 bp and 173bp respectively (Plate 3).

For these MR CoNS isolates, 18(49%) of the total 37 isolates possessed the *mecA* gene and 15 (41%) of the isolates possessed the *blaZ* gene (Table 8).

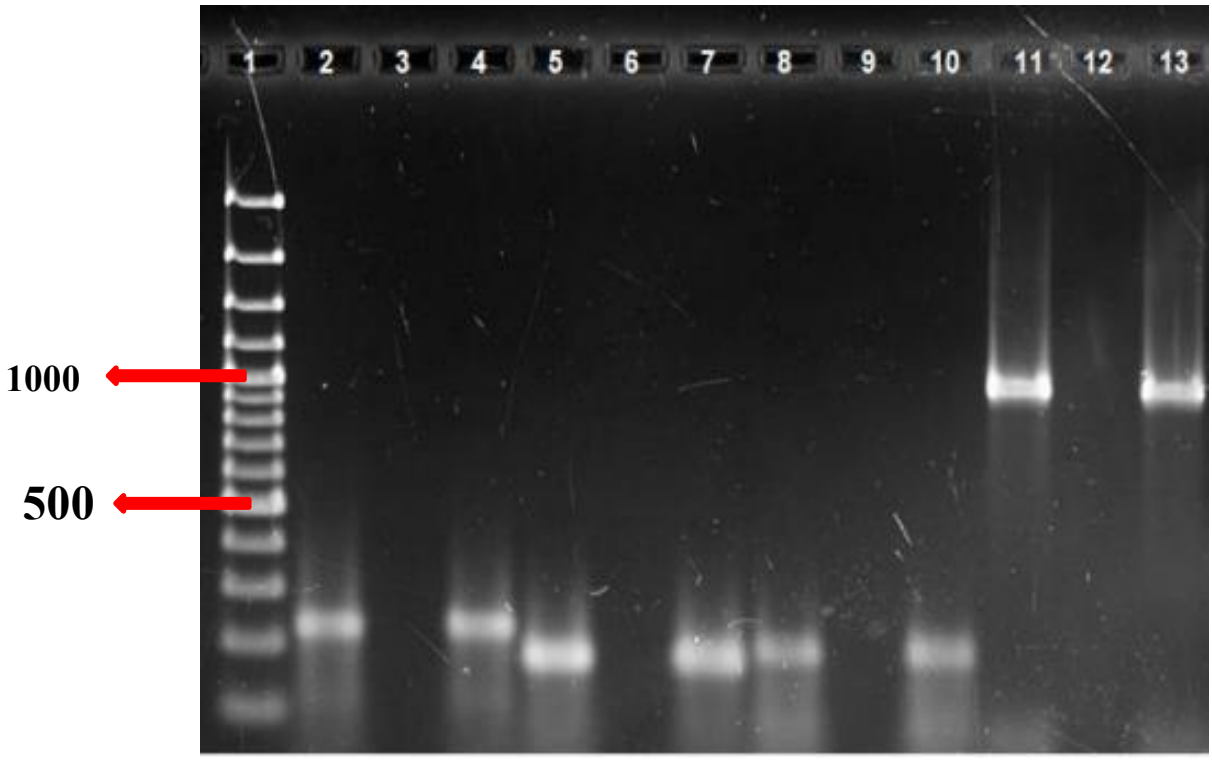


Plate 1. PCR product of representative amplified mecA, blaZ, nuc and pvl genes in the MR CoNS isolates. Lane 1 shows the 100 bp markers. Lane 2 shows the positive control for nuc (227 bp), lane 3 shows the negative control and lane 4 shows nuc detected in the representative MRSA isolate (227 bp). Lane 5 shows the positive control for blaZ (173 bp), lane 6 shows the negative control and lane 7 shows blaZ detected in the representative community isolate (173 bp). Lane 8 shows the positive control for pvl (190 bp), lane 9 shows the negative control and lane 10 shows pvl detected in the representative CoNS isolate (190 bp). Lane 11 shows the positive control for mecA (940 bp), lane 12 shows the negative control and lane 13 shows mecA present in the representative CoNS isolate (940 bp).

Table 10: Distribution of Isolates Possessing the Genes Responsible for Methicillin Resistance and Virulence in the MR CoNS Isolates

Gene	Number of Isolates (%) MR-CoNS (n = 37)
mecA	18 (49)
blaZ	15 (41)
pvl	8 (21)

Key: MR CoNS- Methicillin Resistant Coagulae Negative Staphylococci

Detection and Distribution of the *pvl* Genes

The presence of the *pvl* genes were ampified from the chromosomal DNA of the MR CoNS isolates around bp size of 190 (Plate 4). It was shown from this result that of the total 37 MR CoNS isolates, 8(21%) possessed the *pvl* gene (Table 8)..

Detection and Distribution of the SCC*mec*

Result data from Table 11 shows that for the distribution of *SCCmec* amongst the MR CoNS isolates, 18 (48%) of the isolates possessed the *SCCmec* II, III, IV, but only isolates from Lassa, A&E, Female surgical, Male surgical, Labour and Laundry departments in ISTH possessed SCCmec type IV and of the toatal 18 isolates that possessed the SCCmec, 6 (33%) possessed the SCCmec IV while 12 (67%) possessed the SCCmec type II and III..

Table 11: Detection of Staphylococcal Cassette Chromosome (SCC) mec for MR CoNS Isolates

Source of Sample within ISTH for HA-MRSA	No of MRSA Isolated	MAR Index	SCCmec Type	No of Isolates Possessing SCCmec
SCBU	2	0.2	II	1
Gynecology	1	0.2	–	–
Lassa Ward	2	0.3	IV	1
MCH	1	0.2	–	–
Ophthalmology	3	0.2	II, III	2
Female Surgical	4	0.4	II, IV	3
ICU	1	0.1	–	–
Pediatrics	3	0.2	II	1
Labor Ward	2	0.2	IV	1
Post natal	3	0.2	II	1
A & E	4	0.3	II, IV	2
Male Surgical	5	0.3	II, III, IV	3
Laundry	4	0.4	II, IV	3
Radiology	1	0.2	–	–

Key:

MRSA: Methicillin Resistant *Staphylococcus aureus*, MAR: Multiple Antibiotic Resistance, HA-MRSA: Hospital Acquired Methicillin Resistant *Staphylococcus aureus*, ISTH: Irrua Specialist Teaching Hospital, *SCCmec*: Staphlococcal Cassette Chromosome *mec*; SCBU= Special care baby unit; A&E= Accident and Emergency; MCH= Mother and Child health; ICU= Intensive care unit

Discussion

Methicillin resistant coagulase negative staphylococci (MR-CoNS) is a growing challenge in the treatment of infections caused by Coagulase negative staphylococci and has significantly contributed to the ever-increasing burden of global antimicrobial resistance (Wang et al., 2024).

Prevalence and Distribution

In this study, populations comprising of doctors, nurses, patients, cleaners and fomites in Irrua Specialist Teaching Hospital, Irrua, were used. The prevalence of MR CoNS in the studied populations was 12% of the 299 samples assayed. This is slightly lower than the 18% reported by Shrestha et al. (2020).

Results from this study also revealed that in the distribution pattern of MR CoNS isolated from ISTH, higher proportion of these isolates were recovered from male surgical ward 5 (14%), female surgical ward 4 (11%), Accident and Emmergency Unit 4(11%) and laundry department, 4 (11%) respectively, which allude to the fact these departments in ISTH tend to have a high-risk source of MR CoNS infections within the Hospital environment. This corroborates with findings of Ayepola et al. (2018) in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, where they recorded a prevalence of 21% of MR CoNS from Laundry and Male surgical department in a survey of the distribution pattern of MR CoNS within the hospital. It was also shown that the distribution pattern of the MR CoNS isolates among the various subjects in ISTH (Doctors, Nurse, Cleaners, Patients and Fomites) revealed that of the 37 MR CoNS isolated from ISTH, 14(38%) were isolated from fomites, which had the highest number of MR CoNS isolates. This is in accordance with the findings of Chajecka-Wierzchowska et al. (2023) where they concluded from their findings within hospitals in Denmark that fomites harbored the highest numbers of MR CoNS within the hospital settings. After the 14(38%) MR CoNS from fomites in ISTH, Cleaners had 9(24%) of the isolates, Patient had 6(16%), Nurses had 5(13%) and Doctors had 3(8%). This serial progression suggest that highest numbers of pathogens are always harbored in the hospital fomites, while the cleaners, who are the most frequent handlers of these fomites easily pick them up and spread them across the hospital environment. This is evident from the gradient flow of these MR CoNS isolates where fomites 38% > Cleaners 24% > Nurse 16% > Patients 13% > Doctors 8%. A quick look at this gradient flow puts in mind that fomites which harbored the highest numbers of MR CoNS is stagnated and would be majorly accessed by cleaners and also, nurses had a greater chance of handling these fomites than the doctors and patients. Hence, fomites could be the point source of MR CoNS reservoir in every hospital environment.

Antibiogram

Findings from this study depicted that of all the 37 MR CoNS isolated from ISTH, Vancomycin and Linezolid were the best drug of choice for the treatment of infections caused by these bacteria because all isolates were sensitive to these antibiotics. This is in line with the findings of Igbinosa et al. (2026), where they reported Vancomycin and Linezolid as the most effective antibiotics for treatment of MRSA and other Staphylococcal infections. For these MR CoNS isolates, 96% of the isolates were resistant to Cloxacillin which is not far-fetched from the fact that Cloxacillin is a member of the β-lactam antibiotics hence, the isolates are expected to be resistant to all classes of the β-lactam antibiotics by having a defaulted PBP2a which doesn't conform to the lactam ring or possession of the beta lactamase enzymes that hydrolyse beta lactam rings (Khambhati et al. (2024). Assay of these MR CoNS isolates against Ciprofloxacin, it was shown that 41% of these isolates were resistant to it while for tetracycline, 10% of the isolates were resistant which means that the tetracycline is also good for the treatment of MR CoNS infections. Gentamycin had 45% of isolates resistant to it. It therefore shows that from this study, vancomycin, linezolid and tetracycline are the drug of choice for treating MR CoNS infections.

Multiple Antibiotic Resistant (MAR) Index

Multiple Antibiotic Resistance (MAR) Index, which is a simple, cost effective tool used to evaluate the health risk of contamination sources by measuring bacterial resistance to antibiotics was performed on all MR CoNS isolates and it was shown that isolates from female surgical and laundry had MAR index of 0.4 each, lassa, A&E and male surgical had MAR index of 0.3 which reflects a very high risk source of MR CoNS and where antibiotics are frequently used and misused while isolates from other wards had MAR index of 0.2 This is

in accordance with findings of Xie et al. (2024), where they discovered that some MR CoNS had a higher resistant pattern to most antibiotics used in their research and this partly conforms with findings of Igbinosa et al. (2016) where they showed that Accident, Emergency and surgical ward in University of Benin Teaching Hospital (UBTH) are the high risk source of CoNS infections with MAR index greater than 0.2. The minimum inhibitory concentration (MIC) of oxacillin was also determined in this study and it was shown that the MIC value for all MR CoNS isolates ranged between 0.5 – 4 ug/ml.

Resistant Genes

Molecularly, the genes responsible for methicillin resistance (*mecA* and *blaZ* genes) were also detected using the specific primers. The findings revealed that 49% of the MR CoNS isolates possessed the *mecA* gene, while 41% of the MR CoNS isolates possessed the *blaZ* genes, though other resistance determinants like the novel mec homologues, border-line oxacillin resistance, regulatory gene mutation, where not targeted in this study which could result in differences in the perecentage of resistant genes. This finding partially correlates with findings of Ogefere and Lawrence (2019) where they discovered that some CoNS isolates possessed both *mecA* and *blaZ* genes.

Amplification of the *mecA* gene (which codes for the Penicillin binding proteins (PBP2a) that confers resistance to methicillin), the *blaZ* gene (which encodes the beta lactamse enzyme that hydrolyse the beta lactam ring in beta lactam antibiotics) and the *pvl* genes (which encodes the panton-valentine leucocidin, a virulence factors that lyse leukocytes) in all the isolates revealed that the distribution of these genes among the isolates differs. It was shown that some of the isolates possessed the three genes, some possessed two genes and one gene.

With regards to the *pvl* gene, 21% of the MR CoNS isolates acquired the *pvl* gene, which is suggestive of a drift of these genes from the community to hospital environment, as has been reported by Nurjadi et al. (2020), who reported that possession of the *pvl* gene is a biomarker of methicillin resistant *Staphylococcus aureus* (MRSA) from the community lineage and that *pvl* is mostly associated with community acquired MRSA or community acquired CoNS infections and distinguishable from hospital acquired isolates by multidrug resistance and carriage of the type IV, V and VI Staphylococcal chromosome cassette element (*SCCmec*).

In this present study, the results showed that 24% of the MR CoNS isolates possessed mostly SCC*mec* type II and III, however some isolates, 11% possessed the SCC*mec* type IV and V which is of community origin. And it was also shown that there was correlation between those MR CoNS isolates having the SCC*mec* IV and the same isolates having the *pvl* genes which is also a biomarker of community origin. It was also deduced from these findings that isolates possessing SCC*mec* types IV and the *pvl* genes also had highest MAR index from this study (laundry, accident and emmergency and female surgical ward). Taking a look at these departments in ISTH (laundry, accident and emmergency and female surgical ward) where the isolates had *pvl* genes, SCC*mec* IV and high MAR index, it was clear that they are departments that easily support the access of people from community to the hospital environment and vice versa. This is in correlation with the findings of Ogefere and Lawrence (2019) where they suggested an influx of community associated virulence genes within the hospital environment.

Virulence Factor

Some virulent factors such as Biofilm formation, Haemolysin production and DNase production were also assayed in all the MR CoNS isolates. Findings revealed that 57% of the 37 MR CoNS isolates were biofilm formers (produced biofilm), while 11% produces haemolysin and 8% produces Dnase. This is in confirmation with the findings of Igbinosa et

al. (2026) that CoNS isolates have the capacity to produce biofilm, Dnsae and haemolysin. The production of haemolysin and DNase, as well as formation of biofilms are pointers to the acquisition of some virulence determinant by MR CoNS. The ability for most of these isolates to form biofilm is a characteristic that makes them virulent and it allows them to remain on surface for a long time before being picked up by a potential host (Nasaj et al., 2020). This is not far-fetched from this study where majority of the isolates were obtained from fomites hence, their ability to form biofilm is an adaptive feature for them to remain viable on these fomites till they are being picked up by a biotic host. DNases have often been described as virulence factor in *Staphylococcus* spp because it has been shown that Dnase can help bacteria to escape from neutrophil extracellular traps (NETs) which are structures secreted by neutrophils to trap and kill bacteria (Manandhar et al., 2021). These structures are mainly made of DNA, Protease and Antimicrobial peptides. Hence, the possession of these Dnase enzymes by some isolates from this study shows that some of these bacterial isolates are virulent in nature.

Findings from this study on the isolates shows that, isolates with higher MAR index tend to possess the three genes (*pvl, blaZ* and *mecA*) in them, and also possessed majority of the virulent genes and finally, they had a mixture of SCC*mec* type of hospital and community origin. Therefore, there could be possibility of a new strains of CoNS building up due to crosslinking or influx of community associated genes to hospital associated genes and vice versa.

CONCLUSION AND RECOMMENDATIONS

Conclusion

In the incidence of the threat of Coagulase negative staphylococci (CoNS) acquiring methicillin resistant genes and other virulent biomarkers within Irrua Specialist Teaching Hospital, Irrua, Edo state, an overall prevalence of 12% CoNS isolates which included *Stsaphylococcus scuiri*, *Stsaphylococcus cohnii* and *Stsaphylococcus chromogenes* were isolated. For the distribution of these CoNS isolated from ISTH, Irrua, 38% were from fomites, 24% from cleaners, nurse had 13%, patients had 16% and doctors had 8% of these isolates. From the antibiogram study of these isolates, all CoNS isolated were sensitive to vancomycin and linezolid, though other antibiotics such as tetracycline, gentamycin and erythromycin showed some level of efficacy to these isolates. Most (96%) of isolates were resistant to cloxacillin. The MAR index of the isolates from different wards, department and districts from ISTH shows that Accident and Emmergency ward, female surgical, laundry, lassa institute and male surgical ward had a high MAR index of 0.4 and 0.3 which connotes an environment of high-risk source of antibiotic usage. The occurrence of virulent factors among the CoNS isolates were assayed for, which included biofilm formation, hemolytic activities and DNase production and some of these isolates possessed some of these virulent factors.

Recommendations

This study has revealed the Prevalence, antibiogram patterns and genomes of MR CoNS isolated from ISTH, Irrua. We therefore recommend that

Health care workers should maintain proper hygiene practices by regular washing of hands, cleaning of surfaces with appropriate disinfectants and be mindfull of the fact that most fomites within the hospital could harbor pathogenic microbes.

Laundry departments in hospital environments should be carefully designed and managed because most pathogenic organisms are on the beddings, scrubs etc. Even the waste water from

washing these clothing should be properly treated before being disposed off around the hospital environment if they don't have a well constructed drainage system.

Patients should be educated on the misuse and abuse of antibiotics, and the need for public enlightenment programs on antibiotic stewardship.

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Conflicts of Interest

The authors declare no conflict of interest

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