Molecular characterization of antimicrobial resistance and virulence factors in coagulase – negative staphylococci nasal isolates among adult patients in a tertiary hospital in North-Western Nigeria

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Abstract

Coagulase-negative staphylococci are harmless microorganisms that can become pathogenic when the host resistance is impaired. Their ability to exhibit multidrug-resistance and form biofilm limit treatment options and contributes to the health and financial burden on health care systems worldwide. This study aimed to provide data on the acquisition and expression of genetic elements associated with resistance and virulence in the organisms from nasal colonization of adults admitted to a University Teaching Hospital in Nigeria. One hundred and twenty-three presumptive staphylococci isolates were obtained from the medical microbiology laboratory of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The isolates were characterized, and the antibiotics susceptibility pattern assessed. Biofilm formation was evaluated by the micro-titre plate method, and polymerase chain reaction was used to detect resistance genes (*mecA* and *ermB*), virulence gene (*psm-mec*) and adhesin genes, (*icaAB*, *icaC* and *icaD*) in the isolates.

Out of the 60 coagulase-negative staphylococci isolates, 50 (83.3%) were strong biofilm formers, 5 (8.3%) were moderate biofilm formers and 5 (8.3%) were weak biofilm formers. For the 14 isolates that were characterized by PCR, *mecA* and *ermB* resistance genes were detected in 78% and 71% of the isolates respectively. Twenty-one percent had *psm-mec* virulence gene while biofilm adhesin genes, *icaAB* and *icaD* were detected in 36% and 57% respectively, *icaC* gene was not detected.

This study confirmed formation of biofilm, carriage of resistance and virulence genes which have been shown to play a role in pathogenesis. This study shows the necessity

of the periodical monitoring of the drug resistance pattern and virulence factors of coagulase-negative staphylococci.

Keywords: coagulase-negative staphylococci, phenol soluble modulins, methicillin resistance, macrolide resistance.

Introduction

Antimicrobial resistance poses a growing threat to public health worldwide. Thus, a systematic review and continued surveillance on antibiotic susceptibility is a key strategy in antimicrobial resistance control to inform decisions to assist healthcare providers on the trend of antibiotics and effectiveness (Tacconelli *et al*., 2018). According to WHO, surveillance of antimicrobial resistance tracks changes in microbial populations, permits the early detection of resistant strains of public health importance, and supports the prompt notification and investigation of outbreaks (WHO, 2015).

The ability of coagulase-negative staphylococci (CoNS) to form biofilm on surfaces (mucosal, medical devices) and their multidrug-resistant characteristics play an essential role in their virulence resulting in increased morbidity and mortality in the hospital settings (Becker *et al*., 2014; Soumya *et al*., 2017; Becker *et al*., 2020). This ability to cause diseases and persist in the hospital environments is related to the production of virulence factors - molecules, such as biofilms that add to their effectiveness and enable them to achieve colonization (Solati *et al*., 2015; Franca *et al*., 2021). Coagulase-negative staphylococci are drug resistance and virulence genes reservoir and are becoming important causes of healthcare-associated infections (HAIs) (Becker *et al*., 2020). Because they are characterized by fewer virulence factors than *S. aureus*, especially factors responsible for aggression such as Panton-Valentine leukocidin (PVL) (LukSF-PV), they are generally considered less pathogenic (Michalik *et al*., 2020).

Nevertheless, factors involved in colonization such as phenol soluble modulins (PSMs) and biofilm may successfully support the bacterium-host interaction when the host resistance is impaired (Becker *et al*., 2014). The status of the host immune system influences coagulase-negative staphylococci disease onset and outcome especially among immunocompromised, long-term hospitalized and critically ill patients with prolonged use of implanted medical devices

(Heilmann *et al*., 2018). Virulence factors contributing to the coagulase-negative staphylococci pathogenicity play more essential roles in establishing, maintaining

and persisting of the coagulase-negative staphylococci infections (Solati *et al*., 2015).

The ability to form biofilm is the most important virulence factor of coagulase-negative staphylococci as it allows them to adhere and colonize surfaces (Soumya *et al*., 2017). Biofilms are complex communities of adherent bacteria encased in a matrix of self-produced extracellular polymeric substances (EPS) (Sabaté Brescó *et al*., 2017). Biofilms provide survival advantages to the organism by making bacteria cells less accessible to the host's defense system and by impairing the action of antimicrobials (Michalik *et a*l., 2020). It has been shown that both *S. aureus* and *S. epidermidis* contain the intercellular adhesion operon, *icaADBC* responsible for biofilm formation and plays a significant role in the pathogenic nature of *S. aureus* and *S. epidermidis* (Tacconelli *et al*., 2018; Michalik *et al*., 2020). The ica operon (*icaADBC* gene cluster) encodes the polysaccharide intercellular adhesion (PIA); thus, there is a correlation between the presence of this operon and slime production in staphylococci because the polysaccharides found in both biofilms and slime layer are regulated by the ica operon, especially coagulase-negative staphylococci (Pedroso *et al*., 2016). The *ica* locus is part of the "accessory genes" genome and not a "core" genome, which means that it is not found in all bacterial strains (Arciola *et al*., 2015).

Staphylococci strains, especially CONS, have been reported to secrete the peptide known as PSMs identified as a contributor to the overall pathogenesis of staphylococci (Towle *et al*., 2016). PSMs are family of amphipathic α-helical peptide toxins. They are cytolytic and inflammatory toxins (Queck *et al*., 2009; Cheung *et al*., 2014). All known PSMs are core genome-encoded peptides except for *psm-mec* encoded in the mobile genetic element (MGE) of staphylococci cassette chromosome mec (*SCCmec*), which also contains genes involved in methicillin resistant (*mecA*), recombinase genes, regulatory elements and other accessory genes (Qin *et al*., 2016). Incidence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) causing infections are on the rise and have equally been implicated in clinical infections worldwide (Ibadin *et al*., 2017). Generally, PSMs peptides contribute to the structuring of biofilms and the disseminating of biofilm-associated infections, owing to the significantly increased resistance they provide to antimicrobials and host defenses (Franca *et al*., 2021).

This study aimed to provide data on the characterization of genes associated with resistance and virulence in coagulase-negative

staphylococci from the nares of adult patients admitted to a University Teaching Hospital in North-Western Nigeria.

Materials and methods

Preliminary study

The initial study on the presumptive staphylococci isolate involved the identification of the isolates using various methods. Gram staining was carried out according to Cheesebrough (2006), where a smear was prepared from growth colonies, stained, and microscopically examined. Haemolytic activity was tested using Columbia agar supplemented with 5% sheep blood, and isolates were classified based on the degree of haemolysis observed as described by Jimenez et al., (2008). Biochemical tests, including the catalase and coagulase tests, were performed according to standard protocols (Sue, 2010; Clinical Microbiology Procedures Handbook, 2016). Rabbit plasma was obtained by centrifuging whole blood collected in anticoagulant-treated tubes. A 1-in-6 dilution of the plasma in normal saline was prepared. A colony of catalase-positive cocci was emulsified in the diluted plasma and placed in the incubator. Clot formation was observed at intervals over 24 hours. A positive result was indicated by clot formation within 24 hours, while a negative result was indicated by no clot formation. For Staphylococcus genus identification, the Microgen™ Staph-ID System was used, which involved emulsifying a single colony in the kit's suspending medium, adding the bacterial suspension to microwells, and interpreting reactions based on a color chart and substrate reference table. The PYR and nitrate reduction tests were also performed and interpreted using the Microgen software database. Detection of antimicrobial susceptibility pattern using the Modified Kirby Bauer disk diffusion method (the Cefoxitin disk was used as surrogate marker in the detection of methicillin resistance) and the inducible clindamycin resistance was performed using double disc diffusion test (D-zone test) according to the European committee on antimicrobial susceptibility testing breakpoints (EUCAST) (EUCAST, 2019; Atolagbe *et al*., 2021). This study was carried out on the 60 CONS isolates characterized from the previous study (Atolagbe *et al*., 2021). Out of the isolates, fourteen (14) that met the criteria i.e. phenotypic methicillin resistance, inducible clindamycin resistance, biofilm production and multidrug resistance were molecularly characterized in this study. Ethical clearance was obtained from the Ahmadu Bello University Committee for Research in Human

Subjects with the reference number: ABUCUHR/2019/007.

Detection of biofilm producing coagulase-negative staphylococci

Biofilm production was detected using micro-titre plate assay, following the procedure as modified by Merritt *et al*. 2005. Each identified pure isolate of CONS was inoculated in 10 ml of brain heart infusion (BHI) broth (Oxoid Ltd., Basingstoke, Hampshire, England) supplemented with 2% glucose and 2% sucrose and incubated overnight for 24 h at 37°C. The cultures were diluted 1 µL in 10 ml of the BHI medium, and 150 µL of the diluted cultures, per well, were inoculated into 96-well polystyrene micro-titre plates. After 48 h incubation at 37°C under aerobic conditions, the plates were washed three times with normal saline to remove unfixed microbial cell and then dried in an inverted position. The dried wells were stained with 250 µL of 0.1% crystal violet solution and incubated at 25°C for 20 mins. The excess crystal violet was removed by gently washing the plate thrice with normal saline and dried for 30 mins at 25°C. Positive results were seen as the presence of a layer of stained materials adhered to the inner wall of the wells.

Biofilm was quantified by adding a volume of 250 µL of ethanol-acetic acid (95:5 vol/vol) per well, and 100 µL from each well was transferred to a new micro-titre plate and the absorbance (OD) was measured at 630 nm with micro ELISA auto reader (UNI EQUIP – 2002, Germany). The absorbance of the un-inoculated wells (negative control) was used to calculate the cut off (ODc) as follows:

ODc = Average OD value of negative control + 3 x standard deviations of negative control.

The ODc value was used to classify biofilm producing capability of isolates into four categories: non-adherent (OD < ODc), weakly adherent (ODc < OD < 2 x ODc), moderately adherent (2 x ODc < OD < 4 x ODc), and strongly adherent (4 x ODc < OD) (Stepanovic et al., 2007).

Detection of Resistance and Virulence Genes

Genomic DNA was extracted using Accu prep Genomic DNA extraction kit from Bioneer Inc. (Seoul, South Korea), from an overnight coagulase-negative staphylococci resistance culture according to the manufacturer's instruction.

Detection of mecA and ermB genes: Multiplex polymerase chain reaction for the detection of the *mecA and ermB* genes after an extensive optimization of the reaction to ensure a better amplification was performed

using the AccuPower Gold Multiplex PCR PreMix. Two sets of primers, synthesized by Bioneer, Inc. (Seoul, South Korea) were used to amplify the selected genes that are shown in Table 1 (Murakami *et al*., 1993; Zhang *et al*., 2016). The templates, specific primers and water were added to the PCR premix tube containing the reaction components, the dNTPs and thermostable DNA-polymerase within each tube in a lyophilized premix form. The following components were added for each isolate for the multiplex reaction; 2.0 µL of template DNA, 2.0 µL of forward primer, 2.0 µL of reverse primer and deionized water (14 µL). The final volume of the multiplex polymerase chain reaction was 20 µL. Amplification was performed by a conventional polymerase reaction (Thermal Cycler, PCR System 2700, GeneAmp®, Applied Biosystems). After an initial denaturation step for 5 min at 95^{o}C, 35 cycles of amplification were performed as follows; 95°C for 1 min (denaturation), 47°C for 1 min (annealing) and 72°C for 1 min (extension) and final extension was performed at 72°C for 5 min. Amplified products were analyzed by 1.5% agarose gel electrophoresis containing ethidium bromide and visualized on an ultraviolet trans-illumination gel documentation system (Bio-Rad).

Table: 1: Primers used in this study

Gene	Primer and Description	Primers Sequence	Amplicon Size (bp)	References
mecA	Forward	5'-GTTGTA GTT GTCGGG TTTGG-3'	301	Murakami and Minamide, 1993
	Reverse	5'-CTTCCACAT ACC ATCTTCTTT AA-3'		
ermB	Forward	GAAAAGGTACTCAACCAAATA	639	Zhang *et al.,* 2016
	Reverse	AGA GTT TGA TCC TGG		
icaAB	Forward	5'-TTATCAATGCCGCAGTTGTC-3'	546	Frebourg *et al*., 2000
	Reverse	5'-GTTTAACGCGAGTGCGCTAT-3'		
icaD	Forward	5'-ATGGTCAAGCCCAGACAGAG -3'	198	Petrelli *et al*.,2006
	Reverse	5'-CGTGTTTTCAACATTTAATGCAA -3'		
icaC	Forward	5'- TAACTTAGGCGCATATGTTTT-3'	400	Solati *et al*., 2015
	Reverse	5' -TTCCAGTTAGGCTGGTATTG-3'		
psm-mec	Forward	5'- TGCATATGGATTTCACTGGTGTTA-3'	257	This study
	Reverse	5'CGTTGAATATTTCCTCTGTTTTTAGTTG-3'		

Keys: *mecA* (Methicillin resistance gene), *icaAB* (Intercellular adhesin AB), *icaD* (Intercellular adhesin D), *icaC* (Intercellular adhesin C), *psm-mec* (Phenol soluble modulin mec), *ermB*, (macrolide resistance- target site modification gene)

Detection of psm-mec gene

Amplification of the *psm-mec* gene was performed using primers designed for this study according to sequence type 1 (ST1) DNA Sequence of *S. epidermidis* strain (*psm-mec* positive) (Grace *et al*., 2019). The 20 µL PCR reactions were performed using the AccuPower HotStart PCR PreMix; templates, *psm-mec* primers synthesized by Bioneer, Inc. (Seoul, South Korea) and water were added to the PCR premix tube containing the reaction components, the dNTPs and thermostable DNA polymerase within each tube in a lyophilized premix form. The following components were added for each isolate reaction 2.0 µL of template DNA (genomic DNA), 1.0 µL of psm-mec forward primer, 1.0 µL of psm-mec reverse primer and

deionized water (16 µL). The PCR product size of *psm-mec* gene amplification was 257 bp. After an initial denaturation step for 3 min at 94°C, 35 cycles of amplification were performed as follows: 94°C for 30 s; 51°C for 30 s and 72°C for 45 s. The final extension carried out at 72°C for 5 min. Amplified products were analyzed by 1.5% agarose gel electrophoresis containing ethidium bromide and visualized on an ultraviolet trans-illumination gel documentation system (Bio-Rad).

Detection of ica adhesin genes

The PCR reaction for the biofilm-forming *icaAB*, *icaD* and *icaC* in the isolates were performed using the AccuPower HotStart PCR PreMix. The primer sequence used, the annealing temperature and the PCR program employed are given in Table 1 (Frebourg *et al*., 2000; Petrelli *et al*., 2006; Solati *et al*., 2015). Templates, specific primers synthesized by Bioneer, Inc. (Seoul, South Korea) and water were added to the PCR premix tube containing the reaction components, the dNTPs and thermostable DNA polymerase within each tube in a lyophilized premix form to make a 20 µL reaction. The following components were added for each isolate reaction 2.0 µL of template DNA (genomic DNA), 1.0 µL of forward primer, 1.0 µL of reverse primer and deionized water (16 µL). Amplified products were analyzed by 1.5% agarose gel electrophoresis containing ethidium bromide and visualized on an ultraviolet trans-illumination gel documentation system (Bio-Rad).

Statistical Analysis

The data on production of biofilms by the strains of CONS was analyzed by the IBM Statistical Package for Social Sciences (Version 21, International Business Machines Corporation). P values were calculated using the Chi square test. $P < 0.05$ with a 95% confidence interval was considered to be statistically significant.

Results

The results show varied levels of biofilm formation by the test bacteria. About 50 (83.3%) of the isolates showed strong biofilm formation while 5(8.3%) moderate biofilm formation and 5(8.3%) weak biofilm formation (Figure 1).

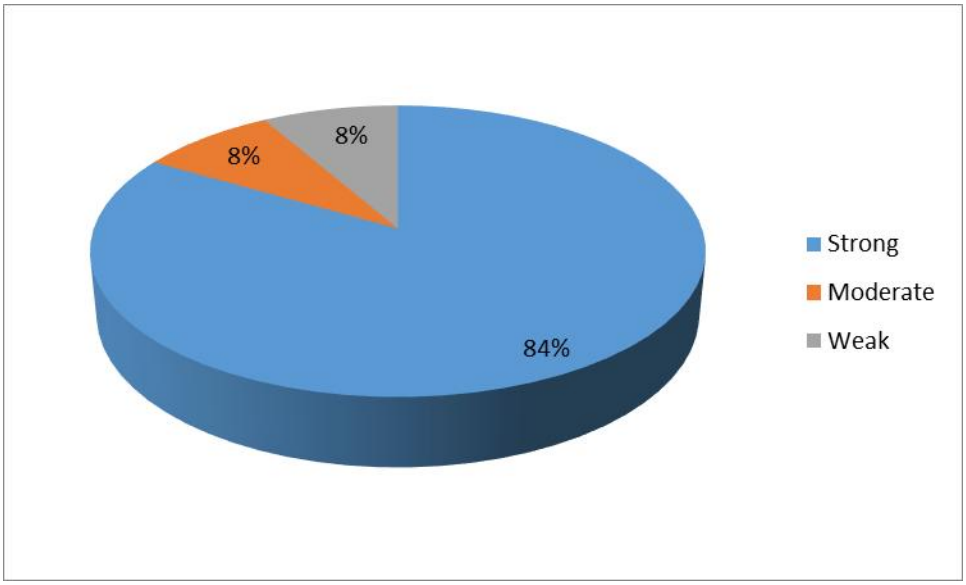


Figure 1: Distribution of the quantity of biofilm produced.

The results of the ELISA readings on 60 isolates of the coagulase-negative staphylococci for biofilms production is shown in Table 2. The results suggest a diverse range of biofilm-forming abilities among the tested coagulase-negative staphylococci isolates. Isolates 40 and 48 stand out with very high absorbance values (0.818 and 0.783, respectively), indicating strong biofilm formation which may have implications for the virulence and persistence of these microorganisms. Isolates with low absorbance values may have weaker biofilm-forming abilities.

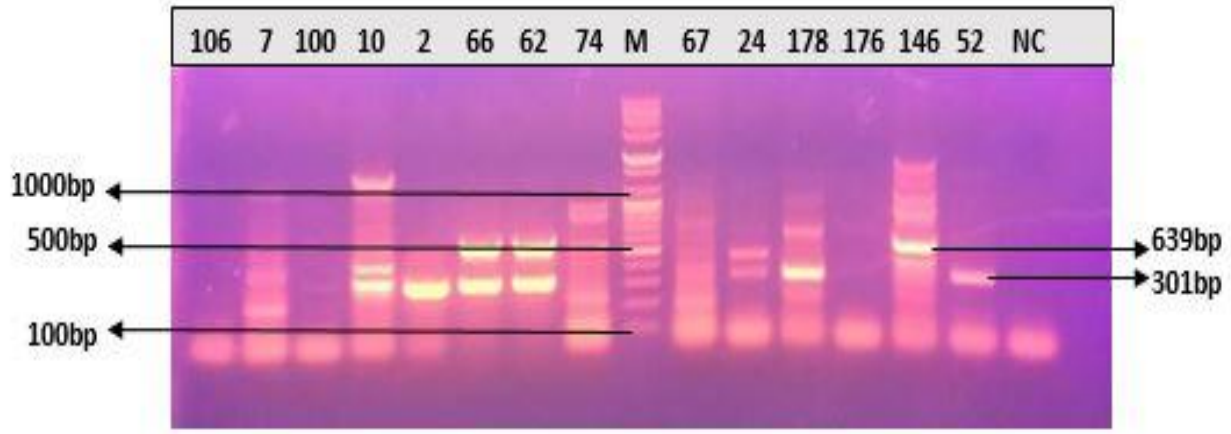
Table 2: The results of the ELISA reading on 60 coagulase-negative isolates for biofilm production

Number of samples	Result	Number of samples	Result	Number of samples	Result
1	0.074	**21**	0.110	**41**	0.150
2	0.172	**22**	0.098	**42**	0.083
3	0.180	**23**	0.070	**43**	0.100
4	0.088	**24**	0.080	**44**	0.578
5	0.094	**25**	0.142	**45**	0.083
6	0.091	**26**	0.201	**46**	0.080

7	0.115	**27**	0.085	**47**	0.095
8	0.108	**28**	0.192	**48**	0.783
9	0.076	**29**	0.122	**49**	0.077
10	0.136	**30**	0.194	**50**	0.150
11	0.207	**31**	0.274	**51**	0.511
12	0.086	**32**	0.085	**52**	0.072
13	0.098	**33**	0.071	**53**	0.090
14	0.078	**34**	0.083	**54**	0.075
15	0.075	**35**	0.074	**55**	0.067
16	0.071	**36**	0.083	**56**	0.075
17	0.163	**37**	0.086	**57**	0.075
18	0.080	**38**	0.075	**58**	0.069
19	0.096	**39**	0.139	**59**	0.077
20	0.068	**40**	0.818	**60**	0.078

The 14 multidrug resistant isolates investigated for the carriage of resistance and virulence genes revealed that 11(78%) harboured the *mecA* gene, 10(71%) carried the *ermB* gene, 3(21%) harboured the *psm-mec* gene, 8(57%) of the isolates were positive for the *icaD* gene, 5(36%) carried the *icaAB* gene and the *icaC* gene was not detected (Figures 3, 4, 5, 6 and 7). *Staphylococcus epidermidis* strains were observed to carry the genes responsible for methicillin resistance and macrolide resistance-target site modification. Two of the *S. epidermidis* strains were also positive for *psm-mec* gene carriage (Table 3).

Statistical analysis using Chi square test showed that there was no significant difference between the intensity of biofilm production and species type (P=0.877).



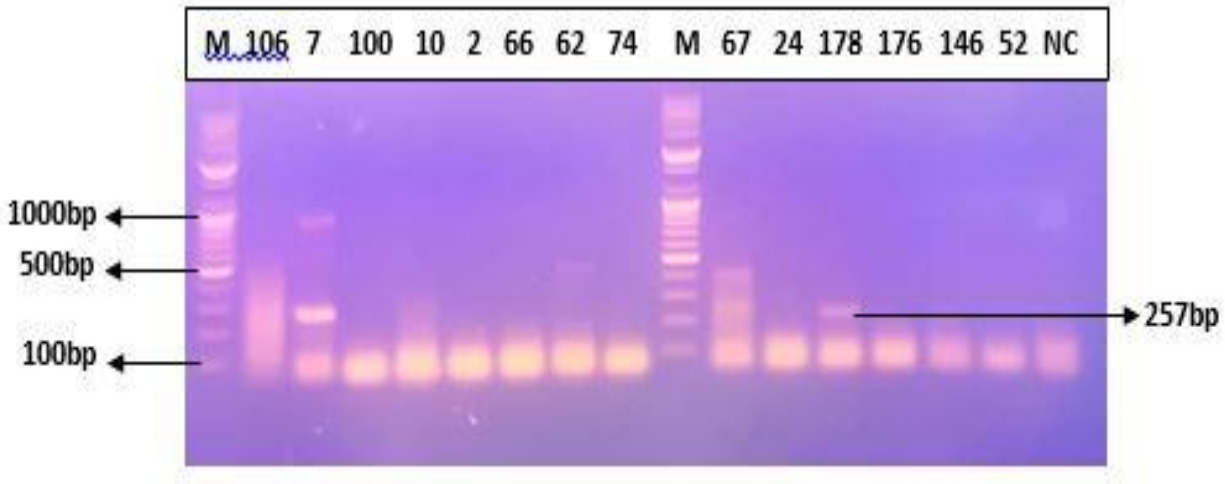
mecA genes in the resistance CoNS isolates

Lane M: 1kb plus DNA ladder, Lane 7: *S. chromogenes*, Lanes 10, 67 & 178: *S. epidermidis*, Lane 2: *S. hyicus,* Lane 66: *S. simulans*, Lane 62: *S. xylosus*, Lane 74: *S. caprae*, Lane 24: *S haemolyticus*, Lane 146: *S. intermedius*, Lane 52: *S. hominis*

ermB genes in the resistance CoNS isolates

Lane 7: *S.* chromogenes, Lanes 10, 67 & 178: *S. epidermidis*, Lane 2: *S. hyicus*, Lane 66*: S. simulans*, Lane 62: *S. xylosus*, Lane 74: *S. caprae*, Lane24: *S. haemolyticus*, Lane 146: *S. intermedius,* Lane NC: Negative Control

Figure 3: PCR amplification for the *mecA* (301bp) and *ermB* (639bp) genes



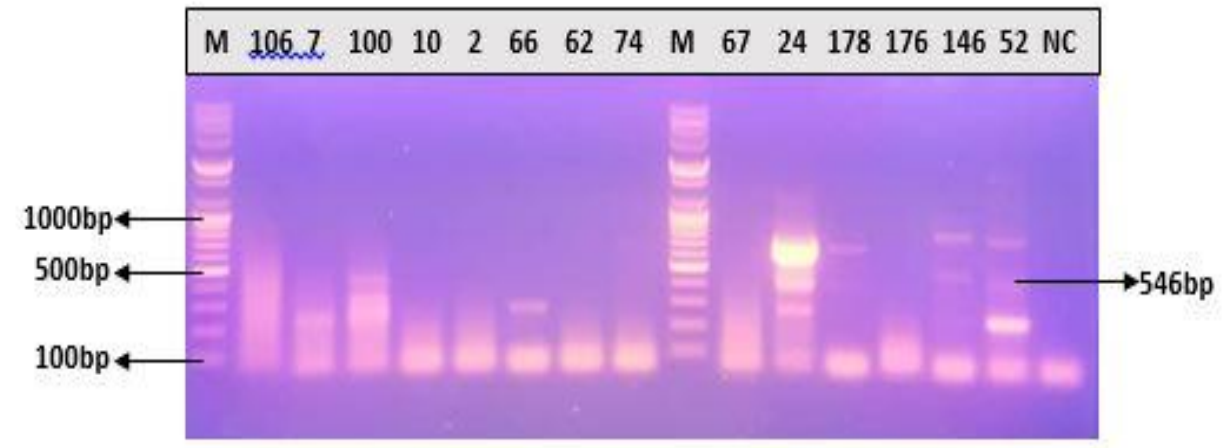
Lane M: 1kb plus DNA ladder

Lane 7: *S. chromogenes*

Lanes 67 & 178: *S. epidermidis*

Lane NC: Negative Control

Figure 4: PCR amplification for the *psm-mec* (257bp) gene



Lane M: 1kb plus DNA ladder

Lanes 106 &100**:** *S. chromogenes*

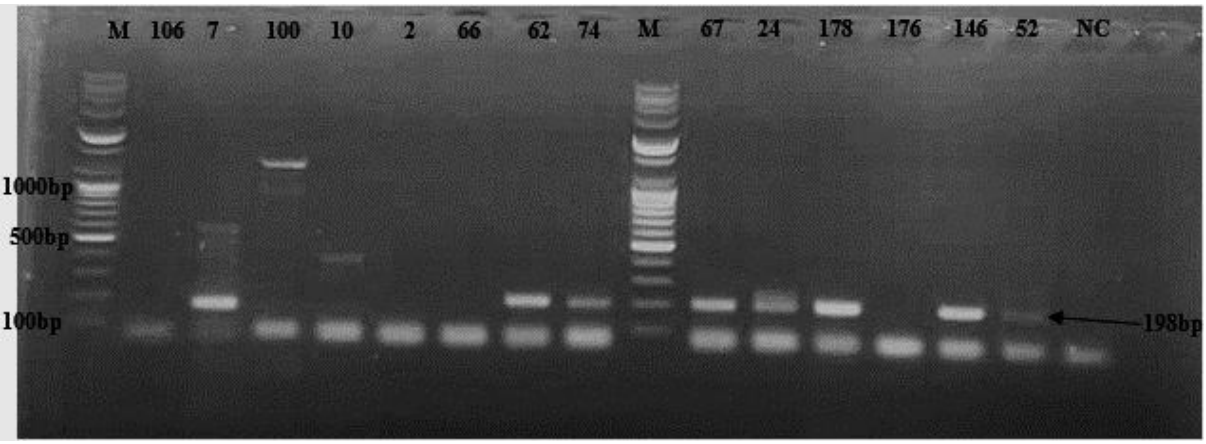
Lane 24: *S. haemolyticus*

Lane 146; *S. intermedius*

Lane 52: *S. hominis*

Lane NC: Negative Control

Figure 5: PCR amplification for the *icaAB* (546bp) gene



Lane M*:* 1kb plus DNA ladder

Lane 7: *S. chromogenes*

Lane 62: *S. xylosus*

Lane 67*&*178*: S. epidermidis*

Lane 74: *S. caprae*

Lane 24: *S. haemolyticus*

Lane 146: *S. intermedius*

Lane 52: *S. hominis*

Lane NC: Negative Control

Figure 6: PCR amplification for the *icaD* (198bp) gene

Table 3: Carriage of *icaAB*, *icaC*, *icaD*, *mecA*, *ermB* and *psm-mec* in Coagulase - Negative Staphylococci isolates

Organisms	**Number positive for gene carriage**					
	icaAB	*icaC*	*IcaD*	*mecA*	*ermB*	*psm-mec*
S. chromogenes (n=03)	02	-	01	01	01	01
S. epidermidis (n= 03)	-	-	02	03	03	02
S. hyicus (n= 01)	-	-	-	01	01	-
S. simulans (n= 01)	-	-	-	01	01	-
S. xylosus (n= 01)	-	-	01	01	01	-
S. caprae (n= 01)	-	-	01	01	01	-
S. haemolyticus (n= 01)	01	-	01	01	01	-
S. coagulans (n= 01)	-	-	-	-	-	-
S. intermidius (n= 01)	01	-	01	01	01	-
S. hominis (n= 01)	01	-	01	01	-	-
Total	05	0	08	11	10	03

Discussion

The public health importance of coagulase-negative staphylococci strains isolated from the hospitals has significantly increased due to the presence of several antibiotics resistance genes and with the increase, comes an increased number of virulence factors identified in these strains (Kateete *et al*., 2020; Franca *et al*., 2021). The majority of the isolates in this study were biofilm producers (98.3%). This is similar to reports from China where most nasal CONS isolates also produced biofilm (Du *et al*., 2013). Statistical analysis found no significant difference in biofilm production intensity based on species type.

However, irrespective of the biofilm formers, susceptibility to antimicrobial was low as higher antibiotics concentration would be required to eradicate the organisms in the biofilm state (Atolagbe, *et al*., 2021). Arciola *et al*. reported that antimicrobials target fast growing cells and cells residing in a biofilm have decreased cellular activities which result in a non-aggressive but persistent, protected mode of growth less sensitive to antimicrobials (Arciola *et al*., 2015). Biofilm production in this study could be as a result of the amphipathic characteristics of the phenol soluble modulin (PSMs) reported to be responsible for biofilm structuring (Becker *et al*., 2020).

The *mecA* gene that codes for the production of modified penicillin binding protein was detected in most of the tested isolates. Similar reports have been made in Iran where nasal coagulase-negative staphylococci isolates possessed the *mecA* gene (Abadi *et al*., 2015). However, some methicillin-resistant isolates did not show amplification of the *mecA* gene, suggesting other mechanisms of resistance. This contrasts with reports from Poland, where all methicillin-resistant coagulase-negative staphylococci isolates possessed the *mecA* gene (Brzychczy-Wloch *et al*., 2013). Methicillin-resistant coagulase-negative staphylococci (MRCoNS) are of concern not only because of their methicillin resistance but also because they are reported to be generally resistant to many other antibacterial agents (Cheung *et al*., 2014).

The presence of the ermB gene in half of the tested isolates indicated constitutive MLSB resistance (Martins *et al*., 2012). The presence of the genes encoding the constitutive MLS resistance and inducible resistance among coagulase-negative staphylococci strains demonstrate the genes→ widespread prevalence (Szemraj *et al*., 2019). Early detection prevents therapeutic failure and

allows physicians to choose appropriate treatment for patients.

The *psm-mec* gene was detected in *S. epidermidis* and *S. chromogenes*. Considering the low presence of *psm-mec* gene, coagulase-negative staphylococci isolates may be carrying the β-type phenol soluble modulins related to biofilm formation and dissemination. This is similar to the findings by Grace *et al*. who reported low presence of *psm-mec* gene in biofilm positive *S. epidermidis* species (Grace *et al*., 2019). Qin *et al*. reported that the *psm-mec* locus influences cytolytic capacity, methicillin resistance, biofilm formation and cell spreading in *S. epidermidis* resulting in significant impacts on immune evasion and disease and these effects are highly strain dependent (Qin *et al*., 2016).

The *psm-mec*, an agr-regulated gene is located on specific staphylococci cassette chromosome mec (*SCCmec*) elements (Berlon *et al*., 2015). There is reported evidence of horizontal transfer of staphylococcal cassette chromosome *mec* between staphylococcal species, implying that coagulase-negative staphylococci could serve as a reservoir for the spread of resistance genes to *S. aureus* (Yang *et al*., 2017). Phenol soluble modulins in *S. epidermidis* is also said to appear to participate primarily in biofilm development however, this process can be blocked by anti-PSM β peptides (Brzychczy-Wloch *et al*., 2013; Otto *et al*., 2014). There have been similar reports of detection of *psm-mec* gene in coagulase-negative staphylococci spp in Germany (Berlon *et al*., 2015). In this study, *psm-mec* gene carrying spp also harbored the *mecA* gene, which was expected considering previous carriage reports of both genes on same genetic elements, *SCCm*ec (Monecke *et al*., 2012).

Screening for the presence of the *psm-mec* gene allows for preliminary assessment of the potential of a given strain to produce the *psm-mec* peptide which contributes to virulence and is primarily influenced by the relative level of production compared to that of other PSMs (Queck *et al*., 2009). It has been suggested that *psm-mec* RNA negatively influences methicillin resistance *Staphylococcus aureus* (MRSA) virulence by inhibition of *agrA* translation in hospital-acquired methicillin *staphylococcus aureus* (HA-MRSA) and the absence of *psm-mec* function in community-acquired methicillin *staphylococcus aureus* (CA-MRSA) confers its high virulence property (Kaito *et al*., 2013). However, the virulence-promoting effect of the *psm-mec* peptide has been suggested to balance or supersede the reported virulence-diminishing effect of the *psm-mec* RNA,

although this is strain- dependent and may not have been observed or different in methicillin resistance coagulase-negative staphylococci (MRCoNS) (Cheung *et al*., 2014).However, psm-*mec* gene expression studies to determine the level of production of the peptide were however, not carried out in this study.

It is evident that the genes responsible for biofilm production are present to a varying degree in the coagulase-negative isolates. Biofilm formation enhances persistence in the environment in which it is produced and could complicate treatment. Ability of coagulase-negative staphylococci to form biofilm is regarded as the major virulence factor (Franca *et al*., 2021). Production of biofilm is one of the strategies coagulase-negative staphylococci have developed to enable them to evade the host immune response and the activity of antimicrobials.

The icaD gene, associated with increased biofilm production, was detected in several isolates, emphasizing its role in virulence. Similar reports of detection of i*caD* operon products from coagulase-negative staphylococci have been made by Zhou *et al.* in China (Zhou *et al*., 2013), Melake *et al*. in Egypt (Melake *et al*., 2016). Co-expression of *icaA* and *icaD* genes has been linked to increased biofilm production (Zhou *et al*., 2013). It has also been suggested that the co-expression of *mecA* and *icaD* in clinical isolates further enhances resistance to antibiotics and their presence could be helpful in rapid determination of the severity of *S. epidermidis* infection (Zhou *et al*., 2013; Heilmann *et al.,* 2018). The icaAB gene, linked to biofilm production, was also prevalent in some isolates. Similar reports have been made in China (Du *et al*., 2013). The absence of the icaC gene in the tested isolates indicated potential alternative mechanisms for biofilm formation. Biofilm formation induced by biofilm-associated protein (bap) gene independent of the polysaccharide intercellular adhesin (PIA), also known as poly-β-(1-6)-*N*-acetylglucosamine (PNAG) PIA/PNAG exopolysaccharide which includes the i*caABCD* operon products has been reported (Becker *et al.,* 2014; Heilmann *et al.,* 2018). Multiple mechanisms could also be responsible for biofilm formation. There has been report of *bap* and *icaA* genes found in the same isolates (Płoneczka-Janeczko *et al.,* 2014). Production of biofilm, carriage of the genes, *icaAB* and *icaD* responsible for biofilm production and those that confer resistance to antibiotics, *mecA* and *ermB* and also carriage of the *psm-mec* virulence gene makes the coagulase-negative staphylococci a major

public health challenge and indicates a need for serious strategies for clinical management.

Conclusion

About 50 (83.3%) of the isolates showed strong biofilm formation while 5(8.3%) moderate biofilm formation and 5(8.3%) weak biofilm formation . *mecA* gene was detected in most of the tested isolates. ermB gene was found in half of the tested isolates while *psm-mec* gene was detected in *S. epidermidis* and *S. chromogenes.*

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Conflict of interest disclosures

The authors declare that there is no conflict of interest.

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