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Detection of *icaD* and *MecA* Genes and Antibiogram Profiling of Ear Swab Borne *Staphylococcus aureus* Isolates

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ABSTRACT: Bacteriological analysis of fifty (50) ear swabs, collected from fifty (50) consenting male undergraduate students was conducted using routine procedures which included pour plating. Thirty one (31) *Staphylococcus aureus* were tentatively identified and subjected to biofilm production test and antibiotic susceptibility assay using Congo red agar and disc diffusion procedure. The presence of *icaD* and *MecA* genes students in the respective bacterial strains was ascertained using polymerase chain reaction and agarose gel electrophoresis. All the isolates were resistant to ceftazidime (100%), cefuroxime (100%), ceftriaxone (100%), cloxacillin (100%), and augmentin (100%), while all the isolates exhibited sensitivity towards gentamicin (100%). Biofilm production was detected in only seven (7) of the thirty one (31) *S. aureus* strains. The amplified *mecA* gene was detected in *S. aureus* isolates coded; 1,2,3, 4, 7 and 8 and also, the amplified *icaD* gene was observed in *S. aureus* isolates coded; 1,2,3, 4, 5, 6, 7 and 8 respectively.

Keywords: Ear swab, Biofilm, Antibiotic resistance, *icaD*.
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Introduction

Biofilm synthesis by bacteria has been regarded by several authors as one of the primary mechanisms of bacterial virulence as well as resistance to antimicrobials (Cucarella *et al*., 2011; Mah and O'Tolle 2011). Several extracellular moieties which include; extracellular polysaccharides and biofilm-associated proteins, enable *Staphylococcus aureus* to produce biofilms. *S. aureus* ability to form biofilm is known to aid the bacterium in tolerating host immune response and is considered responsible for chronicity of the disease and resistant towards antimicrobial agents (Nasr *et al.*, 2012). With respect to *S. aureus* biofilm formation, the synthesis of polysaccharide intercellular adhesin (PIA) is the most essential step that is known to mediate the adhesion of bacterial cells to each other in the biofilm ([Ding *et al.*, 2014). Abbasi and Zamanzad, (2015) opined that the biofilm acts as a barrier to antimicrobial agents and the host immune system, and thus, aids sustained bacterial colonization. *S. aureus* ability to cause a variety of recalcitrant infections has been linked to several interrelated factors which include; acquisition of resistance to multiple antimicrobials, its possession of diverse assortment of virulence factors, and its ability to form biofilm on surfaces (Figueiredo *et al*., 2017). Bacterial biofilm associated infections are often chronic, naturally persistent and have been documented as a primary cause of morbidity and mortality in healthcare settings, including hearing loss in cases of ear infections attributed to *S. aureus* (Moormeier *et al*., 2013). The *icaABCD* genes are known to play a critical role in biofilm formation. *S. aureus* and *Staphylococcus epidermidis* isolates are known to encode the major enzyme,

icaA, which is critical for PIA synthesis (Abbasi and Zamanzad, 2015). Vanderhaeghen *et al.* (2012) observed that this enzyme might require an *icaD* gene product (called *IcaD*) for its activity [12]. The other genes within the *ica* operon are *icaB;* polysaccharide deacetylase, *icaC;* transporter of PIA, and *icaR*; the inhibitor gene (Abbasi and Zamanzad, 2015). Most *S. aureus* strains are known to harbor all four genes of the *ica* operon (Abbasi and Zamanzad, 2015).

Sanchez *et al*. (2013) reported the usefulness of early detection and management of potentially pathogenic bacteria such as biofilm producing drug resistant *S. aureus* and genes responsible for biofilm formation which can result in the reduction of the morbidity and mortality rate in affected patients. This research was aimed at the evaluation of the antibiogram and detection of biofilm formation gene; *icaD* and *MecA* gene in identified *S. aureus* cultured from ear swabs collected from several apparently healthy undergraduate male students attending a private university located in Benin City, Edo State, Nigeria.

Materials and methods

Collection of swab samples: A total of fifty (50) swab samples were collected from the ears of fifty (50) male students using a sterile swab stick moistened by the addition of 2 ml of sterilized peptone water under aseptic conditions. The swabs were taken to the laboratory and left for 24 h prior to bacteriological analysis. The ear swabs were collected during a three (3) month period; February to April, 2021.

Ethical approval and consent to participate: As at the time of sampling, there was no faculty based ethical committee operating in the private tertiary institution where the respective male participants attend and reside. However, informed oral consent was duly obtained from the participants after the purpose for collecting the ear swab samples was painstakingly explained to them.

Isolation and identification of S. aureus from the ear swabs: *Staphylococcus aureus* isolates were cultured using pour plate procedure as described by Harley and Prescott (2002), Pepper and Gerba (2004). Mannitol salt agar was the selective culture medium utilized and the MSA plates were incubated for 24 hours at 37 °C. *S. aureus* isolates were tentatively identified from the agar plates after subjecting the purified sub cultured colonies to a cascade of relevant biochemical and morphological tests which included; Gram staining, tube and slide coagulase production tests and oxidative/fermentation tests as detailed by Aneja (2003) and Cheesebrough (2006).

*Antibiogram profiling of the S. aureus cultures***:** All the identified *S. aureus* isolates were subjected to antibiotic susceptibility test using the modified Kirby-Bauer disc diffusion method as detailed by Vandepitte *et al.* (2003) and the resultant growth inhibitory zones were interpreted with the aid of the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2018). Commercially available antibiotic discs utilized were Ceftazidime (30 µg CAZ), Cefuroxime (30 µg CRX), Gentamicin, (10 µg GEN), Ceftriaxone (30 µg CTR), Erythromycin (5 µg ERY), Cloxacillin (5 µg CXC), Ofloxacin (5 µg OFL), Augmentin (30 µg AUG) and Oxacillin (1 µg OX).

Screening of the S. aureus isolates for biofilm production: The Congo Red Agar (CRA) procedure as described by Karki *et al.* (2019) was utilized in the determination of the ability of the isolates to produce biofilm. The inoculated CRA plates were incubated at 37 °C for 24-48 h. Isolates which produced biofilm produced black coloured colonies while non-biofilm producers developed colonies with red colouration.

Extraction of S. aureus DNA using ZR Fungal/bacterial DNA miniprep: *S. aureus* DNA for PCR assay was extracted from bacterial isolates respectively cultured overnight in Luria-Bertani broth. Bacterial DNA was then extracted using the ZR miniprep (Manufactured by Zymo D6005). Two (2) mL of the *S. aureus* culture broth was added to a ZR BashingTM Lysis Tube. About 750ul of lysis solution was then added to the tube and the mixture was then centrifuged in a microcentirifuge at > 10,000 x g for 1 min. About 400 µl of supernatant was transferred to a Zymo-SpinTM IV Spin filter in a collection tube and centrifuged at 7,000 x g for 1 min. To this filtrate, 1,200 µl of Fungal/Bacterial DNA binding buffer was added and 800 µl of the mixture was transferred to a collection tube containing Zymo-SpinTM IIC column and centrifuged at 10,000 x g for 1 min and the flow through was discarded and the mixture centrifuged again. About 200 µl of DNA Pre-wash buffer was added to the Zymo-Spin TM IIC column and the mixture was centrifuged at 10,000 x g for 1 min. Another 500 µl of bacterial DNA wash buffer was then added to the Zymo-SpinTM IIC Column and centrifuged at 10,000 x g for 1 min. The Zymo-SpinTM IIC Column was transferred to a clean 1.5 ml micro centrifuge tube and 100 µl (35 µl minimum) DNA elution buffer was directly added to the column matrix. This mixture was centrifuged at 10,000 x g for 30 secs to elute the bacterial DNA.

*Polymerase Chain Reaction (PCR) of icaD and mecA genes***:** PCR procedure was utilized to ascertain the presence of both *icaD* and *mecA* genes using forward and reverse primers 5′-ATGGTCAAGCCCAGACAGAG-3′ and 5′-CGTGTTTTCAACATTTAATGCAA-3′ and 5’-TGAGATAGGCATCGTTCCAAAG-3 and 5’-GATAGCAGTACCTGAGCCATAATC-3 respectively. The

PCR cocktail mix comprised of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10 µM forward and reverse primer; 2µL of DNA template using 8.5 µl nuclease free water. PCR conditions were initial denaturation (94 °C for 5 min), followed by thirty six (36) cycles of denaturation (94 °C for 30 sec), annealing (55°C for 30 sec) and elongation (72°C for 45 sec), followed by a final elongation step at 72˚C for 7 min and hold temperature at 10 ˚C. The amplified fragments were visualized on safe view-stained 1.5% agarose electrophoresis. The PCR thermal cycler utilized was GeneAmp PCR system 9700.

*Profiling of the icaD and mecA genes using Gel Electrophoresis***:** The PCR products were separated and characterized using agarose gel electrophoresis. The amplified PCR product alongside the positive control, negative control and ladder was run through 2% agarose gel stained with 10µL EZ vision DNA stain. The gel was visualized under the UV transilluminator and the image was captured.

RESULTS

Thirty one (31) *S. aureus* cultures were isolated from the ear swabs (Table 1). The antibiogram profiles of the respective *S. aureus* isolates indicated that all the isolates were resistant to five (5) antibiotics; ceftazidime (100%), cefuroxime (100%), ceftriaxone (100%), cloxacillin (100%), and augmentin (100%), while all the isolates exhibited sensitivity towards gentamicin (100%) (Table 1). Ninety point three per cent (90.3%) of the isolates were sensitive to two (2) antibiotics; Oxacillin and Ofloxacin (Table 1). Only seven (7) of the thirty one (31) *S. aureus* strains were observed to produce biofilm (Table 2). The amplified *mecA* gene was detected in *S. aureus* isolates coded; 1,2,3, 4, 7 and 8 (Fig. 1). Also, the amplified *icaD* gene was observed in *S. aureus* isolates coded; 1,2,3, 4, 5, 6, 7 and 8 (Fig. 2).

Table 1: Antibiogram of thirty one (31) ear swab borne *S. aureus* isolates

Isolate	CAZ (30µ)	CRX (30µg)	GEN (10µg)	CTR (30µg)	ERY (5µg)	CXC (5µg)	OFL (5µg)	AUG (30µg)	OX (1µg)
1	R	R	14	R	9	R	18	R	14
2	R	R	13	R	7	R	17	R	16
3	R	R	16	R	14	R	18	R	13
4	R	R	11	R	10	R	17	R	15
5	R	R	14	R	9	R	5	R	19
6	R	R	12	R	10	R	18	R	14
7	R	R	14	R	9	R	19	R	16
8	R	R	11	R	7	R	5	R	13
9	R	R	14	R	9	R	18	R	13
10	R	R	15	R	9	R	19	R	14
11	R	R	15	R	11	R	18	R	14
12	R	R	14	R	11	R	18	R	16
13	R	R	14	R	10	R	21	R	R
14	R	R	14	R	9	R	17	R	17
15	R	R	12	R	R	R	17	R	15
16	R	R	13	R	7	R	19	R	16
17	R	R	15	R	R	R	17	R	16
18	R	R	15	R	9	R	18	R	16
19	R	R	13	R	8	R	18	R	16
20	R	R	15	R	10	R	17	R	17
21	R	R	15	R	14	R	18	R	20
22	R	R	13	R	R	R	17	R	16
23	R	R	14	R	15	R	16	R	15
24	R	R	15	R	12	R	19	R	R
25	R	R	16	R	12	R	18	R	16
26	R	R	11	R	9	R	16	R	13

Isolate	CAZ (30µ)	CRX (30µg)	GEN (10µg)	CTR (30µg)	ERY (5µg)	CXC (5µg)	OFL (5µg)	AUG (30µg)	OX (1µg)
27	R	R	13	R	10	R	5	R	R
28	R	R	14	R	R	R	17	R	19
29	R	R	15	R	9	R	18	R	15
30	R	R	12	R	10	R	17	R	15
31	R	R	14	R	11	R	20	R	19
Resistance	31(100)	31(100)	0(0)	31(100)	18(58.1)	31(100)	3(9.7)	31(100)	3(9.7)
Sensitivity	0(0)	0(0)	31(100)	0(0)	13(41.9)	0(0)	28(90.3)	0(0)	28(90.3)

Key: CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, CTR: Ceftriaxone, ERY: Erythromycin, CXC: Cloxacillin, OFL: Ofloxacin, AUG: Augmentin, Ox; Oxacillin; R = Resistant; S = Sensitive.

Table 2: Biofilm producing *S. aureus* isolates cultured on Congo red agar (CRA)

Isolate	Biofilm formation		
	Strong biofilm	Moderate biofilm	Weak biofilm
1	–	–	+
2	–	–	+
3	–	–	+
4	–	–	+
5	–	–	+
6	–	–	+
7	–	–	+

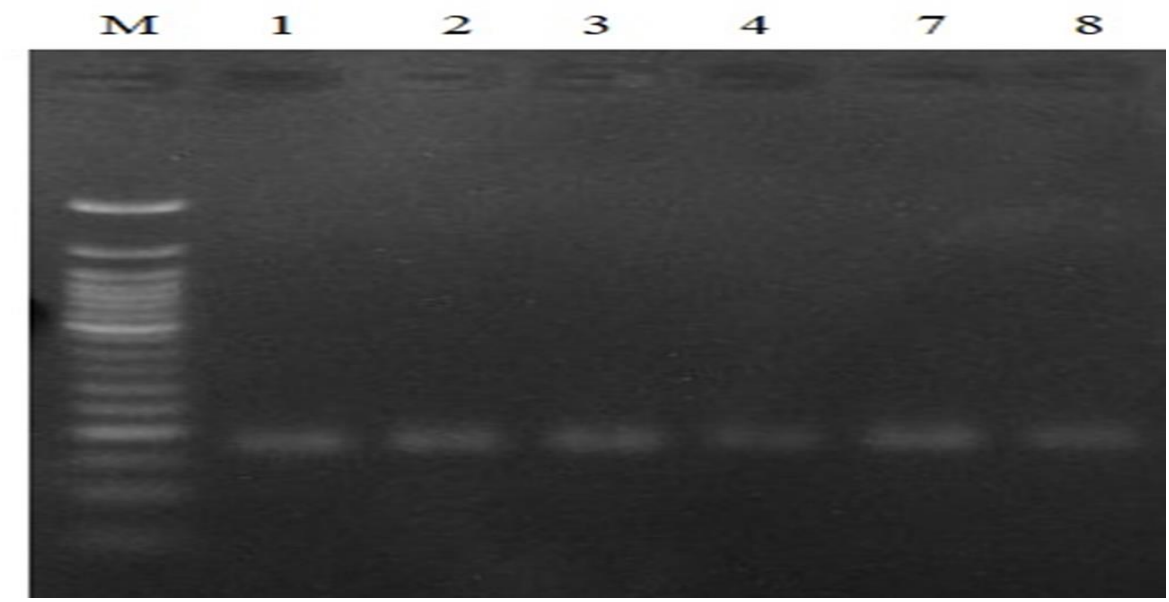

Fig. 1: Amplified *mecA* gene from *S. aureus* isolates, Lane M: DNA Molecular Marker, Lane 1, 2, 3, 4, 7, 8: Positive sample

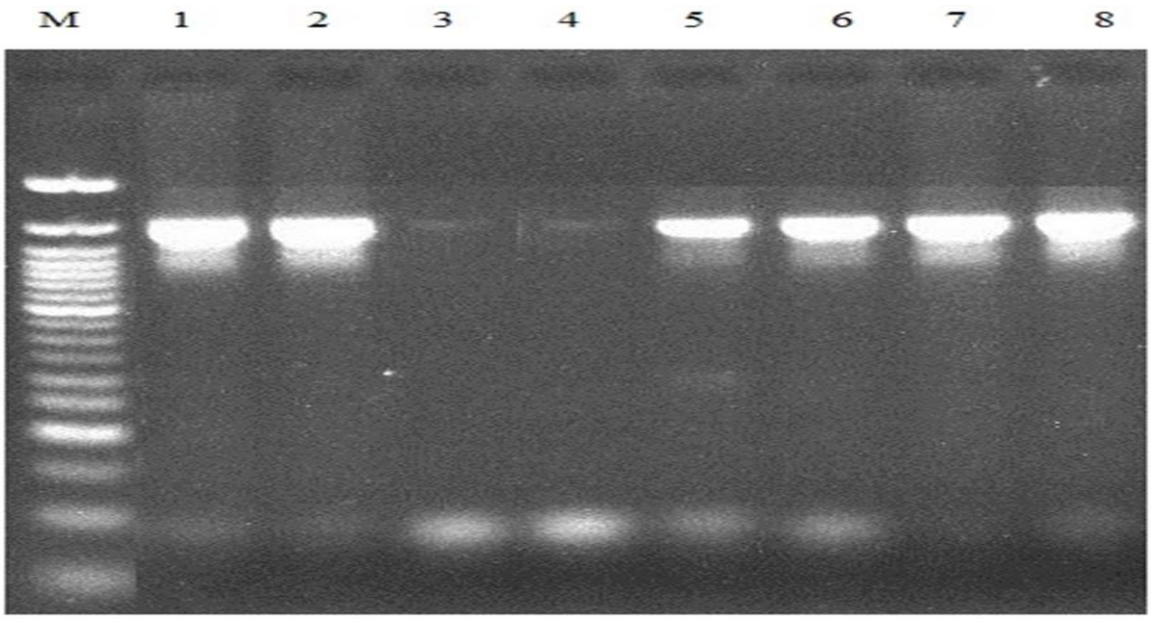

Fig. 2: Amplified *icaD* gene from *S. aureus* isolates, Lane M: DNA Molecular Marker, Lane 1, 2, 3, 4, 5, 6, 7, 8: Positive sample

Discussion

The isolation of *S. aureus* from the ear swabbed ears of apparently healthy students was not surprising, given the fact that the bacterium is a member of the normal body flora. The bacterium is normally present on skin surface and mucous membranes (especially with reference to the nasal region) (Hanif and Hassan, 2019). Its isolation from ear swabs has previously been reported by Gorems *et al.* (2018) which documented the isolation of *S. aureus* from patients with ear discharge in Jimma, Ethiopia. The expressed resistance of all the *S. aureus* isolates to ceftazidime, cefuroxime, ceftriaxone, cloxacillin and augmentin although worrisome was also not surprising given that *S. aureus* is known to possess an anomalous attribute to rapidly develop resistance against every other antibiotic (Hanif and Hassan, 2019). Some of the antibiotic resistance mechanisms include; enzymatic inactivation of antibiotics, target alteration with reduced affinity for the antibiotics, antibiotic trapping and efflux pumps (Hanif and Hassan, 2019). Despite the fact that only eight (8) of the thirty one (31) *S. aureus* isolates elicited biofilms, all the isolates were resistant to a minimum of five (5) antibiotics. This trend would infer that although biofilm formation has been described as a defense mechanism of *S. aureus* (Belbase *et al.*, 2017), without the ability to produce biofilms, the bacterium can still survive the activity of some antibiotics. The *icaD* gene was detected in all the biofilm producing isolates whilst only six (6) out of the eight (8) isolates harbored *mecA* gene. The detection of the *icaD* gene in all the biofilm producing isolates would suggest that the gene might play a role in the expressed biofilm production by the *S. aureus* isolates. The *mecA* gene was present in six (19.35%) of the identified *S. aureus* isolates. Akanbi *et al.* (2017) reported that this antibiotic resistance gene (ARG) was known to encode for penicillin-binding protein 2a (PBP2a), responsible for methicillin resistance in staphylococci and the expression of this ARG invariably led to a decreased affinity for β-lactam antibiotics by the protein.

Conclusion

Thirty one (31) tentative *S. aureus* cultures were isolated from ear swabs collected from apparently healthy male undergraduates and all the isolates were multi antibiotic resistant as they were resistant to a minimum of five (5) antibiotics utilized in the study. A low percentage of the isolates; 22.58% and 19.35% harbored *icaD* and *mecA* genes respectively.

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