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Article

Co-occurrence of Intracellular Adhesion Genes (*icaA* and *icaD*) Confers Higher Multi-Drug Resistance on *Staphylococcus aureus*

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ABSTRACT: The importance of slime factor production in bacterial adherence, colonization and antibiotic resistance emphasizes it as a virulence associated macromolecules. This study was therefore aimed at investigating the presence of slime factor producing *Staphylococcus aureus* from different environmental sources and to correlate slime factor production with methicillin resistance distribution. A total of 384 samples (96 each of toilet seats, sewage samples, poultry droppings and street-vended salads) were collected according to the statistical scheme of the microbiological specification for foods. These samples were processed for *Staphylococcus aureus* prior to screening of the isolated *Staphylococcus aureus* for slime factor production. Slime factor screening was carried out biphasically. First, by Congo Red Agar (CRA) technique and later by a primer specific polymerase chain amplification of *icaA* and *icaD* genes. Methicillin resistance was determined both phenotypically (by screening the susceptibility of *Staphylococcus aureus* to cefoxitin) and genotypically (by amplification of *mecA* gene). The association between slime factor production and antibiotic resistance was estimated using chi-square statistic ($\chi 2$). Result of the Congo red technique depicts 43.9% of the 164 isolated *Staphylococcus aureus* (72) as slime producing strains while 9, 21 and 22 of the 72 *Staphylococcus aureus* show discrete amplification of *icaA*, *icaD* and co-occurrence of *icaA* and *icaD* respectively. Of the 52 molecularly confirmed slime producing *Staphylococcus aureus* strains, 43 (82.7) were methicillin resistant strains while 28(65.1%) exhibits discrete amplification of *mecA* gene. Our results however found significant association between slime factor production and antibiotic resistance.

1. INTRODCTION

The importance of slime factor production in bacterial virulence especially adherence and colonization has long been documented [1-4]. This biological factor which is known to be one of the major virulence associated macromolecules of *S. aureus* and *S. epidermidis* [5-7], plays frequent role in biomaterial centered infections [8-9] and has also been implicated in a wide range of human diseases including skin infections, bone infections, food poisoning, endocarditis, toxic shock syndrome among others [10-12]. Globally, several studies have documented slime producing Staphylococcal species from both veterinary and clinical sources [13-15]. For instance, in the United States of America, Allen *et al.* [16] reported slime producing Staphylococcal species, with 85.0% being strong slime producers and 15.0% moderately to weakly slime producers from patients with atopic dermatitis (AD) attending the Drexel University College of Medicine, Philadelphia, Pennsylvania. In an hospital in San Luis, Argentina, Alcaráz *et al.* [17] recorded a 55.5% and 57.4% slime production by

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Staphylococcal isolates obtained from clinical and environmental specimens respectively. Similarly, Boynukara *et al.* [18] reported 60% slime production by Staphylococci species isolated from various clinical specimens of patients in a university hospital in Turkey. Wenbo *et al*. [19] investigated the slime forming capacity of *Staphylococcus epidermidis* in clinical specimens from Tongren hospital, Beijing with a result of 34.38%. In Mexico, Juárez-Verdayes *et al*. [20] reported 66% slime production by *Staphylococcus epidermidis* strains obtained from patients at the Instituto de Oftalmolgía "Conde de Valenciana", Mexico City. In a study carried out in Rio de Janeiro, Brazil, Marques *et al*. [21] detected 100% slime production by *Staphylococcus aureus* in bovine mastitis. Similarly, Bakheet and Darwish [22] detected 76.19% slime producing *Staphylococcus aureus* from layer's chicken in Assiut Governorate, Eygpt. Recently, Bierowiec *et al*. [23] reported the isolation of slime producing *Staphylococcus pseudintermedius* from sick and apparent healthy cats in Poland, Europe with a frequency of 46.2% and 40.9% respectively.

In Nigeria, similar studies are not unavailable. For instance, in Benin, Edo State, Azih and Enabulele [24] observed 75.95% slime production by Staphylococcal species isolated from clinical samples from the University of Benin Teaching Hospital while Abdulrahim *et al.* [25] detected 81.6% slime production by *Staphylococcus aureus* isolated from clinical specimens from National Orthopaedic Hospital Dala, Kano State, Nigeria. Orjih *et al.* [26] however recorded 78% slime production by the same organism isolated from five hospitals in Lagos State. Ayepola *et al.* [27] also reported slime production in 3 of 5 (60%) *Staphylococcus epidermidis* strains isolated from eight medical centers in Lagos and Ogun State. Furthermore, Shittu *et al.* [28] reported slime producing *Staphylococcus aureus* from West African Dwarf (WAD) goat in Osun State, Southwest Nigeria.

However, most of the documented works on slime factor borders on clinical and veterinary samples [29] and thus there is paucity of information on slime factor producing *Staphylococcus aureus* from environmental sources. Consequently, the fact that slime factor is encoded in the *ica* locus containing *icaA, icaB, icaC* and *icaD* genes in *Staphylococcus aureus* [21-30], makes it ideal to use discrete amplification of any of these genes to identify slime factor producing organisms [31-32]. Of these genes, it has been established that *icaA* and *icaD* are the most predominant in *Staphylococcus aureus*. It is thus crucial to note that the detection of slime factor producing organisms rely more on discrete amplification of *icaA* and *icaD* genes. This study, was therefore, aimed at determining the epidemiology of slime factor producing *Staphylococcus aureus* from different environmental sources and to correlate slime factor production with antibiotic resistance.

2. Materials and Methods

2.1. Sample Collection and Sampling

A total of 384 samples were collected from ninety-six (96) sample collection sites. These samples which included ninety-six (96) each of toilet seat swabs, poultry droppings, street vended salads and sewage samples were collected according to the statistical scheme of the international commission for microbiological specification for foods [33] but with slight modifications. Briefly, the sample collection sites were classified into four different quadrants with each of the quadrant producing 96 samples. The four quadrants however produced a total pool of 384 samples (96 samples×4).

2.2. Sample Transportation and Processing

All the samples were collected in pre-sterilized universal bottles except for the street-vended foods that were collected in pre-sterilized aluminum pans and were transported in different coolers containing ice packs within 4 hours of collection. The samples were processed not later than 24 hours at room temperature (27± 2°C) after getting to the laboratory.

2.3. Microbiological Processing of Specimens

Each sample was processed following standard recommended techniques respectively; street vended salads [34], toilet seats [35], poultry droppings [36] and sewage samples [37]. The samples processed were subsequently inoculated on Mannitol Salt Agar for 24 hours at 37°C [38] and identified by molecular amplification of *nuc* gene (specific for *Staphylococcus aureus*) [5] while other organisms with no discrete amplification were regarded to as other *Staphylococcus* spp.

2.4. Phenotypic Detection of Slime Factor Production in *Staphylococcus aureus*.

Slime factor production was assayed qualitatively by the Congo Red Agar method (CRA), as described by Mathur *et al.* [39]. The bacterial isolates were cultured on Congo Red Agar (CRA) plates containing 0.8g Congo red dye and 36g sucrose and incubated at 37°C in aerobic conditions for 24 hours. After 24 hours, the plates were stored at room temperature for 48 hours. Slime production was interpreted according to colony colours using a four-colour reference scale varying from black to red. Black colonies with rough surface and edges was an indication of strong slime production, almost-black colonies were intermediate or weak slime producers, while red and pink colonies were interpreted to be non-slime producers

2.5. Molecular Detection of *icaA* and *icaD* genes

The genotypic determination of slime production was performed by targeting the *icaA* and *icaD* genes using polymerase chain reaction. Each specimen was stirred directly into 200 ml sterile saline and extracted using a QIAamp DNA mini kit (Qiagen). In brief, each broth culture sample (100 µL) was pre-incubated at 99 ºC for 20 min and then processed as suggested by the manufacturer. After the addition of 5 µL of lysostaphin solution, the sample was incubated again at 99 ºC for 10 min to digest the bacterial cell wall. This was followed by the addition of 5 µL of proteinase K solution and 150 µL of 0.1M Tris-HCl (pH 7.5) and then further incubated for 10 minutes at 37 ºC . Following incubation, the samples were heated for 5 minutes at 100 ºC and the bacterial debris was removed by centrifugation for 5 minutes and then dried at 37 °C. The dried DNA was re-suspended in distilled water and transferred into a new pre-labelled Eppendorf tube by gentle aspiration using a micropipette as a template for PCR. PCR amplification of *icaA* and *icaD* genes were performed as described previously using their specific primers (Table 1). PCR was performed in a DNA thermal cycler containing 10 µL of the extracted DNA, 1 µM of the above-mentioned primers, 100 µM each of deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP), buffer (10mM

Tris-HCl (pH 9.0), 50mM KCl, 0.1% Triton X-100 and 2.5mM $MgCl_2$) and 0.6 U of Taq DNA polymerase. DNA was amplified using the following thermal cycling profile; initial denaturation by incubation at 94 °C for 5 min, followed by 50 cycles each of denaturation at 94 °C for 30 seconds, annealing at 55.5 °C for 30 seconds, extension at 72 °C for 30 seconds and ending with a final extension at 72 °C for 1 min. The amplified products were loaded onto 1.5% Agarose Gel containing 1µg/ml ethidium bromide and the presence and molecular weight of the amplified DNA fragments were visualized under ultraviolet (UV) light.

2.6. Antibiotic susceptibility testing

The susceptibility of *Staphylococcus aureus* to antibiotics was evaluated using the disc diffusion method as described by Finegold and Martin [42]. Briefly, Mueller Hinton (MH) Agar (Merck, Darmstadt, Germany) was prepared according to manufacturer's instruction and with a sterile inoculating loop, a loopful of the 24 hours isolated colonies were picked and suspended in sterile normal saline after which the turbidity of each suspension was adjusted to 0.5 McFarland standard by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy. The Mueller Hinton Agar were subsequently inoculated by dipping a sterile swab into the inoculum tube and rotating the swab against the side of the tube using firm pressure, to remove excess fluid. The swab should not be dripping wet. The dried surface of the MH agar plate was inoculated by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. The plates were left to sit at room temperature for 10 minutes prior to placing the antibiotic-impregnated disks on the surface of the agar with the aid of forceps. The antibiotic-impregnated disks used include the following viz; vancomycin (30µg), gentamicin (10µg), nitrofurantoin (300µg), trimethoprim/sulphamethoxazole (25µg), oxacillin (1µg), ciprofloxacin (5µg), amikacin (30µg), erythromycin (15µg), clindamycin (10µg), chloramphenicol (30µg), cefuroxime (30µg), cephalexin (30µg) and cefoxitin (30µg) and incubated at 37°C for 18–24 hours. The diameter of the zones of inhibition was measured and the results were interpreted as recommended by the Clinical and Laboratory Standards Institute [43].

2.7. Determination of Minimum Inhibitory Concentrations (MIC) of the Intracellular Adhesion gene carrying *Staphylococcus aureus*

Each of the *Staphylococcus aureus* was examined for their susceptibility to different classes of antibiotics. Briefly, the different classes of antibiotics were double fold serially diluted using nine different tubes containing 1 mL each of nutrient broth (Oxoid, England) to achieve final dilutions of 128 µg/ml, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25µg/ml.

Standard bacterial inoculums (10^5) of each of the organism was inoculated into nine different dilutions and incubated overnight at 37 ^oC. The MIC was taken as the lowest concentration of any of the antibiotics that inhibited the growth of *Staphylococcus aureus* strains.

2.8. Molecular Detection of *mecA* (Methicillin Resistance Specific) Gene

The detection of methicillin resistant gene was done using PCR. The genomic DNA was extracted from the phenotypically confirmed slime producing and methicillin-resistant *Staphylococcus aureus* (MRSA) strains by using Qiagen genomic DNA extraction kit. The amplification of the methicillin resistant gene was carried out by using a pair of primers formerly used by Sajith *et al* [40] (Table 1). PCR was carried out in a Perkin-Elmer thermocycler that consist of the following reaction mixtures; 1µl each of the forward and reverse primers, 5µl of the extracted DNA, 0.2mM each of dATP, dTTP, dGTP and dCTP, buffer (50 mM KCl, 10mM Tris- HCl [pH 9.0], 1.5 mM $MgCl_2$), and 2 unit/ml of Taq DNA polymerase (Promega). The DNA was then amplified using the following PCR cycling protocol; initial denaturation by incubation at 94 °C for 5 min followed by 35 cycles each of denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min. The amplicons (PCR products) were detected by electrophoresis using a 2% agarose gel at 80 V for 1 hour after which they were stained with ethidium bromide (0.5 µg/ml) and visualized under ultraviolet (UV) light.

2.9. Statistical Analysis

Data analysis was carried out using the computational software SPSS version 22 (SPSS, Chicago, USA). Chi square test was used to determine significant differences between categorical variables, The statistically significant level was considered as $P < 0.05$.

Table 1: Primers used in this study

Genes	Sequence (5' – 3')	Product size	References
icaA	F: 5'-CCT AAC TAA CGA AAG GTA G-3'	1315 bp	Ciftci *et al*.[5]
	R: 5'-AAG ATA TAG CGA TAA GTG C -3		
icaD	F: 5'-AAA CGT AAG AGA GGT GG-3'	381 bp	Ciftci *et al*.[5]
	R: 5'-GGC AAT ATG ATC AAG ATA-3'		
mecA	F: 5'-TCCAGATTACAACTTCACCAGG-3'	162 bp	Sajith *et al*.[40]
	R: 5' -CAATTCATA TCTTGTAACG-3'		
nuc	F: 5'-GCGATTGATGGTGATACGGTD-3'	279 bp	Dewanand *et al*.[41]
	R: 5' -AGCCAAGCCTTGACGAACTAAAGC-3'		

3. Results

Table 2 depicts the distribution of *Staphylococcus aureus* in the sampled specimens. As shown in this Table 2, poultry droppings yielded more bacterial counts 52 (13.5%), followed by sewage samples 44 (11.5%), street vended foods 36 (9.4%) and toilet samples 32 (8.33%) respectively. In general, 164 (42.7%) of the analysed specimens yielded bacterial colonies. The distribution of the slime producing *Staphylococcus aureus* depicted in Table 3 reveals 72 (43.9%) and 92(56.1%) as slime factor and non-slime factor producing *Staphylococcus aureus* respectively. Of these seventy two (72) isolates that were positive by the Congo red agar technique, 9(5.49%), 21(12.8%) and 22(22%) were found harbouring *icaA*, *icaD* and combination of *icaA* and *icaD* respectively. Consequently, the fifty-two (52) strains of *Staphylococcus aureus* with discrete amplification of *icaA*, *icaD* and/or co-occurrence of *icaA* and *icaD* were screened for methicillin resistance and 43 (82.7%) showed elevated trend of methicillin resistance while 9(17.3%) were found to be sensitive. The polymerase chain amplification of *mecA* gene revealed that 28 out of the 43 resistant isolates (65.1%) shows discrete amplification of *mecA* gene (Table 4). Table 5 connotes the distribution of *Staphylococcus aureus* for antibiotic susceptibility testing. As shown in this table, a total of 52 slime factor gene variants 9(17.3%) of *icaA*, 21(40.4%) of *ica D* and 22 (42.3%) of co-occurred *icaA* and *icaD* were delineated from 164 *Staphylococcus aureus* analyzed. In Table 6, the susceptibility testing data indicated that 100% of the *icaA* producing isolates were resistant to both amikacin and cefoxitin, 88% were resistant to trimethoprim/sulphamethoxazole, cephalexin and chloramphenicol, 77.7% were resistant to ciprofloxacin and erythromycin while 66.6% of these isolates were resistant to nitrofuratoin. 55.5% of *icaA* producing *Staphylococcus aureus* were also found to be resistant to vancomycin. However, resistance to gentamicin, oxacillin and cefotaxime were estimated to be 33.3%. The *icaD* producing isolates connote varying degrees of resistance as follows; 90.5% to chloramphenicol, 76.2% to ciprofloxacin and cephalexin, 85.7% to amikacin, 57.1% to nitrofuratoin and cefotaxime, 52.4% to gentamicin and erythromycin, 61.9% to vancomycin and cefoxitin. Lower levels of resistance were consequently found to oxacillin 9.52%, clindamycin 28.6%, and trimethoprim/sulphamethoxazole 33.3% respectively. The strains of Staphylococcus aureus that harbor both *icaA* and *icaD* exhibited resistance above 70% to vancomycin, gentamicin and amikacin and above 50% to trimethoprim/sulphamethoxazole, cephalexin, cefoxitin and cefotaxime. These isolates also showed 81.8% and 63.6% resistance to clindamycin and nitrofurantoin respectively. Even though resistance to chloramphenicol, erythromycin, oxacillin and ciprofloxacin is relatively lower, their resistance pattern was estimated as 45.5%, 40.9%, 31.8% and 40.9% respectively (Table 6). The Table 7 represents the distribution of MIC of the tested antibiotics against different variants of *Staphylococcus aureus*. As shown in this Table 5, 16 and 18 of the *icaA*, *icaD* and the isolates harboring both *icaA* and *icaD* had breakpoint above Cefoxitin MIC breakpoint while many of these slime factor producing organisms have MIC values above the recommended breakpoints. The resistance to different classes of antibiotics by percentages in slime factor producing *Staphylococcus aureus* ranged from 7.14 to 14.3% (Table 8).

Table 2: Distribution of *Staphylococcus aureus* in the individual specimens sampled

Samples	Distribution		
	n	N	%
Toilet samples	96	32	8.33
Sewage samples	96	44	11.5
Poultry droppings	96	52	13.5
Street vended foods	96	36	9.4
Total	384	164	42.7

Key: n= number of each sample processed, N= number of *Staphylococcus aureus* per each sample, %= percentage distribution of *Staphylococcus aureus* per each sample

Table 3: Distribution of slime producing *Staphylococcus aureus*

Screening methods	Positive	Negative
Phenotypic screening	72(43.9)	92(56.1)
Genotypic screening		
IcaA	9(5.49)	155(94.51)
IcaD	21(12.8)	143(87.2)
icaA+icaD	22(22)	142(78)

Key: Phenotypic = Phenotypic screening on Congo Red Agar, *icaA*= Intracellular adhesion gene A, i*caD*= Intracellular adhesion gene D, *icaA*+ *icaD*= Intracellular adhesion gene A + Intracellular adhesion gene D.

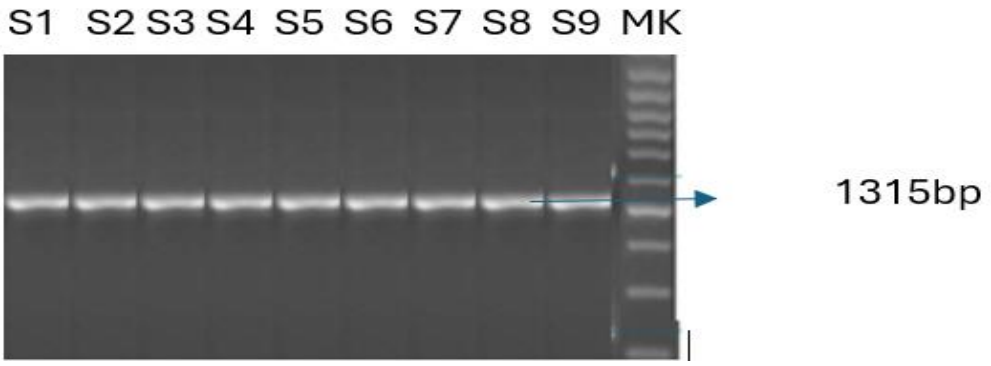

Plate 1B: PCR Amplification of *icaD*

Key: A1-Q1 = Different strains of *icaD* producing *Staphylococcus aureus*
MK = Marker DNA, molecular weight of *icaD* = 381bp

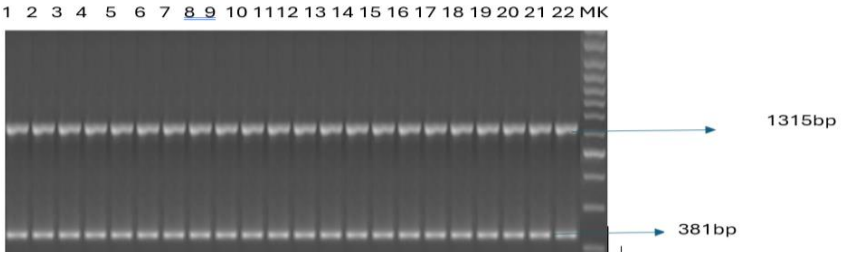

Plate 1C: PCR amplification of *icaD* gene

Key: 1-22 = Different strains of *icaD* + *icaD* producing *Staphylococcus aureus*
MK = Marker DNA, molecular weight of *icaA* = 1315bp, *icaD* = 381bp

Table 4: Occurrence of methicillin resistant *Staphylococcus aureus* and their genes

Screening methods	Resistant organisms		Sensitive organisms	
	n	(%)	N	(%)
Phenotypic screening	43	(82.6)	9	(17.3)
mecA gene	28	(65.1)	0	(0)

Key: n = number of resistant organisms, N = number of sensitive organisms, % = percentage distribution of resistant and sensitive organisms

Table 5: Distribution of *Staphylococcus aureus* for Antibiotic Susceptibility Testing (AST)

Organism	No. of *IcaA*	% of *IcaA*	No. of *IcaD*	% of *IcaD*	Co-occurrence of *IcaA* and *IcaD*	% Co-occurrence of *IcaA* and *IcaD*	Number (%) of slime factor genes for AST/ *IcaA*	*IcaD*	*IcaA* + *IcaD*
SAS	9	17.3	21	40.4	22	42.3	9(100)	21(100)	22(100)

Key: SAS= *Staphylococcus aureus* strains, AST= Antibiotic Susceptibility Testing, % = percentage

Table 6: Percentage distribution of antibiotic resistant slime producing *Staphylococcus aureus* variants

Slime factor variants	Antibiotics (%)												
	Van	Gent	Ami	Nitro	Tri/Su	Cip	Oxa	Cep	Ceft	Cef	Er	Chl	Clin
IcaA	55.5	33.3	100	66.6	88.8	77.7	33.3	88.8	100	33.3	77.7	88.8	33.3
IcaD	61.9	52.4	85.7	57.1	33.3	76.2	9.52	76.2	61.9	57.1	52.4	90.5	28.6
IcaA+IcaD	77.3	77.7	77.3	63.6	54.5	40.9	31.8	59.1	54.5	54.5	40.9	45.5	81.8

Key: Van= vancomycin, Gent= gentamicin, Ami = amikacin, Nitro= nitrofurantoin, Tri/Su= trimethoprim/sulphamethoxazole , Cip= ciprofloxacin, Oxa= oxacillin, Cep= cephalexin, Ceft= cefoxitin, Cef= cefuroxime, Er= erythromycin, Chl= chloramphenicol, Clin= clindamycin, %= percentage

Table 7: Distribution of MIC of the tested antibiotics against slime producing *Staphylococcus aureus* variants

Variants	N0I	Antibiotics Vancomycin	MIC (µg/ml), %									
			128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	22.2	11.9	22.2	-	11.1	22.2	11.1
IcaD	21		-	9.52	38.1	4.76	9.52	-	23.8	4.76	4.76	4.76
IcaA + IcaD	22		22.7	36.4	18.2	4.54	-	-	22.7	-	-	-
		Antibiotics Gentamicin	MIC (µg/ml), %									
			128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	33.3	-	66.6	-	-	-	-	-
IcaD	21		14.4	14.3	23.8	33.3	14.3	-	-	-	-	-
IcaA + IcaD	22		31.8	22.7	18.2	27.3	-	-	-	-	-	-
		Antibiotics Amikacin	MIC (µg/ml), %									
			128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	55.6	44.4	-	-	-	-	-
IcaD	21		28.5	33.3	-	-	23.8	14.4	-	-	-	-
IcaA + IcaD	22		-	63.6	4.5	9.1	-	22.8	-	-	-	-
		Antibiotics Nitrofurantoin	MIC (µg/ml), %									
			128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		66.7	22.2	11.1	-	-	-	-	-	-	-
IcaD	21		57.1	23.8	19.0	-	-	-	-	-	-	-
IcaA + IcaD	22		63.6	-	36.4	-	-	-	-	-	-	-
		Antibiotics Tri/Su	MIC (µg/ml), %									
			128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		22.2	-	11.1	-	33.3	22.2	11.1	-	-	-
IcaD	21		-	-	14.3	-	14.3	4.76	42.9	22.7	-	-
IcaA + IcaD	22		-	-	-	-	54.5	-	31.8	13.6	-	-

Table 7 Contd: Distribution of MIC of the tested antibiotics against slime producing *Staphylococcus aureus* variants

Variants	N0I	Antibiotics Ciprofloxacin	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	-	-	11.1	55.6	11.1	22.2	-
IcaD	21		-	-	-	-	23.8	9.52	14.3	28.6	23.8	-
IcaA + IcaD	22		-	-	-	-	13.6	4.54	9.1	13.6	27.3	31.8
		Antibiotics Oxacillin	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	-	22.2	11.1	33.3	33.3	-	-
IcaD	21		-	-	-	-	9.52	-	90.5	-	-	-
IcaA + IcaD	22		-	-	-	18.2	9.1	4.54	68.2	-	-	-
		Antibiotics Cefoxitin	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	11.1	11.1	11.1	66.6	-	-	-
IcaD	21		-	-	-	19.0	14.3	19.0	38.1	9.5	-	-
IcaA + IcaD	22		-	-	-	36.4	-	18.2	27.3	18.2	-	-
Variants	N0I	Antibiotics Cephalexin	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	11.1	22.2	55.6	11.1	-	-	-
IcaD	21		-	-	28.6	14.3	19.0	9.5	14.3	14.3	-	-
IcaA + IcaD	22		-	-	-	18.2	18.2	27.3	31.8	3.13	-	-
		Antibiotics Cefotaxime	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	33.3	11.1	33.3	-	22.2	-	-	-
IcaD	21		-	-	19.0	14.3	19.0	4.76	9.52	33.3	-	-
IcaA + IcaD	22		-	-	-	18.2	9.1	59.1	18.2	-	-	-

Table 7 Contd: Distribution of MIC of the tested antibiotics against slime producing *Staphylococcus aureus* variants

Variants	N0I	Antibiotics Erythromycin	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	33.3	44.4	-	22.2	-	-	-	-
IcaD	21		-	-	-	-	52.4	14.3	33.3	4.76	-	-
IcaA + IcaD	22		-	-	18.2	18.2	4.54	-	40.9	2.5	-	-
		Antibiotics Chloramphenicol	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	88.9	-	-	11.1	-	-	-	-
IcaD	21		-	9.52	14.3	66.7	-	9.52	-	-	-	-
IcaA + IcaD	22		-	13.6	18.2	13.6	13.6	36.4	4.76	-	-	-
		Antibiotics Clindamycin	MIC (µg/ml), % 128	64	32	16	8	4	2	1	0.5	0.25
IcaA	9		-	-	-	-	-	-	-	33.3	33.3	33.3
IcaD	21		-	-	-	-	-	9.5	14.3	4.8	47.6	23.8
IcaA + IcaD	22		-	-	-	-	-	27.3	31.8	13.6	9.1	18.2

Key: %= percentage resistance in slime producing *Staphylococcus aureus*, MIC=Minimum Inhibitory Concentration, µg/ml=microgram per mil

Table 8: Resistance to classes of antibiotics by percentages in slime producing *Staphylococcus aureus*

Resistance to different classes of antibiotics	Slime producing *Staphylococcus aureus* (%) *IcaA*	*IcaD*	*IcaA + IcaD*
0	14.3	0	0
1	21.4	0	0
2	21.4	14.3	0
3	7.14	14.3	0
4	7.14	0	14.3
5	0	14.3	14.3
6	28.6	0	7.14
7	0	0	0
8	0	14.3	7.14
9	0	14.3	7.14
10	0	21.4	21.4
11	0	0	0
12	0	7.14	14.3
13	0	7.14	7.14

Key: %= percentage resistance in slime producing Staphylococcus aureus, the chi-square statistic is 121.9736. The p-value is < 0.00001. The result is significant at $p < 0.05$.

4. Discussion

The ubiquitous presence of *Staphylococcus aureus* and other *Staphylococcus* species in every concernable environment has been documented [44-45] in different substrates including dried cassava powder [46], sewage [47-48], cockroaches [49], poultry droppings [36], street vended foods [50] and toilet seats [51]. In this study, 42.7% of the analyzed samples yielded visible growth of *Staphylococcus aureus*. This observation indicates that such analyzed samples could be regarded as major reservoir for the spread of these organisms [48, 50-51]. The presence of *Staphylococcus aureus* in poultry droppings is not only dangerous for vegetable consumers where these animal products are commonly applied as manure for planting vegetables [58] but could also pose serious threat to farmers with little or no knowledge of the implication of these organisms [59]. Consequently, such droppings may also contaminate eggs especially the broken ones, thereby serving as source of pathogenic *Staphylococcus aureus* to consumers of improperly cooked eggs or even the raw eggs [62], since some consumers still consume fresh eggs, thereby resulting in staphylococcal food poisoning (SFP) that are known to be associated with nausea, vomiting, retching, abdominal cramps, collapse, and other symptoms [60-61].

The fact that majority of the Nigerian population gives wide acceptance to street vended foods [50, 63-65] probably due to the ease of getting it [66], is an indication that most of the population are at the risk of ingesting *Staphylococcus aureus*. It is therefore important to note that apart from these highlighted sources of *Staphylococcus aureus* observed in this study, it is not surprising to also find these organisms associated with toilet seats [51]. Of more importance in this study, is the confirmation of slime producing strains of *Staphylococcus aureus* among the isolated *Staphylococcus aureus*. These findings though may be uncommon because they are from inanimate sources [67] as against many reports of slime producing organisms from veterinary and clinical samples [15]. We however found that the ability of the organism to produce slime is not only limited to when they are present on living organisms but could also be present on inanimate subjects including foods [50], poultry droppings [36], sewage samples [48] and even toilet seats [51]. In Nigeria, not many studies tracked slime producing organisms from non-clinical and non-veterinary specimens [67-68] but despite this, some of these organisms also show discrete amplification of the slime factor genes (*icaA and icaD*) to further affirm the genotypic expression of these slime factor loci [69-70].

Another interesting observation is the lowest representation of discrete amplification of *icaA* in the molecularly positive slime producing *Staphylococcus aureus*. This observation is like that of Abdulrahim *et al.* [71] where among twelve (12) *Staphylococcus aureus* isolates tested, the lowest prevalence was detected in *icaA* gene (8.3%). Also, in our study, the number of *icaD* (21) amplified by molecular technique was however found to be more than the number captured by the Congo Red Agar technique. This observation is a further attestation to the fact that the CRA technique, while it can capture more than one *ica* loci, may not be enough to cover all *icaD* locus [5]. Our findings however are similar with that of Arslan and Ozkardes [15] who reported that the CRA assay yielded a lower percentage of positive results in clinical staphylococci isolates compared to the staining assay. A high proportion of the isolated *Staphylococcus aureus* that shows elevated trend of oxacillin resistance also exhibited discrete amplification of *mecA* gene. This observation is in conformity with Garcia-Alvarez *et al.* [72] where a novel *mecA* homologue, *mecALGA251*, associated with resistance to β-lactam antibiotics was present in clinical MRSA isolates from the UK and Denmark, and bovine milk samples from the UK.

However, the fact that none of the sensitive organisms reveal *mecA* gene amplification is an indication that conventional susceptibility tests such as agar disc diffusion and broth dilution methods may give reliable results in detecting MRSA [5]. Our observation however agrees with the results of Unal *et al.* [73] and Ribeiro *et al.* [74], where they documented that a significant number of *Staphylococcus aureus* strains that were classified as resistant with an oxacillin MIC of 4 µ/ml shows discrete amplification of *mecA* gene [75]. The fact that most of the slime factor producing *Staphylococcus aureus* shows elevated trend of resistance to different classes of antibiotics emphasize the importance of these genes in upsurging multi-drug resistance in bacterial pathogens. Our findings consequently show that strong association exist between slime factor production and antibiotic resistance [5, 74-75] and this was highly exemplified by the multi-drug resistance of different slime producing organisms to different classes of antibiotics. In conclusion, the results of our study have shown that the phenotypic screening of slime factor production from *Staphylococcus aureus* using Congo Red Agar is able to screen a wide range of slime factor producing strain but lacks the ability to capture all slime factor strains. We however found that oxacillin used for screening methicillin resistance could overestimate numbers as only 50% of the oxacillin disc affirmed methicillin resistance show discrete amplification of *mecA* gene and also provide false negative results, since 46.2% of the organisms affirmed to be methicillin resistant were also found harbouring *mecA* gene. It is therefore important to state that statistical significant association exist between slime factor production and antibiotics resistance.

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