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Comparison of Cefocitin gene with Nuc gene in *Staphylococcus aureus* from three Tertiary Institution in South Western Nigeria

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Abstract

Staphylococcus aureus is a human pathogen of global public health significance. It is implicated in a wide variety of infections. Each *S. aureus* strain is equipped with a diverse repertoire of genes ranging from the determinant genes, virulence genes, and antibiotic-resistant genes e. g. nuc genes, mecA genes, and PVL genes. Nuc genes (nuclease genes) is a heat-resistant gene that serves as a marker for *S. aureus. mecA genes* codes for penicillin binding protein 2a (PBP2a) that codes for methicillin resistance in *S. aureus, while* Panton-Valentine leukocidin genes (PVL) is a virulent gene that codes for toxins which form pores in the membrane of host defense cells. The fatal potentials of these genes mutating in *S. aureus* stirred up the interest to study their genetic variation in these locations. One hundred and thirty isolates of *S. aureus* were collected, cultured and phenotypically identified as *S. aureus* using coagulase test method and their colour change on MSA. Antibiotic susceptibility testing using disk diffusion method were performed on each of the isolates. The DNA was extracted by boiling method and the Polymerase Chain Reaction was performed on the isolates using the conventional PCR technique. The PCR products were tested for *Nuc*, *mecA and PVL* genes by running agarose gel electrophoresis. The study showed that there is a statistically significant relationship for the susceptibility testing of most of the antibiotics used, with the exception of Gentamicin, which had a p-value = 0.11. 63.8% of all the *S. auerus* isolates were MRSA, and 36.2% were MSSA by cefoxitin screening. Many of the isolates have diverse genetic variations. Of the 130 genetically assayed isolates of *S. aureus,* 81(62.3%) of them were Nuc positive, 60(46.1%) were mecA-positive, while 57(44%) were PVL positive. This study reveals a high prevalence of mecA genes and PVL genes which codes for antibiotic resistance and virulence factors in *S. aureus.* Some isolates, 9(6.9%) were coagulase positive nuc negative mecA positive *S. aureus* while 53(63.9%) were mecA negative methicillin resistant *Staphylococcus aureus* by cefoxitin screening. Some nuc gene negative with mecA and PVL positive *S. aureus* were also identified. While nuc positive compared with mecA and PVL positive and negative genes results were also ascertained.

All these reveals a remarkable genetic diversity in the whole isolates of this study and suggests the need for further research and monitoring of the genetic variation and evolution of this organism.

Keywords: *Staphylococcus aureus, pvl gene,* MRSA, NUC genes, MecA and Cefoxitin

Introduction

Staphylococcus aureus is a Gram-positive bacterium that gives a positive reaction to catalase and coagulase test. It is an important pathogen in human infections and is implicated in a wide variety of infections, from mild skin infections to more serious and invasive infections, including sepsis, pneumonia, endocarditis, deep-seated abscesses, and toxinoses including food poisoning and toxic shock syndrome (Chaturvedi, 2022). Staphylococci infections are treated with antibiotics. Over the decades, some strains of

Staphylococcus aureus such as methicillin-resistant *S. aureus* (MRSA) have become resistant to antibiotics that once destroyed it. MRSAs are resistant to beta lactam antibiotics, and many other antibiotics. They are also resistant clinically to all beta-lactam antibiotics, despite apparent in vitro efficacy. Vancomycin has been the antibiotic of choice to treat MRSA infections, and the emergence of vancomycin non susceptible *S. aureus* reported in recent years is a cause of great public health concern and has made therapy of MRSA infections even more challenging for clinicians (Kim *et al.,* 2004).

Recent reports state that acquisition of resistance in Staphylococci is by transposons and plasmids mediated by conjugate transfer, which spreads resistance elements among Staphylococci species. They can also mobilize conjugate plasmids to re-combine and form new plasmids then acquire and transfer potential resistant determinants (Maree *et al.,* 2022, Tao *et al.,* 2022, Urban-Chmiel *et al.,* 2022).

Although MRSA infection emerged in hospitals (Hospital acquired, HA-MRSA) called nosocomial pathogen, then it spread to community (Community acquired, CA-MRSA) and to livestock (Livestock associated, LA-MRSA) (Crespo-Piazuelo *et al.,* 2021). Each *S. aureus* strain is equipped with a diverse repertoire of genes ranging from the determinant gene, virulence genes, and antibiotic-resistant genes. This study is focused on ascertaining the variation in the nuc genes, mec A genes, and PVL genes in each of the isolates, to know their prevalence in each of the locations and compare the genes from the three locations.

Nuc genes (Thermonuclease genes) acts as a marker for *S. aureus* and also the presence of this heat-resistant nuclease gene (nuc) is strongly associated with the production of enterotoxin, and it can be considered an indicator of infection with enterotoxin producer *Staphylococcus aureus* (Nadiya *et al.,* 2023).

PVL genes (Panton-Valentine leukocidin) is a virulent gene in *Staphylococcus aureus*. This gene was discovered in 1932, by Panton and Valentine described PVL as a virulence factor belonging to the family of synergohymenotropic toxins (Snoussi *et al.,* 2023). These toxins form pores in the membrane of host defense cells by synergistic action of 2 secretory proteins, designated LukS-PV and LukF-PV, which are encoded by 2 cotranscribed genes of a prophage integrated in the *S. aureus* chromosome (Thomas *et al.,* 2022).

MecA is a gene on the staphylococcal cassette chromosome *mec* (SCCmec), a mobile genetic element that codes for antibiotic resistance. *mecA* codes for penicillin binding protein 2a (PBP2a) that has a lower affinity for beta-lactams antibiotics; they confer resistance to all available penicillin's and most of the other β - lactam drugs. It is the gene that codes for methicillin resistance in *Staphylococcus aureus,* It is therefore the genetic determinant of methicillin sistance *Staphylococcus aureus* (MRSA) (Sahreena and Kunyan, 2018).

The menace of MRSA colonization and infection has increased from hospitals to the community and further to animals. The surge in the emergence of multi-drugs resistant *S. aureus* particularly methicillin-resistant *S. aureus* has caused serious economic burden, highly increased mortality and morbidity and concerns in public health due to limited options of treatment of MRSA infections. Methicillin resistant *Staphylococcus aureus* being a super bug that is silently infecting and affecting the populace; undertaking this study therefore becomes very crucial. As this will aid the diagnosis of the strains of *S. aureus* in these three tertiary hospitals in Nigeria; it's specified treatment and its eventual defeat. This will in turn reduce the economic burden and reduce the incidence of mortality and morbidity among Nigerians and in the world at large. The objective of this study therefore is to determine the prevalence of *S. aureus* in Nigeria, Methicillin resistance *Staphylococcus aureus* (MRSA), and virulent genes.

Materials and Methodology

Nutrient agar, Manitol salt agar, hydrogen peroxide, rabbit plasma, Mueller-Hinton agar, HCl (1N), Gentian violet, Lugol's iodine, acetone, safranin, antibiotic discs (Oxoid), PCR water, buffer, primers, DNA laCdder, 1.5% agarose gel.

Cultured samples were collected from hospital laboratories in each of the six geopolitical zones of Nigeria. The MRSA isolates were identified by phenotypic method and amplification of the mecA gene.

Study Design
This was a cross-sectional study.

Sample Size
A total of 130 clinical isolates of MRSA were collected using a random sampling technique

Study Location
The clinical isolates were collected from University College Hospital Ibadan, Ladoke Akintola University of Technology Ogbomoso, and Enugu State University of Science and Technology, Enugu.

Study Site
The samples assembled were processed at the Medical Microbiology Department of the University College Hospital (UCH) Ibadan, and FOWN Laboratory Lagos.

Study Duration
This study was carried out between July 2021 and December 2022.

Isolates Identification using Phenotypic Methods
Transport
The assembled putative *S. aureus* isolates were inoculated in a nutrient agar slant, refrigerated at 4°C until delivery to the laboratory.

Culture
The isolates were confirmed by sub-culturing in a freshly prepared Mannitol Salt Agar (Oxoid Ltd., United Kingdom) and incubated overnight at 37° (Colonies presumptive of *S. aureus* would appear golden yellow colonies with a smooth glistening surface, 2-4mm in diameter, circular, convex, shiny and easily emulsifiable. These were confirmed using the Gram staining technique and conventional biochemical tests (Cheesbrough *et al.,* 2005).

Gram Staining Reaction
Young colonies were emulsified on a clean grease complimentary glass slide, air dried, and heat fixed. The smear was flooded with Gentian violet for 1 minute, rinsed and flooded with Lugol's iodine for 1 minute, rinsed and decolorized briefly using acetone, rinsed and counterstained with safranin for half a minute and rinsed. The slide is then blotted; air dried and examined using an oil immersion objective lens. *S. aureus* would appear Gram positive in clusters (Cheesbrough *et al.,* 2005).

Biochemical Tests
Conventional tests were used to distinguish *S. aureus.* These tests include Catalase; 1-2 ml of 3% hydrogen peroxide solution was put into a test tube, using sterile wire loop some colonies of the 24 hours test organism growth was immersed in the hydrogen peroxide solution. Observation of bubbles was made. Slide coagulase: Two drops of saline on a slide, using a sterile inoculating loop, a few colonies of organism were emulsified in the saline to make thick suspension of bacteria. A loopful of plasma was added to the suspension. Coarse clumping of the mixture was checked within 10-15 seconds. *S. aureus* exhibit positive catalase and coagulase. (CLSI, 2021).

Antibiotic Sensitivity Testing
This was performed using the Kirby-Bauer disc Diffusion method. The 24-hour fresh colonies of the organisms were inoculated into peptone using a sterile wire loop and observation of 0.5 McFarland standard. The suspension was then **seeded** on Mueller-Hilton agar using a sterile wire loop, and antibiotic disks containing known amounts of an antimicrobial agent are placed on the surface of an agar plate containing a nonselective medium that has been inoculated with a suspension of a strain of *S. aureus* to give a confluent lawn of growth. The antimicrobial agent diffuses into the medium, causing a zone of inhibition of growth of the strain around the disk corresponding to the susceptibility of the strain to the agent. Interpretative inhibition zone diameters have been established for susceptibility test results to permit classification of an isolate as being susceptible, intermediate (or exhibiting decreased susceptibility), or resistant to an antimicrobial agent (CDC, 2022)

The following antibiotics were used: Amoxicillin (30µg), Clindamycin (10µg),

Ciprofloxacin10µg), Erythromycin (10µg), Gentamycin10 µg), Linezolid 10 µg), Penicillin10 µg) Vancomycin 30 µg) and cefoxitin 30 µg). The plates were incubated for 18-24 hours, and the zone of inhibition was measured to ascertain the susceptible, Intermediate and resistant organisms (CLSI, 2021).

Detection of MRSA

Methicillin resistant *S. aureus* (MRSA) was determined by disc diffusion test using cefoxitin disc on Mueller-Hilton agar as stated above in (Antibiotic Sensitivity Testing). *S. aureus* isolates resistant to cefoxitin (30µg) were considered MRSA and with PCR technique for the presence of MecA gene

Molecular Characterization

DNA Extraction

The boiling technique was employed to isolate genomic DNA. In 1000µL of sterile water, the cells were collected and vortexed until they were completely dissolved. The mixture was then centrifuged at 10,000 rpm for 5 minutes. The supernatant was then discarded, 1000µl of sterile water was added, vortexed, and centrifuged. The supernatant was decanted again and 200µl of sterile water was added. The solution was then vortexed until thoroughly mixed, then boiled for 10 minutes at 100°C. It was then cooled immediately on ice and vortexed. The solution was centrifuged for 5 minutes at 10,000rpm, the supernatant transferred into fresh Eppendorf tubes, and the pellet was discarded. The isolated DNA was then stored at 4°C

Polymerase Chain Reaction

The PCR reaction was carried out using the Solis Biodyne 5X FIREPol Master mix. PCR was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1 X concentration containing 1 X Master mix -buffer Buffer (Solis Biodyne), 1.5 mM MgCl2, 200µM of each deoxynucleosidetriphosphates (dNTP)(Solis Biodyne, Tartu, Estonia), 25pMol of each primer (Stab Vida, Portugal), 2unit of Hot FIREPol DNA polymerase (Solis Biodyne, Tartu, Estonia), Proofreading Enzyme, 5µl of the extracted DNA and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in a Techne Prime thermal cycler for an initial denaturation of 95°C for 1 5 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute as indicated in the primer table, and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 1 0 minutes at 72°C.

Primers

Primer Name	Sequence	Annealing Temp	BasePair	Reference
MecA	F-AAAATCGATGGTAAAGGTTGGC R-AGTTCTGCAGTACCGGATTTGG	52°C	533bp	Merlino *et al.,* 2002
Luk-pvl	F-ATCATTAGGTAAAATGTCTGGACAT R-GCATCAAGTGTAT TGGATAGCAAAAGC	55°C	433bp	Jaraud *et al.,* 2002
NUC	F:GCGATTGATGGTGATACGGTT R:AGCCAAGCCTTGACGA-ACTAAAGC	55°C	279bp	Puah *et al.,* 2016
ArcA-ACME	F:CTAGGTGCATAAATGTACGTG R:CCAGAAGTACGCGAGAAC	55°C	577bp	Dehnad *et al.,* 2020

Gel electrophoresis

The amplification product was separated on a 1.5% agarose gel, and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. DNA ladder 100bp was used as DNA molecular weight standard.

Results

One hundred and thirty isolates of *Staphylococcus. aureus* from human patients were used for this study. These *S. aureus* isolates showed the highest susceptibility to Vancomycin 130(100%) Gentamycin 89(68.5%) Linezoid 80(61.5%) Clindamycin 62(47.7%) Erythromycin 45(34.6%) Cefoxitin 35(26.9%) Ampicillin 28(21.5%) Ceftriaxone 13(10.0%) Penicillin 4(3.1%). The highest degree of resistance was obserevd in Penicillin 125(96.2%) Ampicillin 102(78.5%) Cefoxitin 83(63.8%) Ceftriaxone 101(77.7%) Linezoid 50(38.5%) Erythromycin 43(33.1%) Gentamycin 35(26.9%) Clindamycin 29(22.3%) Vancomycin 0(0%). Cefoxitin resistance is the phenotypic determinant of methicillin resistant *Staphylococcus. aureus*. 83(63.8%) of the 130 isolates were methicillin resistant *Staphylococcus. Aureus* (MRSA).

This evaluation was also performed for Clindamycin with respect to the three locations, it was found that there is no statistical significant relationship between ESUTH and LTH which a P value of 0.684, but there was a statistical significant relationship between LTH and UCH with the P value of 0.005 and between ESUTH and UCH with the P value 0.05. Linezoid was also studied with respect to the three locations, there is no statistical significant relationship between ESUTH and LTH which a P value of 0.691 and between ESUTH and UCH with the P value 0.196. But there was a statistical significant relationship between LTH and UCH with the P value of 0.030. Ceftriaxone was studied also with respect to the three locations, There is no statistical significant relationship between ESUTH and LTH which a P value of 0.617, but there was a statistical significant relationship between LTH and UCH with the P value of 0.001 and between ESUTH and UCH with the P value of <0.003. Ampicillin was also evaluated with respect to the three locations, there is no statistical significant relationship between ESUTH and LTH which a P value of 0.847, but there was a statistical significant relationship between LTH and UCH with the P value of 0.036 and between ESUTH and UCH with the P value 0.007. Cefoxitin also was evaluated with respect to the three locations, There is statistical significant relationship between ESUTH and LTH with the P value of <0.003 and between ESUTH and UCH with the P value of <0.003 but there is no between LTH and UCH with the P value of 0.719.

Table 1: Result of the *S. aureus* genetic studies from the three tertiary institutions

Location	S. aureus	Fox (SENS)	Fox (RES)	NUC (POS)	NUC (NEG)	MEC A (POS)	MEC A (NEG)	PVL (POS)	PVL (NEG)
UCH	46	34	12	35	11	31	15	37	9
ESUTH	43	8	35	26	17	20	23	15	28
LAUTECH	41	5	36	20	21	9	32	5	36
TOTAL	**130**	**47**	**83**	**81**	**49**	**60**	**70**	**57**	**73**

Coagulase positivity is a phenotypic determinant of *Staphylococcus. aureus.*

The results in Table 3 shows that these130 isolates were coagulase positive *Staphylococcus. aureus*. The table highlights their methicillin susceptibility and resistance and molecular results. Cefoxitin resistance is the phenotypic determinant of methicillin resistance in *S. aureus*. So from the result it was seen that 83(63.8%) of the isolates were methicillin resistant *Staphylococcus. aureus* (MRSA). Nuc

genes is the genetic determinant of *S. aureus* organisms. The result here shows that 81(62.3%) of the 130 isolates are Nuc genes positive while the remaining 49 were Nuc gene Negative. Whereas coagulase test puts it that 130 isolates were *S. aureus*, genotypic confirmation using PCR validates that it is only 81 of those isolates that were true Staphylococcus. *aureus*. The result from this table also shows that 60(46%) and 57(44%) of the isolates were positive for mecA and PVL genes respectively; while 70 and 73 of them were negative for the mecA and PVL genes. PVL genes are virulent gene in *S. aureus* that causes critical pathologic conditions in patients infected with the organism.

Table 2: Comparison of *S. aureus* (cefoxitin screening) with nuc gene by location

				NUC				
LOCATION				**NEG**	**POS**	**Total**	**Chi square**	**P-value**
ESUTH	***S. aureus* (Cefoxitin screening)**	Resistance	Frequency	13	22	35	0.45	0.5
			%	30.2%	51.2%	81.4%		
		Sensitive	Frequency	4	4	8		
			%	9.3%	9.3%	18.6%		
		Total	Frequency	17	26	43		
			%	39.5%	60.5%	100.0%		
LTH	***S. aureus* (Cefoxitin screening)**	Resistance	Frequency	19	17	36	0.29	0.59
			%	46.3%	41.5%	87.8%		
		Sensitive	Frequency	2	3	5		
			%	4.9%	7.3%	12.2%		
		Total	Frequency	21	20	41		
			%	51.2%	48.8%	100.0%		
UCH	***S. aureus* (Cefoxitin screening)**	Resistance	Frequency	4	8	12	0.95	0.62
			%	8.7%	17.4%	26.1%		
		Sensitive	Frequency	7	27	34		
			%	10.9%	37.0%	47.8%		
		Total	Frequency	11	35	46		
			%	23.9%	76.1%	100.0%		
Total	***S. aureus* (Cefoxitin screening)**	Resistance	Frequency	36	47	83	3.98	0.14
			%	27.7%	36.2%	63.8%		
		Sensitive	Frequency	13	34	47		
			%	8.5%	18.5%	36.2%		
		Total	Frequency	49	81	130		
			%	37.7%	62.3%	100.0%		

NB: Significance is at the 0.05 level

Table 3 highlights the relationship between the Methicillin resistance and sensitive *S. aureus* with Nuc genes. It shows that there is no statistical significant difference in their relationship in the three locations. From the table we can see that there 47(36.2%) MRSA'S that were Nuc gene positive while 36(27.7%) of the MRSA'S were negative for Nuc genes. And as such can be labeled as Nuc negative methicillin resistant *StaphylococcuS. aureus*. While 34(8.5%) of the MSSA'S were positive for Nuc genes while 13(8.5%) of the methicillin isolates were negative for Nuc genes.

Table 3: Comparison of S. *aureus* (Cefoxitin screening) with mec A gene by location is shown in

LOCATION				MEC A NEG	MEC A POS	Total	Chi square	P-value
ESUTH	*S. aureus* (Cefoxitin screening)	Resistance	Frequency	20	15	35	1.0	0.3
			%	57.1%	42.9%	100.0%		
		Sensitive	Frequency	3	5	8		
			%	37.5%	62.5%	100.0%		
		Total	Frequency	23	20	43		
			%	53.5%	46.5%	100.0%		
LTH	*S. aureus* (Cefoxitin screening)	Resistance	Frequency	28	8	36	0.0	0.9
			%	77.8%	22.2%	100.0%		
		Sensitive	Frequency	4	1	5		
			%	80.0%	20.0%	100.0%		
		Total	Frequency	32	9	41		
			%	78.0%	22.0%	100.0%		
UCH	*S. aureus* (Cefoxitin screening)	Resistance	Frequency	5	7	12	1	1
			%	41.7%	58.3%	100.0%		
		Sensitive	Frequency	10	24	34		
			%	31.8%	68.2%	100.0%		
		Total	Frequency	15	31	46		
			%	32.6%	67.4%	100.0%		
Total	*S. aureus* (Cefoxitin screening)	Resistance	Frequency	53	30	83	0.1	0.0
			%	63.9%	36.1%	100.0%		
		Sensitive	Frequency	17	30	47		
			%	36.2%	63.8%	100.0%		
		Total	Frequency	70	60	130		
			%	53.8%	46.2%	100.0%		

Table 3. Shows the relationship between the Methicillin resistance and sensitive *S. aureus* with mecA genes. It shows that there is no statistically significant difference in their relationship in the three locations. From the table we can see that there 30(36.1%) MRSA'S that were mecA gene positive while 53(63.9%) of the MRSA'S were negative for mecA genes. mecA is the genotypic determinant of methicillin resistance in *S. aureus* while cefoxitin disc is used as a phenotypic determinant of *S. aureus*. This result reveals that some isolates were cefoxitin sensitive and mecA negative. These isolates can be regarded as mecA negative methicillin resistant *StaphylococcuS. aureus*. While 30(10.1%) of the MSSA'S were positive for mecA genes while 17(40.0%) of the methicillin sensitive *S. aureus* isolates were negative for Nuc genes. The 10.1% Methicillin susceptible isolates which were determined by cefoxitin screening had mecA genes, which is the gene that confers methicillin resistance in *S. aureus.* This reveals that some mecA homologues are present in the isolates but they do not confer methicillin resistance in *S. aureus*.

Table 4: The comparison of *S. aureus* (Cefoxitin screening) with PVL genes by location

				PVL				
LOCATION				**NEG**	**POS**	**Total**	**Chi square**	**P-value**
ESUTH	***S. aureus* (Cefoxitin screening)**	RESISTANCE	Count	23	12	35	0.03	0.86
			%	65.7%	34.3%	100.0%		
		SENSITIVE	Count	5	3	8		
			%	62.5%	37.5%	100.0%		
		Total	Count	28	15	43		
			%	65.1%	34.9%	100.0%		
LTH	***S. aureus* (Cefoxitin screening)**	RESISTANCE	Count	32	4	36	0.32	0.57
			%	88.9%	11.1%	100.0%		
		SENSITIVE	Count	4	1	5		
			%	80.0%	20.0%	100.0%		
		Total	Count	36	5	41		
			%	87.8%	12.2%	100.0%		
UCH	***S. aureus* (Cefoxitin screening)**	RESISTANCE	Count	1	11	12	2.43	0.29
			%	8.3%	91.7%	100.0%		
		SENSITIVE	Count	8	26	34		
			%	18.2%	81.8%	100.0%		
		Total	Count	9	37	46		
			%	19.6%	80.4%	100.0%		
Total	***S. aureus* (Cefoxitin screening)**	RESISTANCE	Count	56	27	83	11.99	0.002
			%	67.5%	32.5%	100.0%		
		SENSITIVE	Count	17	30	47		
			%	36.1%	63.9%	100.0%		
		Total	Count	73	57	130		
			%	56.2%	43.8%	100.0%		

This Table highlights the relationship between the Methicillin resistance and sensitive *S. aureus* with PVL genes. It shows that there is no statistical significant difference in their relationship in the three locations. From the table we can see that there were 27(32.5%) MRSA'S that were PVL gene positive while 56(67.5) %of the MRSA'S were negative for PVL genes. While 30(63.9%) of the MSSA'S were positive for PVL genes while 17(36.1%) of the methicillin isolates were negative for PVL genes.

Table 5: NUC Positive genes with mecA gene and PVL genes

LOCATION	**NUC POS**	**mecA POS**	**mecA Neg**	**PVL Pos**	**PVL Neg**
UCH	35	**23**	**12**	**29**	**6**
ESUTH	26	19	7	14	12
LAUTECH	20	9	11	5	15
Total	**81**	**51**	**30**	**48**	**33**

Nuc positivity is the true genetic determinant of *StaphylococcuS. aureus*. So genotypically it can be said that only 81 of these isolates are *S. aureus*. This tables tries to highlight the antibiotic resistant genes (mecA) and the virulent genes present in this genotypically confirmed *S. aureus*. So from this data, it is seen that 51(62.9%) of the isolates were mecA positive with 30(37%) of them negative for mecA. and 48(59.2%) of the isolates were PVL gene positive and the remaining 33(40.7%) isolates were PVL negative.

Table 6: NUC Negative gene with positive mecA and PVL genes

LOCATION	Nuc NEG	mecA POS	mecA NEG	PVL POS	PVL NEG
UCH	11	8	3	8	3
ESUTH	17	1	16	1	16
LAUTECH	21	0	21	0	21
Total	**49**	**9**	**40**	**9**	**40**

Table 6 presents another interesting finding in this research. The presence of coagulase positive Nuc negative and mecA positive StaphylococcuS. aureus which were positive for mecA and PVL genes.It reveals that out of the 49 coagulase positive Nuc negative S. aureus, 9(18.4%) of the isolates were mecA positive. This implies that these isolates which are S. aureus by coagulase test but genotypically non S. aureus are able to confer methicillin resistance.

Table 7: Interactions between Mec A Genes (Pos and Neg) and Nuc Genes (Pos and Neg) By Location and Their *Post Hoc Tests*

				MEC A				
LOCATION				NEG	POS	Total	Chi square	P-value
ESUTH		NEG	Frequency	16(37.2%)	1(2.3%)	17(39.5%)		
	NUC	POS	Frequency	7(16.3%)	19(44.2%)	26(60.5%)	18.66	<0.001
		Total	Frequency	23(53.5%)	20(46.5%)	43(100..0%)		
LTH		NEG	Frequency	21(51.2%)	0(0.0%)	21(51.2%)		
	NUC	POS	Frequency	11(%26.8)	9(22.0%)	20(48.8%)	12.11	0.001
		Total	Frequency	32(78.0%)	9(22.0%)	41(100.0%)		
UCH		NEG	Frequency	3(6.5%)	8(17.4%)	11(23.9%)		
	NUC	POS	Frequency	12(26.1%)	23(50.0%)	35(76.1%)	0.19	0.67
		Total	Frequency	15(32.6%)	31(67.4%)	46(100.0%)		
Total		NEG	Frequency	40(30.8%)	9(6.9%)	49(37.7%)		
	NUC	POS	Frequency	30(23.1%)	51(39.2%)	81(62.3%)	24.43	<0.001
		Total	Frequency	70(53.8%)	60(46.2%)	130(100.0%)		

Significance is at 0.05

Table 7 highlights the interaction between Nuc genes and mec A genes in the three locations. It shows that 30(23.1%) of isolates that were Nuc positive were mecA negative while 40(30.8%) of isolates that were Nuc negative were mecA negative. So these isolates had neither Nuc genes nor mecA genes. While 51(39.2%) of isolates that were Nuc positive were mecA positive but 9(6.9%) of isolates that were Nuc negetive were mecA positive. This implies that these 6.9% isolates are genotypically unconfirmed *S. aureus* that had mecA genes; Nuc negative mecA positive *S. aureus*. There was a statistical significant relationship in the interaction between mecA and Nuc genes in ESUTH and LTH while there was no statistical significant relationship in UCH.

Table 8: Interactions between Pvl Genes (Pos and Neg) and Nuc Genes (Pos and Neg) By Location and their *Post Hoc Tests*

LOCATION				PVL			Chi square	P-value
				NEG	POS	Total		
ESUTH		NEG	Frequency	16(37.2%)	1(2.3%)	17(39.5%)		
	NUC	POS	Frequency	12(27.9%)	14(32.6%)	26(60.5%)	10.41	0.001
		Total	Frequency	28(65.1%)	15(34.9%)	43(100%)		
LTH		NEG	Frequency	21(51.2%)	0(0.0%)	21(51.2%)		
	NUC	POS	Frequency	15(36.6%)	5(12.2%)	20(48.8%)	5.98	0.014
		Total	Frequency	36(87.8%)	5(12.2%)	41(100.0%)		
UCH		NEG	Frequency	3(6.5%)	8(17.4%)	11(23.9%)		
	NUC	POS	Frequency	6(13.0%)	29(63.0%)	35(76.0%)	0.55	0.46
		Total	Frequency	9(19.6%)	37(80.4%)	46(100.0%)		
Total		NEG	Frequency	40(30.8%)	9(6.9%)	49(37.7%)		
		POS	Frequency	33(25.4%)	48(36.9%)	81(62.3%)		
	NUC						20.74	<0.001
		Total	Frequency	73(56.2%)	57(43.8%)	130(100.0%)		

Significance is at 0.05

Table 9 highlights the interaction between Nuc genes and PVL genes in the three locations. It shows that 33(25.4%) of isolates that were Nuc positive were PVL negative while 40(30.8%) of isolates that were Nuc negative were PVL negative. And 48(36.9%) of isolates that were Nuc positive were PVL positive while 9(6.9%) of isolates that were Nuc negative were PVL positive. There was a statistically significant relationship in ESUTH and LTH while there was no statistically significant relationship in UCH. But the overall data also shows that there is a statistical relationship between Nuc genes and PVL genes.

Table 9: Interactions between PVL Genes (Pos and Neg) and Mec A Genes (Pos and Neg) By Location and Their *Post Hoc Tests*

				PVL				
LOCATION				**NEG**	**POS**	**Total**	**Chi square**	**P-value**
ESUTH	**Mec A**	NEG	Frequency	23(53.5%)	0(0.00%)	23(53.5%)	26.49	<0.001
		POS	Frequency	5(25.0%)	15(75.0%)	20(46.5%)		
		Total	Frequency	28(65.1%)	15(34.9%)	43(100.0%)		
LTH	**Mec A**	NEG	Frequency	32(78.0%)	0(0.0%)	32(78.0%)	20.25	<0.001
		POS	Frequency	4(44.4%)	5(55.6%)	9(22.0%)		
		Total	Frequency	36(87.8%)	5(12.2%)	41(100.0%)		
UCH	**Mec A**	NEG	Frequency	3(20.0%)	12(80.0%)	15(32.6%)	0.003	0.96
		POS	Frequency	6(19.4%)	25(80.6%)	31(67.4%)		
		Total	Frequency	9(19.6%)	37(80.4%)	46(100.0%)		
Total	**Mec A**	NEG	Frequency	58(82.9%)	12(17.1%)	70(53.8%)	43.93	<0.001
		POS	Frequency	15(25.0%)	45(75.0%)	60(46.2%)		
		Total	Frequency	73(56.2%)	57(43.8%)	130(100.0%)		

Table 9 highlights the interaction between mecA genes and PVL genes in the three locations. It shows that 15(25.0%) of isolates that were mecA positive were PVL negative while 58(82.9%) of isolates that were mecA negative were mecA negative. While 45(75.0%) of isolates that were mecA positive were PVL positive but 12(17.1%) of isolates that were mecA negative were PVL positive. These 45 isolates that had both mecA genes and PVL genes have both antibiotic-resistant genes and virulent genes also. There was a statistically significant relationship in the interaction between mecA and PVL genes in ESUTH and LTH while there was no statistical significant relationship in UCH.

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence observed in previous studies by Alli *et al*. (2022) and Peter *et al*. (2022) reported MRSA prevalence rates of 61.8% and 66.9%, respectively. Alli and colleagues focused on the nasal carriage of Staphylococcus aureus and its antibiogram among medical undergraduate students at a private university in Ogun State, Nigeria. In contrast, Peter et al. studied the prevalence of livestock-acquired methicillin-resistant Staphylococcus aureus (LA-MRSA) strains in southeastern Nigeria.

On the other hand, Gaddafi (2021) reported a prevalence of 19.4% of pigs that tested positive for MRSA, and 1.4% of farm attendants were MRSA positive. Igbinosa *et al*. (2023) also reported that 29.9% of the isolates tested positive for MRSA. Their study focused on the prevalence, multiple antibiotic resistance, and virulence profile of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail poultry meat from Edo, Nigeria.

Vazquez-Rosas *et al.* (2021) noted a decrease in the frequency of MRSA between 2011 and 2019 in their research on the molecular characterization of *Staphylococcus aureus* obtained from blood cultures of pediatric patients treated in a tertiary care hospital in Mexico. This aligns with the report by Edgeworth and his team in the UK (2020), which revealed a significant decline in methicillin-resistant *Staphylococcus aureus* and bloodstream infections acquired in intensive care units. They observed that between 2007 and 2016, the number of ICU MRSA acquisitions per 1,000 patients fell due to the implementation of a national infection control campaign, with MRSA acquisitions dropping from 25.4% to 4.1%.

Dai *et al*. (2019) conducted a survey on the decline of MRSA infections in Shanghai from 2008 to 2017. They found that, despite a steady decline in the prevalence of MRSA infections in China, the molecular mechanisms underlying this decline are still poorly understood. Their findings indicated a notable decrease in the previously pervasive HA-MRSA ST239 clones, contributing to a significant drop in MRSA prevalence over the past decade. However, it remains unclear what specifically caused this decline.

The decline in methicillin-resistant *Staphylococcus. aureus* (MRSA) infections in Shanghai from 2008 to 2017. They found that although there has been a steady decline in the prevalence of MRSA infections in China, the molecular mechanisms underlying the decline was still poorly understood, according to their report. Their study findings showed a notable decline in the previously pervasive HA-MRSA ST239 clones, which contributed to a notable drop in MRSA prevalence over the previous ten years. But it is still unclear what caused this decline, in terms of its process.contrast with this study, which reports a high prevalence of MRSA (sixty-three point one percent of the one hundred thirty *S. aureus* isolates). This may be due to Nigeria's substandard infection control surveillance program. Additionally, the continued misuse of antibiotics contributes to increased horizontal gene transfer of mobile genetic elements, further raising the prevalence of MRSA in the population.

There was also variation in the prevalence of MRSA among these three locations. The reasons behind this disparity are not yet known. Although the studies by Rakonjac *et al.* (2022) and Zhang *et al*. (2022) indicate that the epidemiology of MRSA strains is constantly changing, it is understood that their prevalence and characteristics can differ between hospitals within the same country or even between wards in the same hospital. Consequently, the results of this research show a notable difference in the prevalence of MRSA isolates from the three hospitals.

The coagulase test from this study is not highly specific for phenotypic identification of *S. aureus. It* highlights that Sixty-two-point three percent of the one hundred and thirty isolates of *S. aureus* with phenotypic confirmation were Nuc positive and were genotypically verified to be *S. aureus*, while thirty-seven-point six percent were Nuc negative and were therefore determined to be other species of Staphylococci. This is true because *S. aureus* may be distinguished from other species of the same organism by the Nuc gene. A similar result by Sheet *et al. (*2021) corroborated this data. They worked on the direct detection of *Staphylococcus aureus* in camel milk in the Nineveh governorate by using the PCR technique. They reported that seventy percent of *S. aureus* was isolated from their total isolates through nuc genes detection. Rao and his team in 2022 even reported a higher prevalence; that ninety-eight-point six percent of their isolates were confirmed to be *S. aureus* (nuc gene positive) in the study they did on the Antimicrobial resistance and genetic diversity of *Staphylococcus. aureus* collected from livestock, poultry and humans. In a study on Coagulase-positive, methicillin-resistant *Staphylococcus. aureus* that was conducted in Bangladesh in 2020, Rana and his Colleagues found that of the forty-nine samples that tested positive for Staphylococci, thirty-four-point six percent of the isolates carried the nuc gene (positive). Contrary to this study, Deddefo *et al. (*2022) reported that their pooled prevalence of *S.*

aureus in locally produced soft cheese and traditional fermented milk was eighteen-point six percent and fourteen-point nine percent, respectively. This Prevalence is quite lower than that seen in this study and that of Sheet *et al. (*2021) and molecular characteristics of *Staphylococcus aureus* in raw milk and milk products in Ethiopia.

On the other hand, Gaddafi in 2021 reported a prevalence of nineteen-point four percent of pigs tested positive for MRSA and one point four percent of farm attendants were MRSA positive. Igbinosa *et al. (*2023) also reported that twenty-nine-point nine percent of the isolates are positive for MRSA. Prevalence, multiple antibiotic resistance and virulence profile of methicillin-resistant Staphylococcu*s aureus* (MRSA) in retail poultry meat from Edo, Nigeria

Staphylococcus. aureus strains capable of producing PVL are more likely to cause severe infections such as necrotizing pneumonia and skin and soft tissue infections. In a study by Kwapisz *et al. (*2020) on MRSA and MSSA strains, the detection of the PVL gene was much more common in MRSA than in MSSA isolates.

In the three tertiary hospitals used in the study, highlights the NUC-positive genes together with the mecA positive and negative genes and the PVL positive and negative genes.

This study found that nine (six-point nine percent) of the isolates that were nuc negative (nuc deficient) were positive for mecA gene. This implies that these isolates are genotypically not *S. aureus* but had mecA genes; Nuc negative mecA positive *S. aureus*. A researcher, Jiang *et al. (2021)* reported the finding of a *nuc*-deficient methicillin-resistant *Staphylococcus. aureus* strain in 2008. They observed that, primarily in younger adults, PVL-positive MRSA had a high frequency in Australia. Rakonjac and his team also did a work on the Predominance of among PVL-positive MRSA isolates in a tertiary care hospital in Belgrade, Serbia. They reported from their work that one hundred and thirty-one PVL-positive MRSA isolates were identified in five hospital sites in that region within the period of their study. From this study it was observed that some of these isolates have both virulent gene and antibiotic-resistant genes while some lacked both mecA and PVL genes and thus lacks the antibiotic-resistant genes but has PVL genes.

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Competing Interests
Authors have declared that no competing interests exist.

Conclusion
Staphylococcus aureus remains the most common organism causing microbial infections. Antibiotic testing, phenotypic identification, and genotypic identification were performed on 130 isolates from three tertiary institutions. A total of 63.8% of the organisms were MRSA (Methicillin-Resistant Staphylococcus aureus), while 36.2% were MSSA (Methicillin-Susceptible Staphylococcus aureus). The genotypic studies revealed the presence of Nuc genes, mecA genes, and PVL genes in the isolates. The identified genes were analyzed alongside the phenotypic results: coagulase test results were correlated with Nuc genes, and MRSA results were compared with mecA genes. Furthermore, the antibiotic-resistant gene mecA was compared with the virulence gene PVL.

The results of this study revealed significant genetic variation among the isolates from the three tertiary institutions. This indicates that there are notable genetic differences in the composition of the organisms analyzed. The study has highlighted the genetic variation in *Staphylococcus aureus* isolates across these three locations, showing the presence of coagulase-positive, Nuc-negative S. aureus, Nuc-negative mecA-positive S. aureus, cefoxitin-resistant mecA-positive S. aureus, among others.

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