

Molecular Epidemiological Study on Panton Valentine Leukocidin (PVL) Positive Staphylococcus Aureus Isolates from Wound Samples in Some States of North-Western Nigeria

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Abstract- The aim of this study was to molecularly detect *mecA* gene and panton valentine leukocidine (*pvl*) gene in *Staphylococcus aureus* isolates from wound samples in some states of north-western Nigeria. **Methodology:** A total of 380 *Staphylococcus aureus* isolates from wound samples were studied for *Staphylococcus aureus* specific gene, *mecA* gene and *pvl* gene using multiplex PCR assays. **Molecular analysis** was performed on these *Staphylococcus aureus* isolates. **Results:** Out of the 380 *Staphylococcus aureus* that were subjected to multiplex PCR to detect *Staphylococcus aureus* specific gene all 380 isolates (100%) were positive for *Staphylococcus aureus* specific gene, 10 (6.4%) were *mecA* positive and 1 (0.64%) was *pvl* positive. The 10 *mecA* positive *Staphylococcus aureus* were sequenced and Blastn and the result showed a phylogenetic analysis of our 10 *mecA* positive *Staphylococcus aureus* when compared with 11 isolates from other parts of the world, submitted to the gene bank by other researchers. **Conclusion:** Based on the presentation of our phylogenetic tree it was shown that the isolates were grouped into two. One of the groups (clades) include four of our studied isolates, while the other group (clade) include six of our study isolates. The second group which content six of our isolates grouped together with an isolate from Tami Nadu India, the remaining grouped with other isolates that are from USA, Netherland, Tami Nadu India and Japan. **Recommendations:** Screening for MRSA for patients with wound infection is highly suggested in our clinics to mitigate the spread of this bacterium. Considering the relatedness of our (MRSA) that is, the 10 *mecA* positive *Staphylococcus aureus* isolates with isolates from other parts of the world, it is therefore, necessary to take serious action in treating an infection caused by this dangerous organism in any part of the world in order to stop its spread to other parts of the world.

Indexed Terms: *Staphylococcus Aureus*, MRSA, Meca Gene, Pvl Gene, Phylogenetic Tree.

I. INTRODUCTION

The genus *Staphylococcus* consists of both pathogenic and non-pathogenic organisms (Andreacarola et al., 2024). They are gram positive cocci and do not produce spore, but are highly resistant to dehydrating, hence highly versatile and adoptable pathogen, causing a range of infections of variable severity, affecting the skin; this can manifest in various ways, including small benign boils, folliculitis, impetigo, cellulitis, and more severe, invasive soft-tissue infections, respiratory tract infections, bones, joints and endovascular tissue infections (Tabaja et al., 2021).

There are 32 species and 15 subspecies of *Staphylococcus* (Shahmoradi et al., 2019). The three major species include *S. aureus*, *S. epidermidis* and *S. saprophyticus*. The three species can be separated from each other based on colonial morphology, Gram's stain reaction, cultural and biochemical characteristics (Li et al., 2018). The biochemical tests include catalase, coagulase, growth characteristics and fermentation of mannitol, and resistance or susceptibility to antibiotics (Tabaja et al., 2021). *Staphylococcus aureus* is considered the pathogenic strain causing different range of diseases in humans (Fu et al., 2020). The most notable feature of *S. aureus*

is its ability to acquire resistance to antibiotics; many resistance genes are acquired by plasmid mediated gene transfer and some may be transferred to the chromosome as mobile genetic elements (Li et al., 2019).

Between 25% and 35% of healthy individuals carry *S. aureus* on the skin or mucous membrane especially anterior nares, any injury to the epithelial integrity, trauma, medical or surgical interventions as well as viral infections, can predispose to tissue invasion by *S. aureus* (Abdullahi and Iregbu, 2018). A major difficulty for the management of infections caused by *S. aureus* is the development of antibiotic resistance in the isolates (Li et al., 2018). This resistance tendency is as a result of methicillin-resistance by *S. aureus* bringing about the term methicillin-resistant *Staphylococcus aureus* (MRSA), are bacteria that are resistant to penicillinase stable semi-synthetic penicillins such as methicillin, nafcillin, oxacillin and cloxacillin that are used for treatment of infection due to *S. aureus* (Fu et al., 2020). Methicillin-resistant *Staphylococcus aureus* first emerged in 1961 and for the first 25 to 35 years was endemic as hospital associated (HA-MRSA) (Idris et al., 2019). Colonized and infected patients are the most important reservoir of MRSA strains in hospitals. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a largely nosocomial pathogen, but later on, it has been seen with increasing frequency in the community, which report came about in 1982 and in the early 1990s giving rise to what is called the community acquired methicillin resistant-*Staphylococcus aureus* (CA-MRSA) (Turner et al., 2019). The deaths from necrotizing pneumonia of four young children without underlying healthcare-associated (HCA) risk factors that brought CA-MRSA to world-wide fame in the late 1990s (Turner et al., 2019).

The resistance to antibiotic happens when the organism has a *mecA* gene producing an altered penicillin binding protein (PBP 2a) (Cheung et al., 2021). The presence of *MecA* gene and either an oxacillin MIC of >2mg/L and methicillin MIC of >4mg/L, or a cefoxitin MIC of >4mg/L, are accepted criteria for resistance (Tabaja et al., 2021). The *mecA* gene encodes resistance, and expression of this gene results in production of altered penicillin binding protein. This binding protein has little affinity to

methicillin making bacteria that produce it resistant to all beta lactam antibiotics (Tabaja et al., 2021). Methicillin resistant strains usually have more than four genes encoding different resistant mechanisms (Andreacarla et al., 2024). *MecA* is a 2,130 bp fragment of DNA coding for a penicillin binding protein. The *mecA* gene is part of a mobile genetic element the staphylococcal cassette chromosome *mec* (SCC*mec*) designated I, II, III, IV and varying in size 20kb to 68kb have been described (Abdullahi and Iregbu, 2018).

Molecular techniques for the detection of *mecA* are viewed as the “gold standard” for determining resistance, polymerase chain reaction (PCR) amplification of staphylococcal *mecA* gene is thus very important (Kronman et al., 2017). Agarose Gel Electrophoresis (AGE) has been reported to be the most reliable strain typing method available, particularly for MRSA, and is the preferred reference method (Abubakar and Suleiman, 2018). Conventional and molecular methods are used for complete identification of *Staphylococcus*. In general, a combination of two or more conventional typing techniques is used for strain identification. AGE appears to be the most objective and reliable molecular techniques that can be used (Adeiza et al., 2020).

The expression of panton valentine leukocidin (PVL) a cytotoxin produced by *Staphylococcus aureus* causes leukocyte destruction and tissue necrosis (Garoy et al., 2019). Although produced by <5% of *Staphylococcus aureus* strains, the toxin is detected in large percentage of isolates that cause necrotic skin lesions and severe necrotizing pneumonia although commonly associated with community acquired MRSA (CA-MRSA) (Garoy et al., 2019). Several outbreaks due to MSSA have also been reported. PVL, a bacteriophage encoded, bi-component, hetero- oligomeric, pore forming cytolytic toxin was initially described in 1932 (Ghanbari et al., 2017). The PVL toxin consists of two synergistic proteins, LUKS-PVL and LUKF-PVL, encoded by the PVL genes *lukS* and *lukF*, which are carried on a temperate bacteriophage (Nas et al., 2018). The PVL genes encode a cytotoxin that has multiple functions. It causes concentration-dependent necrosis and death (apoptosis) of human polymorphonuclear neutrophils (PMWs), Lysis of human monocytes and macrophages, activation of

calcium channels, and global changes in gene transcription (Fu et al., 2020).

The considerable interest in PVL is due to its involvement in severe disease among children and adults with no known exposure to health care establishment (Borg and Camilleri, 2021).

PVL gene has been associated with community acquired pneumonia (Li et al., 2018). It was also significantly linked with strains causing invasive skin syndrome like furunculosis (93%) and cutaneous abscess (50%), compared with superficial folliculitis (0%) (Li et al., 2018). PVL was observed in strains associated with infective endocarditis, urinary tract infections, toxic shock syndrome, or media stinitis, although only few strains were tested (Shahmoradi et al., 2019). There was a report of a similar relationship of PVL with skin and soft tissue infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) (Christina et al., 2020).

Molecular characterization of PVL positive *Staphylococcus aureus* have been reported worldwide, in US, in Europe, in Japan (Timothy et al., 2017). Characterization of some Egyptian strains has been studied by Christina and co-workers, (2020) but information from Africa is still very limited, although a molecular detection of PVL was reported in Southern Nigeria (Hussain et al., 2022).

Structurally, and in terms of sequence similarities, PVL is related to other leukocidins, like: LukE-Luk D and LukM-LukF-P83 in *Staphylococcus aureus* and luk F- int-luk S- int in *Staphylococcus intermedius/pseudointermedius*, and to the hlgA and hlgC related to luk S gamma- hemolysin/leukocidin locus and hlg B related to luk F (Hanitsch et al., 2020; Dangari et al., 2024).

Sequence variations within the lukSF-PV gene, as well as several PVL protein isoforms bearing non-

synonymous mutations, have been described (Ogefere et al., 2019), although their functional implications are still not clear. Several studies have explored the distribution of PVL bacteriophages and gene variants in different *Staphylococcus aureus* clones.

A variant of the PVL toxin containing an amino acid substitution has been reported by Hussain et al. (2022). Saeed et al. (2018) in their study showed the single clonality of ST 8 PVL+MRSA and the heterogeneity of CC5 PVL-MRSA including the novel ST 764 MRSA.

Wound could be a surgical wound, diabetic wound, and burn wound, ulcers, purulent skin and soft tissue wounds, traumatic wound infection (Li et al., 2018). Wound can be classified as clean wound, clean-contaminated wound, contaminated wound and dirty wound (Li et al., 2019).

II. METHODS

Staphylococcus aureus isolates and analysis. Three hundred and eighty (380) *Staphylococcus aureus* isolates were obtained from wound samples from the service laboratories of the health facilities of the three states of north-western Nigeria, were examined for *Staphylococcus aureus* specific gene, *mecA* gene and *pvl* gene. Primers targeting these three genes. *S. aureus* Forward: 5'-AATCTTTGTCGGTACATATTCACG-3, Reverse: 5'CGTAATGAGATTTTCAGTAGATAAATACA ACA-3' , *mecA* gene Forward: 5'-AAAATCGATGGTAAAGGTTGGC-3', Reverse: 5'-AGTTCTGCAGTACCGGATTTGC-3', and *pvl* gene Forward: 5'ATCATTAGGAATGTCTGGACATGATCC A-3' , Reverse: 5'-GCATCAASTGTATTGGATAGC-3', were also used as an amplification control for each reaction. After the PCR, amplicons was separated and detected by gel electrophoresis.

Primers and probes for PCR

Gene	Nucleotide sequence (primer)	Nt.. No	Nucleotide position	CG Ratio	Tm (°C)	Amplicon Size (bp)
<i>S.aureus</i>	F: 5'-AATCTTTGTCGGTACATATTCACG-3	30	3-34	10S	72°C	107

	R: 5'-CGTAATGAGATTTTCAGTAGATAATACA ACA-3'	30	112-82	45S	72°C	
<i>MecA</i>	F: 5'-AAAATCGATGGTAAAGGTTGGC-3'	22	1282-1301	5Min	95°C	532
	R: 5'-AGTTCTGCAGTACCGGATTTGC-3'	22	1814-1793	30S	95°C	
<i>PVL</i>	F: 5'-ATCATTAGGAATGTCTGGACATGATCC A-3'	32	LukS/F	80S	55°C	433
	R: 5'-GCATCAASTGTATTGGATAGC-3'	32	LukS/F	80S	55°C	

(Hanitsch et al., 2020)

III. RESULTS

A multiplex Polymerase Chain Reaction (PCR) consisting of *Staphylococcus aureus* chromosomal DNA-specific primer, specific primers targeting *Staphylococcus aureus* *mecA* gene and *Staphylococcus aureus* *PVL* gene all forward and reverse primers as indicated in table 3.2 above, was set up. A 20µl total reaction volume was prepared for each isolate's DNA samples with 5X HOT FIREPol Blend Master mix with 7.5mM MgCl₂ (Solis Biotyne) which was brought down to 1X concentration containing 1X Blend Master Mix buffer (Solis Biotyne), 1.5mM MgCl₂, 200µM of each deoxynucleotide triphosphates (dNTP) (Solis Biotyne), 25pMol of each forward and reverse primer (BIOMERS, Germany), 2 unit of HotPol DNA polymerase (Solis Biotyne), Proofreading Enzyme, 2µL of the extracted DNA, and sterile distilled water was used to make up the reaction volume mixture. Thermal cycling was conducted in a PTC 200 gradient thermal Cycler Eppendorf for an initial denaturation at 95°C for 5minutes followed by 35 amplification cycles of 30seconds at 95°C; 30 seconds at 58°C and 45 seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C.

Statistical analyses: All statistical analyses were performed using the SPSS version 22.0 software package. Data were expressed as percentage mean (%). Pearson correlation was applied to compare the association between *S. aureus* gene, *mecA* gene, *pvl* gene and gender, clinics and states. The Chi-square test was used to compare the distribution of MRSA in some states of the Northwestern Nigeria, age, gender and some other socio-demographic variables. A p-value of less or equal to 0.05 ($p \leq 0.05$) was considered statistically significant (Fu et al., 2020).

Results from the multiplex PCR of Mix1 and Mix2 showed an overall *S. aureus* specific gene of 380 (100%), *mecA* gene 10 (6.4%) and *pvl* gene 1 (0.64%). The presence of these genes detected by simultaneous presence of band in both Mix1 and Mix2. There was an overall prevalence of 6.4% MRSA from wound isolates of *S. aureus* from the three states of north-western Nigeria. The distribution among the states of *mecA* gene were as follows 5 (50.00%) *mecA* from Kano, 4 (40.00%) *mecA* from Kaduna and 1 (10.00%) *mecA* from Jigawa state making the total of 10 *mecA* positive. From the hospital the hospitals that the samples were collected, National orthopedics hospital Dala had 4 (80.00%) *mecA*, AKTH had 1(20.00%) *mecA*, ABUTH had 3 (75.00%) *mecA*, Barau Dikko Teaching Hospital Kaduna had 1(25.00%) *mecA* and FMC Birnin- Kudu Jigawa state had 1(100%) *mecA*. From Kano state, Dala had 1 (20.00%) *mecA* from SOPD, 2 (40.00%) from BPLW (Burn and Plastic Ward) and 1 (20.00%) from MMW and AKTH 1 (20.00%) *mecA*. From Kaduna ABUTH had 1 (25.00%) *mecA* from MMW, 2(50.00%) *mecA* from MSW and 1(25.00%) *mecA* from Barau Dikko Teaching Hospital FSW. From Jigawa state 1(100%) from MSW. Looking at the above, the distribution of *mecA* was not evenly among the three states, the more densely populated state had more number of *mecA*, and those that are less densely populated had less number of *mecA* gene

Table 1: Showing primers used against *Staphylococcus aureus* specific gene (107bp), MRSA, *mecA*(532bp) and *PVL*gene(433bp)

<i>Staph.aureus</i> (107bp)	<i>mecA</i> gene(532bp)	<i>PVL</i> gene(433bp)
MRSA(107bp)		
(%)	(%)	(%)

Pos380 (100)			Pos 1(0.64)
Neg 0 (0.0)	Pos156 (41.1)	Pos10 (6.4)	Neg155(99.6)
	Neg224 (58.9)	Neg146 (93.6)	

Table 2: Showing MRSA, *mecA* positive isolates, states, Hospitals and clinics where they were isolated

VARIABLES	STATES	NUMBER POSITIVE(%)
MRSA		156(41.1)
<i>MecA</i>		10(6.4)
STATES <i>MecA</i>	KANO	5(50)
	KADUN	4(40)
	A	
	JIGAWA	1(10)
HOSPITAL <i>MecA</i>	DALA	4(40)
	AKTH	1(10)
	ABUTH	3(30)
	BDTH	1(10)
	FMCBK	1(100)
CLINICS/WARD	KANO	DALA(SOPD)
S IN STATES		1(10)
<i>MecA</i>		DALA(BPLW)
		2(20)
		DALA(MW)
		1(10)
		AKTH(PSW)
		1(10)
	ADUNA	ABUTH(MMW)
		1(10)
		ABUTH(MSW)
		2(20)
		BDTH (FSW)
		1(10)
	JIGAWA	FMCBK(MSW)
		1(10)

Key: SOPD-Surgical out patients department; BPLW- Burn and plastic ward; MW- Male ward; PSW- Pediatrics surgical ward; MMW-Male medical ward; MSW-Male surgical ward; FSW-Female surgical ward.

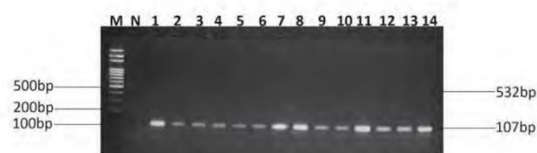


Fig.1: A representative of Agarose gel Electrophoresis showing PCR amplification Products for Staphylococcus aureus specific gene and *mecA* gene in Staphylococcus specie isolates, Lane M: A molecular weight size marker: Lane N: A negative control. Lane 1-14 are positive for Staphylococcus aureusSpecific gene as indicated by 107 base pairs. Lane 1-14 are negative for *mecA* gene.

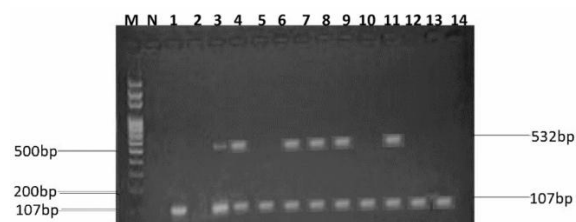


Fig.2: A representative of Agarose gel Electrophoresis showing PCR amplification Products for Staphylococcus aureus specific gene and *mecA* gene in Staphylococcus specie isolates, Lane M: A molecular weight size marker. Lane N: A negative control. Lane 1,3,4,5,6,7,8,9,10,11 and 12 are positive for Staphylococcus aureus specific gene as indicated by 107 base pair. Lane 3,4,6,7,8 and 11 is positive for *mecA* gene as indicated by 532 base pair.

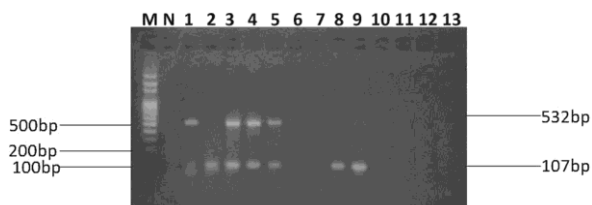


Fig.3: A representative of Agarose gel Electrophoresis showing PCR amplification Products for Staphylococcus aureus specific genes and *mecA* in Staphylococcus specie isolates. Lane M: A molecular weight size marker. Lane N: A negative control. 1,3,4 and 5 are positive for *mecA* as indicated by 532 base pair and Lane 1,2,3,4,5,8 and 9 are positive for Staphylococcus aureus specific gene as indicated by 107 base pair.

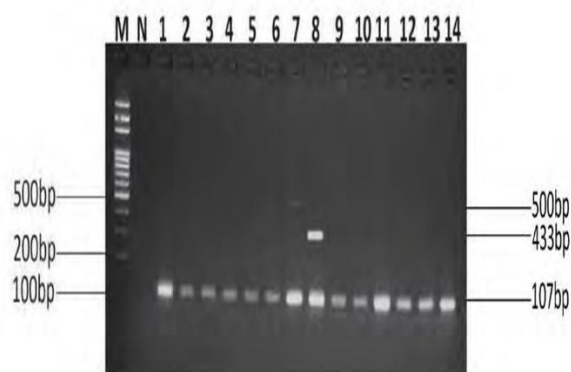


Figure 4: A representative of Agarose gel Showing PCR amplification Products for Staphylococcus aureus Specific gene and PVLgene in Staphylococcus specie isolates. Lane M: A molecular weight size marker. Lane N: A negative control. Lane 1-14 are positive for Staphylococcus aureus Specific gene as indicated by 107 base pairs. Lane 8 is PVLgene as indicated by 433 base pair

Table 3: Blastn Result for the informed MRSA Nucleotide sequences obtained from wounds isolate from some states of North-Western Nigeria

Sample Code	Best hit organism	Query cover (%)	Sequence Identity (%)	Sequence Length	Query Length	Expected Value	Gaps (%)	Accession number
I/PW/AKTH	Staphylococcus aureus 98 strains TN/CN/1/12	99.38	99.38	2107	498	0.0	0	PP_449375.1
3/A&E/NOPHD	Staphylococcus aureus 96 strains TN/CN/1/12	96.45	96.45	2107	499	0.0	0	PP_44938.1
54/A&E/NOPHD	Staphylococcus aureus 97 strains TN/CN/1/12	99.59	99.59	2107	501	0.0	0	NG_047939.1
5/O/PD/NOPHD	Staphylococcus aureus 98 strains TN/CN/1/12	95.49	95.49	2107	499	0.0	0	LC_727174.1
61/MN/NOPHD	Staphylococcus aureus 96 strains TN/CN/1/12	97.48	97.48	2107	500	0.0	0	KC_243783.1
101/MW/ABUTH	Staphylococcus aureus 98 strains TN/CN/1/12	98.99	98.99	2107	498	0.0	0	NG_047945.1
109/MW/ABUTH	Staphylococcus aureus 96 strains TN/CN/1/12	99.54	99.54	2107	501	0.0	0	PP_44938.1
112/FW/BDTH	Staphylococcus aureus 98 strains TN/CN/1/12	95.59	95.59	2107	501	0.0	0	PP_449379.1
118/MW/ABUTH	Staphylococcus aureus 97 strains TN/CN/1/12	97.48	97.48	2107	499	0.0	0	NG_047947.1
142/MW/FMCBR	Staphylococcus aureus 96 strains TN/CN/1/12	99.49	99.49	2107	498	0.0	0	NG_047937.1

Key: Blastn: Basic Local Alignment Search tool for nucleotides

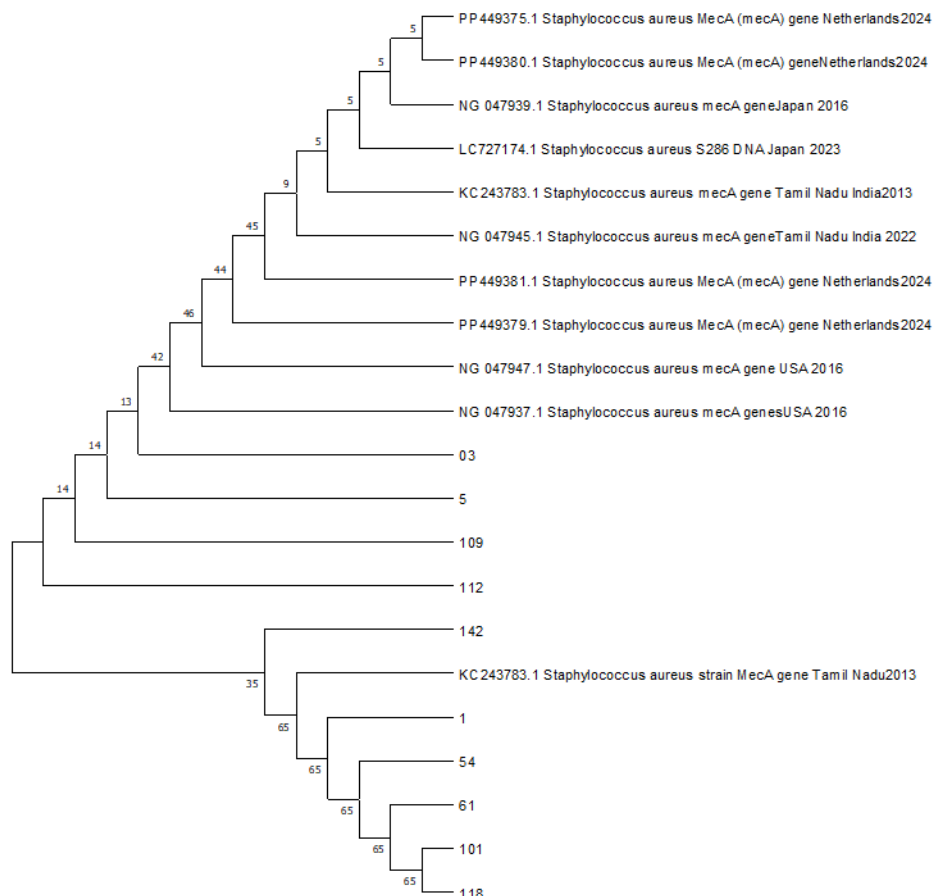


Fig 5: Phylogenetic tree presentation of the study.

Phylogenetic tree presentation of the study isolates. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (2004) and are in the units of the number of base substitutions per site. This analysis involved 21 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 466 positions in

the final dataset. Evolutionary analyses were conducted in MEGA11 (2021).

IV. DISCUSSION

Out of the 10 confirmed MRSA isolates that were sent for sequencing 10 (100%) were successfully sequenced. The nucleotide sequences of the 10 mecA positive MRSA are shown on Appendix I. The distribution of mecA among the three states was not evenly made some state had higher number of mecA positive than others for example in Kano state we had 5(50%), Kaduna state 4(40%) and the least 1(10%) came from Jigawa state (Table 2). The distribution of

mecA within the clinics in the facilities of the states studied was almost evenly distributed (Table 2). The sample codes of 10 mecA positive *Staphylococcus aureus* isolates, the best hit MRSA strains, Query cover, sequence identities, sequence length, Query length, Expect values Gaps and Accession numbers are shown on Table 3. The DNA nucleotide sequences of the confirmed MRSA isolates showed high sequence identity of 95.49-99.59%, this finding is in agreement with the findings of Soliman et al. (2020), where they had high sequence identity in their study in Cairo, Egypt of 95.45-99.67%. Pantone-valentine Leukocidin (PVL) encoding gene was detected in only one (0.64%) of our MRSA isolates this is not in agreement with the findings of Soliman et al. (2020) where they had 4 (13.5%) of MRSA carrying the PVL gene in Cairo Egypt, but in agreement with the work of Lakhundi and Zhang (2018) where they had only one (08%) isolate with PVL gene in China. Studies in some Northern parts of Nigeria shows relative increase in prevalence of *S. aureus* with PVL 10.9% of *S. aureus* isolates are discovered in Gombe state North-Western Nigeria which is much higher than what we found in this study and not in agreement with our findings. They also found out in their study that all PVL producing isolates were MDR (multidrug resistant) this observation was not made in our study owing to the few number of PVL positive case recorded Onanuga et al. (2020). In another study in Jos North Central Nigeria by Essien et al. (2021), it was also noticed that isolates from different clinical samples including wound, blood, and urine, had PVL gene detected as high as 31.1% in the study they carried out in 2021 which shows a marked increased recorded as compared to our study and another study in Gombe state North eastern Nigeria by Abubakar et al. (2020) had PVL of 26.6%, the two studies had sample sizes of 263 and 214 respectively almost similar to our sample size but, their results not in conformity with our findings, this disparity could be as a result of methodology, while they used ELISA to detect PVL antibodies, we carried out PCR assay to detect the PVL gene.

Panton-valentine Leukocidin (PVL) encoding gene was detected in only one of our MRSA isolates this is not in agreement with the findings of Soliman et al. (2020) where they found four MRSA carrying the PVL gene, but in agreement with the work of

Lakhundi and Zhang (2018) where they had only one isolate with PVL gene.

The phylogenetic study of our isolates showed 21 isolates in all, 10 are our isolates and the remaining were isolates submitted to the gene bank by other researchers. Based on the presentation of our phylogenetic tree it showed the isolates were grouped into two. One of the groups (clades) includes four of our study isolates, while the other group (clade) includes six of our study isolates. The second group which contained six of our isolates grouped together with an isolate from Tami Nadu India, the remaining grouped with other isolates that are from USA, Netherland, Tami Nadu India and Japan.

CONCLUSION

In conclusion, MRSA prevalence of 6.4% (mecA) and PVL of 0.64% were obtained in this study which agrees with the rates obtainable in other studies in this area by many researchers. Pantone Valentine Leukocidine (PVL) was positive in one (0.64%) of MRSA isolates in this study, showing that there is danger signal in confronting necrosis of the skin and soft tissues if this virulent gene keeps spreading among patients suffering from wound infections with MRSA with PVL. There could be more of PVL MRSA, when similar research is carried out in future, considering larger sample size and more study area. In this study evolutionary relatedness was carried out through phylogenetic tree, we found out that some of the *Staphylococcus aureus* strains that were confirmed to possess mec A gene had evolutionary relationship with strains in some other parts of the world example Tami-Nadu in India, USA, Netherlands, and Japan. That shows how the world has become a global village; therefore, an outbreak of disease in one part of the world should be a thing of concern to everybody in other parts of the world

REFERENCES

- [1] Abdullahi, N. and Iregbu, K.C. (2018).Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital. *Annals of Tropical Pathology*, 9:6-10.

- [2] Abubakar, S. and Sulaiman, A. S. (2018): Prevalence, trend and antimicrobial susceptibility of Methicillin resistant *Staphylococcus aureus* in Nigeria. A systematic review, *Journal of Infection and Public Health*. 11 (2018): 763-770. [Crossref]
- [3] Adeiza, S. S., Onalapo, J. A. and Olayinka, B. O. (2020): Prevalence, risk factors and antimicrobial susceptibility profile of Methicillin resistant *Staphylococcus aureus* obtained from nares of Patients and staff of Sokoto state owned Hospitals in Nigeria. *G. M. S. Hygiene and Infection Control*. (15): 2196-5226. [Crossref]
- [4] Andreacarola, U., Ian, R. M., Ying-Tsun, C., Camilla, P. and Alice, S.P. (2024) *Staphylococcus aureus* adapts to exploid collagen-derived proline during chronic infection. *Nature Microbiology*. (9):2506-2521.
- [5] Borg, M. A. and Camilleri, L. (2021): What is driving the Epidemiology of Methicillin resistant *Staphylococcus aureus* Infections in Europe? *Microbiological Drug Resistance*, 27: 889-994. [Crossref]
- [6] Cheung, G.Y.C., Bae, J. S. and Otto, M. (2021) Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*. 12(1):547–569.
- [7] Christina, J. H., Gracen, R. G., Lucas, D. S., Vishwash, P., and Tara C. S. (2020) *Staphylococcus aureus* Epidemiology in Wildlife: A Systematic Review. *Antibiotics* 9(2): 89.
- [8] Dangari, M. A., Sunusi, A. A., Salihu, K. M. and Ado, A. (2024): Phenotypic and Molecular Detection of MRSA isolated from clinical samples in some selected Hospitals in DutsinMa and Kurfi Local Government Area, Katsina State. *Arid Zone Journal of Basic and Applied Research*. Volume 3 (3): 133-144.
- [9] Fu, Y., Xiong, M., Li, X. (2020) Molecular characteristics, antimicrobial resistance and virulence gene profiles of *Staphylococcus aureus* isolates from Wuhan, Central China. *Infection and Drug Resistance*. ;13:2063–2072.
- [10] Garoy, E. Y., Gebreab, Y. B., Achila, D. G. T., Keset, R., Ghirmay, R. and Yesfu, T. (2019): Methicillin resistant *Staphylococcus aureus* Prevalence and antimicrobial sensitivity pattern among Patients – A multicenter study in Asmara, Eritrea. *Canadian Journal of Infectious Diseases and medical Microbiology*. Vol. 2019,
- [11] Ghanbari, F., Saberianpour, S., Zarkesh-Eshfahani, F., Ghambari, N., Taraghian, A. (2017): *Staphylococcal Cassette Chromosome mec (SCCmec) Typing of Methicillin-Resistance Staphylococcus aureus Strains isolated from Community and Hospital-acquired Infections*. *Avecenna Journal of Microbial Infections*, 2017; 4 (2): 42244. [Crossref]
- [12] Hanitsch, L. G., Kruger, R., and Hoppe, P. A. (2020): Out-patient decolonization after recurrent skin infection with panton valentine leucocidin (PVL) producing *S. aureus*: The importance of treatment repetition, *PLoS one* 2020; 15 (4): e0231772 [Journal]
- [13] Hussain, K., Bandyopadhyay, A., and Roberts, N. (2022): Panton-valentine leucocidin producing *Staphylococcus aureus*: a clinical review *Experimental Dermatology*. 2022; 47 (12): 2150-8
- [14] Idris, M. A., Kumurya, A. S., Mohammed, Yusuf. And Mustapha, M. H. (2019). Phenotypic determination of methicillin-resistant *Staphylococcus aureus* in Aminu Kano Teaching Hospital, Kano, Nigeria. *Nigerian Journal of Experimental and Clinical Biosciences* DOI 10.4103/njecp.njecp-20-18.
- [15] Kronman, M. P., Zaoutis, T. E., Haynes, K., Feng, R., Coffin, S.E. (2017). Antibiotic Exposure and IBD Development among Children: A Population-based Cohort Study. *Pediatrics*, 130 (4): 794-803
- [16] Li, X., Fang, F., Zhao, J. (2018) Molecular characteristics and virulence gene profiles of *Staphylococcus aureus* causing bloodstream infection. *Brazilian Journal of Infectious Diseases*. ;22(6):487–494.
- [17] Nas, F. S., Yahaya, A., Zage, A. U., Garba, K. A. and Ali, M. (2018): Characterization and Evaluation of Antibiotic Susceptibility Pattern of Clinical isolates of Methicillin resistant *Staphylococcus aureus* at some Tertiary Hospitals in Kano, Nigeria. *International Journal of Research Studies in Microbiology and*

Biotechnology, Volume 4, Issue 3: 38-44.
[Crossref]

- [18] Ogefere, H. O., Umaru, G., Ibadin, E. E. and Omoregie, R. (2019): Prevalence of Methicillin resistant Staphylococci among apparently healthy Students attending a tertiary Institution in Benin City, Nigeria. *Nigerian Journal of basic and applied Science* (June, 2019), 27 (1): 114-121. [Crossref]
- [19] Saeed, k., Gould, I., and Esposito, S. (2018): Panton-valentine leucocidin-positive Staphylococcus aureus: a position statement from the international society of chemotherapy. *International Journal of antimicrobial agents*. 2018; 51 (1):16-25
- [20] Shahmoradi, M., Faridifar, P., Shapouri, R. (2019) Determining the biofilm forming gene profile of Staphylococcus aureus clinical isolates via multiplex colony PCR method. *Rep Biochem Mol Biol*. 7(2):181–188.
- [21] Tabaja, H., Hindy, J. R., Kanj, S. S. (2021) Epidemiology of methicillin-resistant Staphylococcus aureus in Arab Countries of the Middle East and North African (MENA) Region. *Mediterranean Journal of Hematology and Infectious Diseases*. Timothy J. F. (2017) Antibiotic resistance in Staphylococcus aureus. Current status and future prospects *FEMS Microbiology Reviews*. 41 (3): 430–449
- [22] Turner, N.A., Sharma-Kuinkel, B.K., Maskarinec, S. A. (2019) Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *National Review Microbiology*. 17(4):203–218.