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Research Article

Prevalence and Antibiogram Studies of Hospital Aquired Methicillin Resistant *Staphylococcus aureus* from Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main causative agents of nosocomial and environmental infections which pose a major threat to health-care delivery. The aim of this study was to determine the prevalence and antibiogram studies of Hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) isolates from Irrua Specialist Teaching Hospital (ISTH). A total of 310 nasal, fomite and wound swabs were collected from different departments in ISTH. Swabs were cultured on Mannitol salt agar and incubated at 37°C for 24 hours for presumptive growth of *Staphylococcus aureus*. Gram staining and biochemical tests were conducted and the isolates were subcultured on Oxacillin Resistant Screening Agar Base for growth of MRSA. These were further screened for methicillin resistance by subjecting isolates through Oxacillin single disc and other classes of antibiotics. Molecular studies was done using the polymerase chain reaction to target some genes, using specific primers to detect, *nuc, mecA, blaZ, pvl* and SCC*mec*. Isolates were assayed for some virulent factors comprising biofilm, haemolysin and DNase. Results from this study revealed that HA-MRSA had a prevalence of 27%. On the distribution of isolates within ISTH and according to specific source, the prevalence in decreasing order were fomites (32%), cleaners (24%), nurses (19%), patients (13%), and doctors (12%). The results revealed that 38% of HA-MRSA possessed the *nuc* gene. Of the three genes amplified on all isolates, 62% possessed the *mec*A gene, 43% had *blaZ,* gene while 10% had *pvl* gene. Findings from this study shows that 12% of the isolates had CA-MRSA associated SCC*mec* IV. From the distribution of the SCC*mec* and *pvl*, it is evident that there is a drift of genetic material influx from CA-MRSA to HA-MRSA strains. Inference from this study also shows that some MRSA isolates possessing the targeted genes correlates with HA-MRSA isolates with higher MAR index. Results revealed that 96% of the HA-MRSA were resistant to cloxacillin, while varying percent of the isolates were resistant to ciprofloxacin, gentamincin, tetracycline and erythromycin. Vancomycin and Linezolid were the best drug of choice. The multiple antibiotic resistant (MAR) index shows that some isolates had greater MAR index ranging between 0.2 - 0.4 and MAR index > 0.2 is a high risk source of antibiotic usage. In conclusion, most of the isolates recovered in this study had high MAR index, which is an indication of antibiotic overuse within the hospital sampled. Hence, the need for strict antibiotic stewardship.

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Keywords

Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA), Antibiogram, *mecA* Gene, SCC*mec*

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium, which are commonly found on the surfaces of the human skin and mucous membranes, and it can also be carried asymptomatically for a long time on mucous membranes. It is a major cause of hospital acquired infections particularly in colonized humans [1].

Some strains of S. aureus have acquired resistance to antimicrobial agents and their prevalence within the hospital is of potential epidemiological threat. Accumulation of resistance factors by S. aureus has rendered the bacterium resistant to a variety of commonly used antibiotics, thus increasing the ability of S. aureus to survive in hostile environments [2]. S. aureus was initially reported resistant to penicillin when penicillinase-producing S. aureus strains were detected. This transited to the development of penicillinase-resistant semi-synthetic penicillins, such as methicillin and oxacillin in 1961 to treat Penicillin resistant S. aureus. Unfortunately, Methicillin-resistant S. aureus (MRSA) was reported 2 years after its introduction in the United Kingdom [3]. MRSA refers to strain of S. aureus that is resistant to a large group of antibiotics, called "beta-lactams' and even strains resistant to vancomycin and ciprofloxacin have equally emerged [4]. MRSA is one of the major causes of infections in humans, occurring in both the community and hospital. It causes skin infections, osteoarthritis and respiratory tract infections. They also causes abscess in deep organs, and is responsible for toxin mediated diseases.

Infections caused by MRSA have been found to be responsible for increased length of hospital stays, rising health care costs, and a high mortality rate. Carriers of MRSA are also prone to septicemia, wound infections and the occasional toxic shock syndrome [5]. The researcher reported that acquisition of MRSA has been associated with two different environments: Community-acquired MRSA (CA-MRSA) and healthcare-acquired MRSA (HA-MRSA) [6]. MRSA poses a serious therapeutic problem in Nigeria because there is limited information on mechanism of their emergence and spread in healthcare environments. Therefore, the characterization of MRSA isolates provides base line information needed to implement strategies for the effective management of its carriage. HA-MRSA is a major global challenge due to the presence of multiple drug resistant genes and colonization has been identified as a major risk factor which influences the rapid spread of this MRSA. This present study was conducted to evaluate the prevalence, antibiogram and virulence factors associated with hospital acquired-methicillin resistant *Staphylococcus aureus* (HA-MRSA) from fomites, staff and patients of Irrua Specialist Teaching Hospital, Irrua, Nigeria.

2. Materials and Methods

2.1. Study Site

The study was carried out inIrrua Specialist Teaching Hospital, Irrua, Edo State.

Irrua Specialist Teaching Hospital (ISTH) is a Federal Government of Nigeria Teaching Hospital located in Irrua, Edo State Nigeria. Irrua is administrative headquarters of Esan Central Local Government Area. It is located at Latitude 5°15 48°E. It has a population of about 40,000 people whose major occupation includes Farming, Teaching, Civil services and Trading [7].

The hospital is less than 2km from Ekpoma town and serves as the teaching hospital to Ambrose Alli University Ekpoma medical programme.

2.2. Study Population

The study population for the isolation of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) comprised Doctors, Nurses, Patients, Cleaners, and fomites from ISTH, Irrua.

2.3. Determination of Sample Size

According to [8], sample size was determined using the formula;

$$N = \frac{P(100-P)Z}{E^2}$$

Where:

N= required sample size

P= Percentage occurrence of the event (30%; Ogefere and Lawrence, 2019)

E= Percentage error required (5%)

Z= Statistical value corresponding to confidence level (95%) which is 1.96

Inputing this Figures into the formula,

$$N = \frac{30(100-30)\ 1.96}{5^2}$$

N= 164 samples for the study population.

However, a total number of 310 samples was used for this study.

2.4. Ethical Approval

Before the commencement of this study, ethical approval was obtained from the ethical committee of ISTH, Irrua.

2.5. Sample Collection

A total number of 310 nasal, wound and fomite samples were collected from ISTH, Irrua for HA-MRSA. Samples were collected from personnels and fomites in 14 major clinic departments/wards of the hospital. From each department, 20-25 samples were collected from doctors, nurses, cleaners, patients and fomites, depending on the shift running at the time of sample collection.

From each department/ ward, sterile swab sticks (oxoid) were used to aseptically collect Nasal and wound samples from Doctors, Nurses, Cleaners, Patients and also samples were swabbed from Fomites. These samples were immediately taken to the laboratory for culture.

2.5.1. Isolation of *Staphylococcus Aureus*

Nasal, wound and fomite samples were streaked aseptically on Mannitol salt agar and incubated at 37°C for 24 hours. Presumptive growth of *Staphylococcus aureus* (isolates that changed color of the media from pink to yellow) were further subjected to Gram staining and biochemical tests, which included Catalase, Coagulase, Citrate, Indole, Oxidase, Urease and Sugar utilization test.

2.5.2. Isolation of MRSA/Antibiogram

The *Staphylococcus aureus* isolated following biochemical identification were smeared on the surface of Oxacillin Resistant Screening Agar Base (ORSAB) medium which is selective for the isolation of MRSA. MRSA isolates were identified on ORSAB by change in the colour of the medium from pale blue to deep blue.

Presumptive MRSA isolates from ORSAB were further probed for methicillin resistance by using the disk diffusion method. Briefly, a 0.5 McFarland turbidity standard match of the isolates were flooded on a 5% NaCl Mueller Hinton agar, it was allowed to dry a little and the 1µg of Oxacillin single disc was placed on the surface of the plates and incubated at 37°C for 24 hours. After 24 hours' incubation, zones of clearance were measured according to guidelines of [9] interpretative chart to screen for MRSA.

The HA-MRSA isolates were further screened through a panel of other antibiotics comprising of single disk Vancomycin (1µg) and Linezolid (1µg), multiple disk of Cloxacillin (5µg), Ciprofloxacin (5µg), Gentamycin (5µg), Tetracycline (10µg) and Erythromycin (5µg). This was carried out by preparing a 0.5 McFarland standard match of the MRSA isolates which was used to flood the surface of 5% NACL Mueller Hinton agar. It was shaken around and the excess fluid was decanted into disinfectant jar. It was allowed to dry before the antibiotic disks were placed on the surface of the media and incubated at 37°C for 24 hours. After 24hours incubation, plates showing zones of inhibitions were read and measured according to guidelines of [9] interpretative chart to screen for resistant isolates.

2.5.3. Determination of Multiple Antibiotics Resistant (MAR) Index

Research revealed that MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to [10]. A MAR index greater than 0.2 means that there is high risk source of resistance where antibiotics are frequently used. Therefore, the formula for calculating MAR index which is a/bxc.

Where "a" represents the number of antibiotics to which an isolate was resistant to, "b" represents the total number of antibiotics tested and "c" represents total number of isolates collected from the sample source. All isolates were subjected to this ratio.

2.6. Assay for Biofilm Formation

All HA-MRSA isolates were assayed for biofilm formation. Biofilms are aggregates of microorganisms suspended in a matrix of extracellular polymeric substances attached to a surface. Possession of biofilm by bacteria gives them survival chances by making them resistant to antibiotics and evading host cells.

Adopting the technique used by [10], the Congo red agar method was used in this study to detect biofilm formation. Briefly, Congo red stain was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 min. It was then added to autoclaved Brain heart infusion agar with sucrose at 55°C. Plates of these reparations were inoculated with MRSA isolates and incubated at 37°C for 24 hours aerobically. After incubation, black colonies that looked crystalline indicated biofilm formers, while colonies that were not too dark indicated weak biofilm formers and finally, colonies without colour change were indicated as non-biofilm formers.

2.6.1. Assay for Haemolysin Production

Haemolysin are lipids and proteins produced by some bacteria that causes lysis of the red blood cells by disrupting the cell membrane.

Some pathogenic bacteria produce this haemolysin to derive nutrient from lysing of red blood cells of the host and evade host destruction, hence enhancing their virulent characteristics.

The blood agar test as reported by [11] was perform to detect haemolysin production in the MRSA isolates. Briefly, 5% sheep blood agar (Oxoid) plates were prepared according to

the manufacturer's instructions. The HA-MRSA isolates were streaked on the surface of each blood agar and the plates were then incubated at 37°C for 24 hours. After 24 hours' incubation, plates showing a clear zone of clearance around the streaked bacterium, indicated positeve for haemolysin production.

2.6.2. Assay for Dnase Production

Deoxyribonuclease (Dnase) is a short group of glycoprotein endonucleases which are enzymes that catalyzes the hemolytic cleavage of phosphodiester linkage in the DNA backbone, thus degrading DNA.

According to methods used by [12], the test was carried out by aseptically streaking the test isolates on Dnase agar (prepared according to manufacturer's instruction) using a sterile wire loop. Thereafter, plates were incubated at 37°C for 24 hours. After incubation, plates were observed for color change. Medium that appeared colorless around the test organism were reported as being positive to Dnase production while medium that remained green around the test organism were reported to be negative to Dnase production.

2.7. Genomic Deoxyribose Nucleic Acid (DNA) Extraction Protocol

Genomic DNA extraction was carried out as described by [12]. Single colonies of Methicillin Resistant *Staphylococcus aureus* isolates grown on Brain Heart Infusion agar was transferred to 1.5 mL of Brain Heart Infusion broth and cultures were grown on a shaker water bath for 48 hours at 28°C. Thereafter, cultures were centrifuged at 4600 rpm for 5 minutes. The resulting pellets were each re-suspended in 520 µL of TE buffer, and 5 µL of 20% SDS and 3 µL of Proteinase K (20mg –ML^{-1}) were added. The mixture was incubated for 1 hour at 37°C, then 100 µL of 5M NaCL and 80 µL of a 10% cetyltrimethyl ammonium bromide (CTAB) solution in 0.7M NaCl were added and mixed. The suspension was incubated for 10 minutes at 65°C and kept on ice for 15 minutes before centrifugation at 7200 rpm for 20 minutes. The aqueous phase was transferred to a new tube followed by addition of isopropyl (1: 0: 6) and DNA was precipitated at -20°C for 16 hours. DNA was collected by centrifugation at 7200 rpm for 10 minutes, washed with 500 µL of 70% ethanol, air dried at room temperature for approximately 3hours and finally dissolved in 50 µL of TE buffer. The genomic DNA was stored at -20°C and used for molecular identification and bacterial typing protocols.

2.8. Amplification of mecA, blaZ, nuc, and pvl Genes

The detection of these genes was carried out as described by [12]. The 25.0 µL volume of PCR reaction mixture contained a 1.0 µL of template genomic DNA, 12.5 µL of PCR master mix, 7.5 µL PCR H_2O and 2 µL each of the primers. DNA amplification was carried out for 40 cycles according to the following protocols: denaturation at 94°C for 30 seconds and annealing at 55°C for 30 second; extension at 72°C for 1 minutes with a final extension at 72°C for 5 minutes and cooling at 4°C. Electrophoresis of amplicons were performed with 1% agarose gel containing ethidium bromide (Et Br) 0.5 mg L^{-1}, for 1 hour at 100 V in 0.5 x TAE buffer (40mM Tris-HCL, 20 mM Na-acetate, 1mM EDTA, pH 8.5) and visualized under a UV trans illuminator.

2.9. Amplification of Staphylococcal Cassette Chromosome (SCC) mec I, II, III, IV, and V Genes

The PCR detection of the SCC *mec* I, II, III, IV and V was carried out as described by [10]. The 25.0µL volume of PCR reaction mixture contained a 1.0µL of genomic DNA, 12.5µL of PCR master mix, 7.5µL PCR H_2O and 2µL each of the SCCmec primers. DNA amplification was carried out for 45 cycles according to the following protocols; denaturation at 94℃ for 30s, extension at 72℃ for 1mins with final extension at 72℃ for 5mins and cooling to 4℃. Electrophoresis of amplicons was performed with 1% agarose gel containing ethidium bromide (EtBr) 0.5$mgL^{-1,}$ for 1 hour at 100V in 0.5×TAE buffer (40Mm Tris-HCL, 20Mm Na-acetate, 1mm EDTA, PH8.5) and visualized under a UV transilluminator.

Table 1. *Primers Used for the Amplification of Relevant Genes.*

Gene Target	Sequence (5'⟶3')	Product Size (bp)	Reference
nuc- F	TAATCCAAGAGCAATAAGGGC	227	[12]
nuc-R	GCCACACTATCATAACCACTA		
blaZ-F	ACTTCAACACCTGCTGCTTT	173	[12]
blaZ-R	TGACCACTTTTATCAGCAAC		
pvl-F	CTTGTTGATCACGATAATTTCC	167	[12]
pvl-R	ATCTTTTAGCAAACCCGTATTC		

Gene Target	Sequence (5'⟶3')	Product Size (bp)	Reference
mecA-F	GGCACAATAAGAGTGTTTAAAGG	940	[12]
mecA-R	AGTTATATCATGAATAGATTGTCCTGTT		

Table 2. *Primers Used for the Amplification of the Staphylococcal Cassette Chromosome (SCC) mec I, II, III, IV, and V Genes.*

SSCmec TYPE	ORIENTATIONS	OLIGONUCLEOTIDE SEGMENTS (5' -3')	SIZE OF BASE PAIR
Type I	Forward	GCTTTAAAGAGTGTCGTTACAGG	613
	Reverse	GTTCTCTCATATAGTATGACTCC	
Type II	Forward	GATTACTTICAGAACCAGGTCAT	287
	Reverse	TAACTGTGTCACACGATCCAT	
Type III	Forward	CATTIGTGAAACACAGTACG	243
	Reverse	GTTATTGAGACTCCTAAAGC	
Type IV	Forward	GCCTTATTCGAAGAAACCG	776
	Reverse	CTACTCTTCTGAAAAGCTCG	
Type V	Forward	GAACATTGTTACTTAAATGAGCG	325
	Reverse	TGAAAGTGTATAAATGAGCG	

3. Results

3.1. Prevalence of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) in the Studied Population

Table 3. *Prevalence of HA-MRSA among the Studied Population.*

Sample obtained from: n = 310	No of Samples	No (%) positive for HA-MRSA
Hospital HA-MRSA	310	82 (27)

Key:
HA-MRSA: Hospital Acquired Methicillin Resistant *Staphylococcus aureus*

An overall prevalence of 27% HA-MRSA isolate was recorded in this study comprising of 14 different wards and clinics in ISTH. That is of the 310 samples obtained from the hospital, HA-MRSA isolates were 27% (Table 3).

3.2. Identification of MRSA Isolates

The results showed that all MRSA isolates grew as yellow colonies on mannitol salt agar, were Gram positive cocci in clusters and were resistant to oxacillin. Biochemical characteristics of these isolates shows that all were catalase positive, coagulase positive, oxidase negative, citrate positive, indole variable, urease positive and were able to utilize the sugars glucose, mannitol and lactose (Table 4).

Table 4. *Characterization of the Staphylococcus aureus Isolates.*

Identification Criteria	Result
Gram reaction	+
Catalase	+
Coagulase	+

Identification Criteria	Result
Oxidase	-
Citrate	±
Indole	+
Urease	+
Mannitol	+
Glucose	+
Lactose	+
Growth on MSA	+
Resistance to Oxacillin	+

Key:
+ = positive; - = negative; ± = variable; MSA = Mannitol Salt Agar

3.3. Occurence of Virulent Factors and House Keeping Genes of HA-MRSA Isolates

Table 5 shows the occurence results of the MRSA isolates on haemolysin production 75 (56%), DNAse production 44 (33%), biofilm formation 69 (52%) as well as presence of *nuc* gene 62 (47%), *pvl*gene 18 (14%), *mecA* 75 (56%), *blaZ* 51 (38%) and *SCCmec* types I, II, III (Hospital lineage), 78 (59%) and*SCCmec* IV-V (Community lineage) 38 (29%) genes.

Table 5. *Occurrence of virulent factors and house keeping genes in HA-MRSA Isolates (n=82).*

Characteristic feature	Number of positive isolates (%)
Production of Haemolysin	52 (63)
Prouction of Dnase	27 (33)
Biofilm formation	46 (56)
Presence of *mecA* gene	51(62)
Presence of *blaZ* gene	34 (42)
Presence of *pvl* gene	8 (10)
Presence of *nuc* gene	27 (33)
SCCmec I – III	69 (84)
SCCmec IV – V	31 (36)

Key:
SCC*mec*: Staphlococcal Cassette Chromosome *mec*
Dnase: Deoxyribonuclease

3.4. Distribution of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) According to Subjects and Hospital Departments

A total of 82 (27%) of the 310 nasal, wound and fomite samples yielded HA-MRSA isolates. Of the 82 isolates, 11 (13%) was recovered from male surgical ward, 9 (11%) each from female surgical ward and laundry, while the least number of MRSA isolates 3(4%) were recovered each from Mother and Child Health (MCH) department and Intensive Care Unit (ICU) (Table 6). In addition, the highest number of the MRSA isolates were recovered from fomites (32%), followed by cleaners (24%), nurses (19%), patients (13%) and the least from doctors (12%) (Figure 1).

Table 6. *Distributions and Prevalence of Hospital Acquired Methicillin Resistant Staphylococcus aureus (HA-MRSA) Isolated in ISTH.*

	Number of MRSA Isolates Recovered from:					
DepartmentNo of set	Doctors	Nurses	Patients	Cleaners	Formites	Total No (%)
SCBU	0	1	0	1	2	4 (5)
Gynecology	1	1	1	0	2	5 (6)
Lassa	1	0	2	2	1	6 (7)
MCH	0	0	1	0	2	3 (4)
Ophthalmology	1	2	0	0	3	6 (7)
Female Surgical	2	1	2	2	2	9 (11)
ICU	0	1	0	1	1	3 (4)
Pediatrics	1	1	1	0	1	4 (5)

Number of MRSA Isolates Recovered from:						
DepartmentNo of set	**Doctors**	**Nurses**	**Patients**	**Cleaners**	**Formites**	**Total No (%)**
Labour wards	1	2	1	1	2	6 (7)
Post-natal	1	0	1	1	1	4 (5)
A & E	1	2	1	1	2	7 (9)
Male surgical	1	3	1	3	2	11 (13)
Laundry	-	-	-	6	3	9 (11)
Radiology	0	2	0	1	2	5 (6)
Total (%)	10 (12)	16 (19)	11 (13)	19 (24)	26 (32)	82 (100)

Key: SCBU =Special Care Baby Unit; MCH = Mother and Child Health; ICU = Intensive Care Unit; A&E= Accident and Emergency; MRSA: Methicillin Resistant *Staphylococcus aureus*

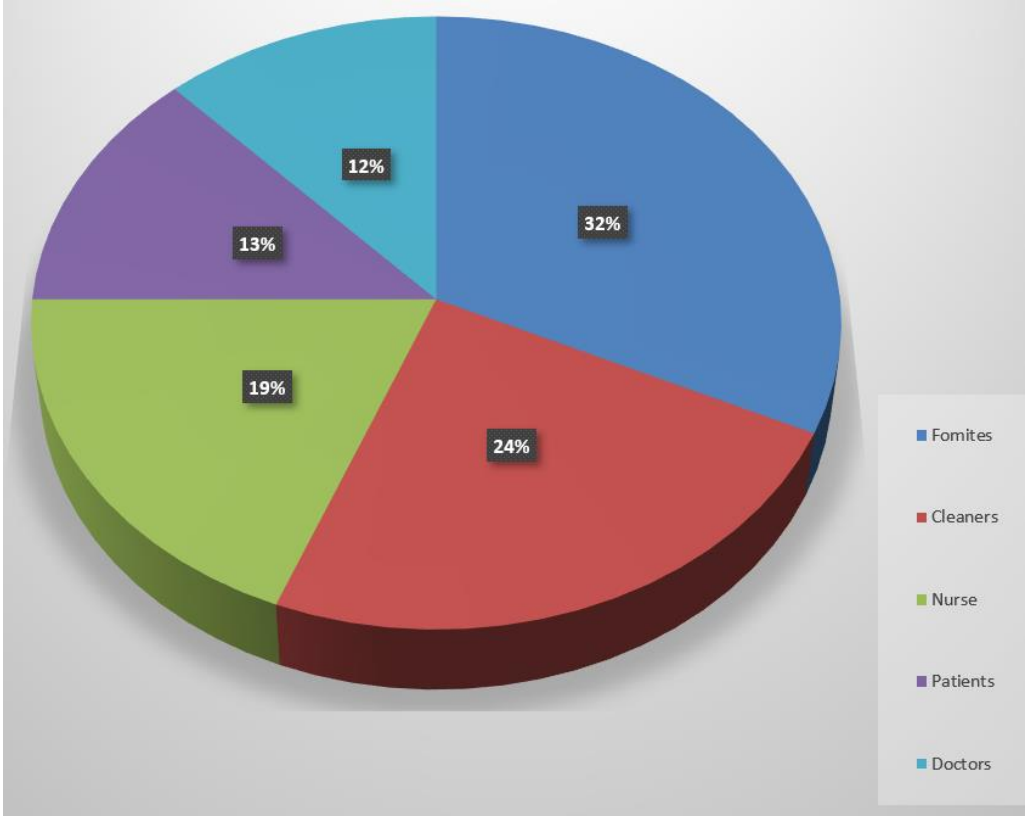

Figure 1. *Distribution of HA-MRSA amongst the Subjects in ISTH.*

3.5. Antibiogram of HA-MRSA Isolates

The antibiogram of all the 82 HA-MRSA isolated from ISTH revealed that vancomycin and linezolid are the best drugs of choice for the treatment of HA-MRSA infections. All 82 isolates were sensitive to 1*ug* of vancomycin and linezolid, while for cloxacillin, 92% of the isolates were resistant. The results show that forciprofloxacin, 41% of the isolates were resistant to the antibiotic, while for Tetracycline only 9% of the isolates were resistant to the antibiotic. Gentamycin had a total of 45% of isolates resistant to it and, Erythromycin had 26% of isolates resistant (Table 7).

Table 7. Antibiogram of HA-MRSA Isolates.

No of MRSAN=82	Antibiotics Susceptibility to;						
		Van	Lin	Clox	Cipro	Tetra	Gent	Ery
	S	82	79	3	47	73	42	61
	I	0	3	0	1	1	3	4
	R	0	0	79	34	8	37	17
Percentage of Resistant isolates		0	0	96	41	10	45	21

Keywords:
S= Sensitive, I= Intermediate, R=Resistant, Van=Vancomycin, Lin= Linzolid, Clox= Cloxacillin, Cipro= Ciprofloxacin, Tetra=Tetracyclin, Gent=Gentamycin, Ery=Erythromycin, MRSA: Methicillin Resistant *Staphylococcus aureus*.

3.6. Minimum Inhibitory Concentration (MIC) Value and Multiple Antibiotic Resistsnce (MAR) Index of HA-MRSA

It was shown from the results (Table 8) that HA-MRSA isolates recovered from SCBU, Lassa ward, pediatrics had the lowest MIC value 0.5-2ug/ml, while isolates from female surgical ward, had the highest MIC value ranging from 2-8ug/ml. Female surgical ward and laundry isolates had a MAR index of 0.4, Lassa ward, A&E and male surgical ward isolates had MAR index of 0.3. Isolates from other departments and wards from this study recorded a MAR index of 0.2.

Table 8. MIC value and MAR index of HA-MRSA Isolates.

Department / Source of Isolates	No of MRSA	MIC Value (ug/ml)	No of Resistant Antibiotics	No of Antibiotics tested	MAR index
SCBU	4	1 – 2	6	7	0.2
Gynecology	5	1 – 4	12	7	0.2
Lassa	6	0.5 – 2	13	7	0.3
MCH	3	2 – 4	6	7	0.2
Ophthalmology	6	0.5 – 4	10	7	0.2
Female Surgical	9	2 – 8	13	7	0.4
ICU	3	2 – 4	5	7	0.1
Pediatrics	4	0.5 – 2	6	7	0.2
Labour Wards	6	1 – 4	14	7	0.2
Post natal	4	2 – 4	7	7	0.2
A&E	7	0.5 – 4	14	7	0.3
Male Surgical	11	2 – 4	26	7	0.3
Laundry	9	0.5 – 4	26	7	0.4
Radiology	5	2 – 4	7	7	0.2

Keywords:
SCBU= Special care baby unit; A&E= Accident and Emergency; MCH= Mother and Child health; ICU= Intensive care unit; MRSA: Methicillin Resistant *Staphylococcus aureus*; MIC: Minimum Inhibitory Concentration; MAR: Multiple Antibiotic Resistance

3.7. Detection of Genes Coding for Methicillin Resistance

For the detection of genes coding for methicillin resistance in the HA-MRSA isolates, the two genes targeted for amplification, included the *mecA* gene and the *blaZ* gene and are amplified products of 940 bp and 173bp respectively (Plate 2).

For the HA-MRSA isolates, 51(62%) of the total 82 isolates possessed the *mecA* gene and 34 (42%) of the isolates possessed the *blaZ* gene (Figure 2).

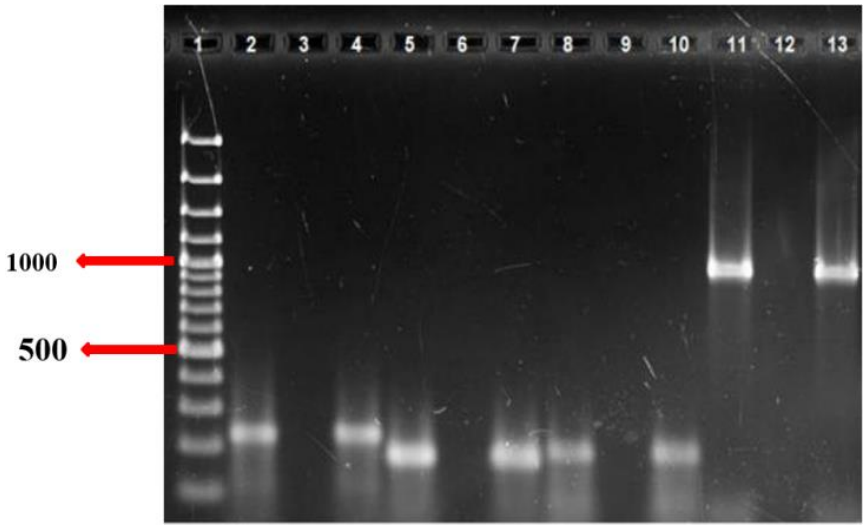

Figure 2. *PCR Product of Representative Amplified mecA, blaZ, nuc and pvl genes in the MRSA Isolates.*

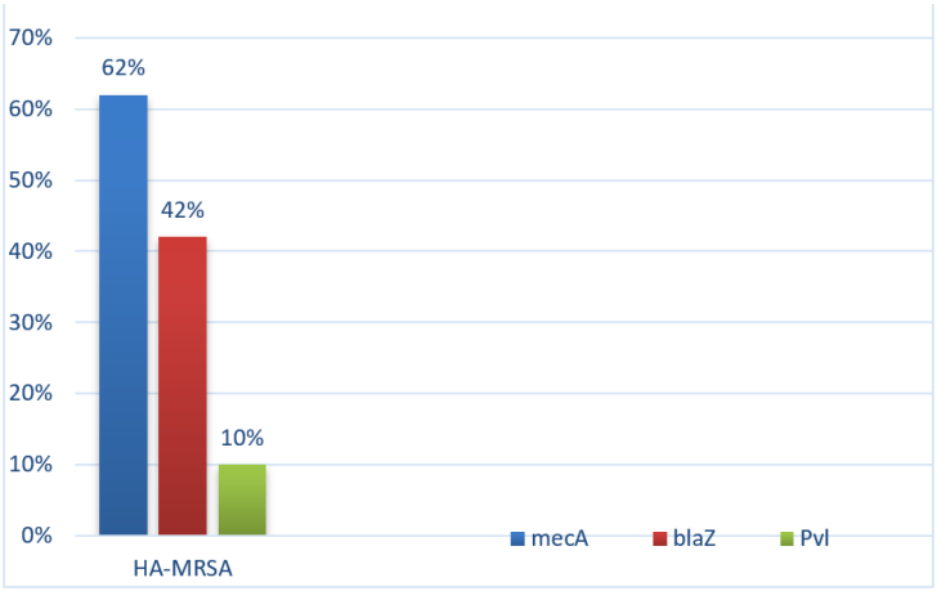

Figure 3. *Percentage Distribution of HA-MRSA Amplified Genes.*

3.7.1. Detection and Distribution of the pvl Genes

The presence of the *pvl* genes were ampified from the chromosomal DNA ofHA-MRSA isolates around bp size of 190 (Plate 2). It was shown from this result that for the HA-MRSA isolate, 9 (10%) of the total 82 HA-MRSA isolates possessed the *pvl* gene.

3.7.2. Detection and Distribution of the SCCmec

For the distribution of *SCCmec* amongst the HA-MRSA isolates, 49 (60%) of the isolates possessed the *SCCmec* (II, III, IV) but isolates from Female surgical, A&E and Laundry departments in ISTH possessed SCCmec type IV.

Table 9. Detection of Staphylococcal Cassette Chromosome (SCC) mec for HA-MRSA Isolates.

Source of Sample within ISTH for HA-MRSA	No of MRSA Isolated from each Department	MAR Index	SCC*mec*Type	No of Isolates Possessing SCC*mec*
SCBU	4	0.2	II	3
Gynecology	5	0.2	II	2
Lassa Ward	6	0.3	II	4
MCH	3	0.2	II, III	3
Ophthalmology	6	0.2	II	4
Female Surgical	9	0.4	II, IV	6
ICU	3	0.1	II	2
Pediatrics	4	0.2	II	2
Labor ward	6	0.2	II	3
Post-natal	4	0.2	II	2
A & E	7	0.3	II, IV	4
Male surgical	11	0.3	II, III	8
Laundry	9	0.4	II, IV	5
Radiology	5	0.2	II	2
	82			50

Key:
MRSA: Methicillin Resistant *Staphylococcus aureus*, MAR: Multiple Antibiotic Resistance, HA-MRSA: Hospital Acquired Methicillin Resistant *Staphylococcusaureus*, ISTH: Irrua Specialist Teaching Hospital, *SCCmec*: Staphlococcal Cassette Chromosome *mec*; SCBU= Special care baby unit; A&E= Accident and Emergency; MCH= Mother and Child health; ICU= Intensive care unit

3.7.3. Distribution of SCCmec and Other Amplified Genes in the HA-MRSA Isolates

The results in Table 10 revealed that for the HA-MRSA isolates, only isolates from male surgical, A&E, female surgical and laundry department that possessed the *pvl* gene, while isolates from other wards did not possess the *pvl* gene. Isolates from MCH, ICU, pediatrics did not possessed the *blaZ* gene but isolates from other wards did possessed the *blaZ* gene and finally, isolates from MCH did not possessed either of *mecA*, *blaZ* nor *pvl* genes but isolates from all wards and departments in ISTH possessed the *SCCmec* genes.

Table 10. Distribution of Staphylococcal Cassette Chromosome (SCC) mec and the Amplified Genes of HA-MRSA.

Source of Sample within ISTH for HA-MRSA	No of MRSA Isolated	MAR Index	SCCmecType	Percentage of Isolates with gene		
				MecA	*blaZ*	*pvl*
SCBU	4	0.2	II	75	50	-
Gynecology	5	0.2	II	80	40	-
Lassa	6	0.3	II	33.3	50	-
MCH	3	0.2	II, III	-	-	-
Ophthalmology	6	0.2	II	66.6	33.3	-
Female Surgical	9	0.4	II, IV	66.6	55.5	22.2
ICU	3	0.1	II	33.3	-	-

Source of Sample within ISTH for HA-MRSA	No of MRSA Isolated	MAR Index	SCCmecType	Percentage of Isolates with gene *MecAblaZpvl*		
Pediatrics	4	0.2	II	50	-	-
Labor ward	6	0.2	II	50	66.6	-
Post-natal	4	0.2	II	50	25	-
A & E	7	0.3	II, IV	85.7	57.2	28.6
Male surgical	11	0.3	II, III	72.3	36.7	9.1
Laundry	9	0.4	II, IV	66.6	55.5	33.3
Radiology	5	0.2	II	80	60	-
TOTAL		82		62%	42%	10%

Key:
MRSA: Methicillin Resistant *Staphylococcus aureus*, MAR: Multiple Antibiotic Resistance, HA-MRSA: Hospital Acquired Methicillin Resistant *Staphylococcus aureus*, ISTH: Irrua Specialist Teaching Hospital, SCC*mec*: Staphlococcal Cassette Chromosome *mec;* SCBU= Special care baby unit; A&E= Accident and Emergency; MCH= Mother and Child health; ICU= Intensive care unit

3.8. Occurrence of Other Virulent Determinants Among HA-MRSA Isolates

The occurrence of some virulent determinants among the HA-MRSA isolates were assayed for including biofilm formation, hemolytic activities and DNase production, and the results showed that 56% of these isolates were biofilm formers and of these 56% biofilm formers, 78% of them were strong biofilm formers, while 22% of them were weak biofilm formers. For their ability to produce hemolysin, 63% of these isolates were positive to hemolysin production, while 33% of these isolates were positive to DNase production. The result from this study revealed that those isolates with higher MAR-index correlates with those with higher virulence factors assayed (Table 11).

Table 11. *Occurrence of Virulent Factors among HA-MRSA Isolates.*

Sample source within ISTH	MAR Index	No of MRSA Isolated	No (%) of Isolates with Biofilms	Biofilmproduction		Haemolysin production	Dnase production
				Strong	Weak		
SCBU	0.2	4	2(50)	1	1	2	1(25)
Gynecology	0.2	5	3(60)	3	-	4	3(60)
Lassa	0.3	6	5(83.3)	4	1	4	2(33.3)
MCH	0.2	3	1(33.3)	1	-	2	-
Ophthalmology	0.2	6	2(33.3)	1	1	4	1(16.6)
Female Surgical	0.4	9	6(66.6)	4	2	5	3(33.3)
ICU	0.1	3	1(33.3)	-	1	2	1(33.3)
Labor ward	0.2	6	3(50)	2	1	3	1(25)
Post-natal	0.2	4	1(25)	-	1	3	1(25)
A & E	0.3	7	4(57.1)	4	-	4	2(28.5)
Male surgical	0.3	11	8(72.7)	8	-	8	3(27.2)
Laundry	0.4	9	7(77.7)	6	1	6	4(44.4)

Sample source within ISTH	MAR Index	No of MRSA Isolated	No (%) of Isolates with				
			Biofilms	Biofilmproduction		Haemolysin production	Dnase production
				Strong	Weak		
Radiology	0.2	5	2(40)	1	1	2	4(80)
Pediatrics	0.2	4	1(25)	1	-	3	1(25)
		82	46(56)	36(78)	10(22)	52(63)	27(33)

Key:
MAR: Multiple Antibiotic Resistance, ISTH: Irrua Specialist Teaching Hospital, SCC*mec*: Staphlococcal Cassette Chromosome *mec,* HA-MRSA: Hospital Acquired Methicillin Resistant *Staphylococcus aureus,* SCBU= Special care baby unit; A&E= Accident and Emergency; MCH= Mother and Child health; ICU= Intensive care unit

4. Discussions

Methicillin resistant *Staphylococcus aureus* (MRSA) is a growing challenge in the treatment of infections caused by *Staphylococcus aureus* and has significantly contributed to the ever increasing burden of global antimicrobial resistance. The isolation of MRSA from livestock (Livestock acquired, LA-MRSA), apparently healthy humans in the community (Community acquired, CA-MRSA) and from the hospital (Hospital acquired, HA-MRSA), has exacerbated the problem caused by MRSA [13].

4.1. Prevalence and Distribution

In this study, populations comprising of doctors, nurses, patients and fomites in Irrua Specialist Teaching Hospital, Irrua were used in the isolation of HA-MRSA. The prevalence of HA-MRSA from this study revealed a prevalence rate of 27%, which is slightly lower than the prevalence of HA-MRAS obtained from studies conducted by [12] at the University of Benin Teaching Hospital (UBTH), where a prevalence rate of 31% was recorded. Studies also conducted by [7] within hospitals in Ekpoma had a prevalence rate of 29%, which is slightly higher than but within the same rangr of the prevalence rate obtained from this study.

Results from this study also revealed that in the distribution pattern of HA-MRSA isolated from ISTH, significant number of the isolates were recovered from male surgical ward 11 (13%), female surgical ward 9 (11%), and laundry department, 9 (11%) respectively, which suggests these departments in ISTH as a high risk source of HA-MRSA infections within the Hospital environment. This is in line with findings by [14] in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, where they recorded a prevalence of 21% of HA-MRSA from Laundry department in a survey of the distribution pattern of HA-MRSA within the hospital. It was also shown that the distribution pattern of the HA-MRSA isolates among the various subjects in ISTH (Doctors, Nurse, Cleaners, Patients) and Fomites revealed that of the 82 HA-MRSA isolated from ISTH, 27(32%) were isolated from fomites, which had the highest number of HA-MRSA isolates. This is in accordance with the findings of [15] where they concluded from their findings within hospitals in Denmark that fomites harbored the highest numbers of HA-MRSA within the hospital settings. After the 27(32%) HA-MRSA from fomites in ISTH, Cleaners had 19(22%) of the isolates, Patient had 11(13%), Nurses had 15(18%) and Doctors had 10(12%). This series suggest that highest numbers of HA-MRSA were harbored in the hospital fomites and the cleaners, who are the most frequent handlers of these fomites and can easily pick up the HA-MRSA and spread them across the hospital environment. This could easily be evident from the gradient flow of these HA-MRSA isolates where fomites 32% > Cleaners 24% > Nurse 19% > Patients 13% > Doctors 12%. A quick look at this gradient flow will suggest in mind that fomites which harbored the highest numbers of HA-MRSA is stagnated and would be majorly accessed by cleaners of every departments and also, nurses had a greater chance of handling these fomites than the doctors and patients. Hence, fomites could be the point source of HA-MRSA reservoir in this hospital environment.

4.2. Antibiogram

Findings from this study shows that of all the 82 HA-MRSA isolated from ISTH, Vancomycin and Linezolid were the best drug of choice for the treatment of MRSA infections because all isolates were sensitive to these antibiotics. This is in line with the findings of [10], where they reported Vancomycin and Linezolid as the most effective antibiotics for treatment of MRSA infections. For these HA-MRSA isolates, 96% of the isolates were resistant to Cloxacillin which is not far-fetched from the fact that Cloxacillin is a member of the β-lactam antibiotics hence, the HA-MRSA isolates are expected to be resistant to all classes of the β-lactam antibiotics by having a defaulted PBP2a which doesn't conform to the lactam ring or possession of the beta lactamase enzymes that hydrolyse beta

lactam rings [16]. Assay of these HA-MRSA isolates against ciprofloxacin, showed that 41% of these isolates were resistant to it while for tetracycline, 10% of the isolates were resistant which means that the tetracycline is also good for the treatment of HA-MRSA infections. Gentamycin had 45% of isolates resistant to it. It therefore shows that from this study, vancomycin, linezolid and tetracycline are the drug of choice for treating HA-MRSA infections.

4.3. Multiple Antibiotic Resistant (MAR) Index

However, some of the HA-MRSA isolates possessed a high MAR index 0.2 - 0.4 which inferred that HA-MRSA infections take a longer time and more difficult to treat. The minimum inhibitory concentration (MIC) of oxacillin was also determined in this study and it was shown that the MIC for HA-MRSA isolates ranged between 0.5 – 8ug/ml, which reflects that the higher MIC value of HA-MRSA indicate that they require higher concentrations of oxacillin to be susceptible and this also points to the fact that HA-MRSA possess higher resistance to antibiotics.

Sudies has shown thatMAR index greater than 0.2, depicts a high risk source and high use of antibiotics [17]. From the results of this study, it was shown that HA-MRSA isolates recovered from female surgical ward and laundry department had the highest MAR index of 0.4 which reflects a very high risk source of HA-MRSA and where antibiotics are frequently used and misused. Isolates from Lassa ward, Accident and Emergency ward, and Male surgical ward had a MAR index of 0.3 which is also indicativeof a high risk source and high rate of antibiotic consumption. All other department and wards from this study had a MAR index of 0.2 which according to [18] is not a high risk source. Therefore, finding from this study showed that female surgical ward, laundry, Accident and Emmergency ward, Male surgical and Lassa ward had a MAR index greater than 0.2 which reflects a high risk source of MRSA infection and this partly conforms with findings of [12] where they showed that Accident and Emergencyand surgical ward in University of Benin Teaching Hospital (UBTH) are the high risk source of HA-MRSA infections with MAR index greater than 0.2.

4.4. Resistant Genes

Molecularly, the genes responsible for methicillin resistance (*mecA* and *blaZ* genes) were also detected using the specific primers. The findings revealed that 62% of the HA-MRSA isolates possessed the *mecA* gene, while 42% possessed the *blaZ* gene, and 7% of HA-MRSA possessed both the *mecA* and *blaZ* genes. This finding partially correlates with findings of [13] where they discovered that HA-MRSA isolates had higher percentage of the genes coding for methicillin resistance.

Amplification of the *mecA* gene (which codes for the Penicillin binding proteins (PBP2a) that confers resistance to methicillin), the *blaZ* gene (which encodes the beta lactamse enzyme that hydrolyse the beta lactam ring in beta lactam antibiotics) and the *pvl* genes (which encodes the panton-valentine leucocidin, a virulence factors that lyse leukocytes) in all the HA-MRSA isolates revealed that the distribution of these genes among the isolates differs. It was shown that some of the isolates possessed the three genes, some possessed two genes and one gene, while others had none. For these HA-MRSA isolates, 62% of the isolates had *mecA* gene, 42% of the isolates had *blaZ* gene.

With regards to the *pvl*and *nuc*gene, 10% of the HA-MRSA isolates acquired the *pvl* gene, which is suggestive of a drift of the genes from the community to hospital isolates of MRSA, as has been reported by [15], who reported that possession of the *pvl* gene is a biomarker of MRSA from the community lineage and that *pvl* is mostly associated with CA-MRSA infections and distinguishable from nosocomial MRSA by multidrug resistance and carriage of the type IV, V and VI Staphylococcal chromosome cassette element (*SCCmec*), while 33% of the HA-MRSA isolates possessed the *nuc* gene which is indicative of the fact that some of the isolates were *nuc* deficient *Staphylococcus aureus*.

In this present study, the results showed that majority (88%) of the HA-MRSA isolates possessed mostly SCC*mec* type II and III, however few isolates (12%) possessed the SCC*mec* type IV which is of community origin. And it was also shown that there was correlation between those HA-MRSA isolates having the SCC*mec* IV and the same isolates having the *pvl* genes which is also a biomarker of community origin. It was also shown from these findings that isolates from HA-MRSA possessing SCC*mec* types IV and the *pvl* genes also had highest MAR index from this study (laundry, accident and emmergency and female surgical ward). Taking a look at these departments in ISTH (laundry, accident and emmergency and female surgical ward) where the isolates had*pvl* genes, SCC*mec* IV and high MAR index, it was clear that they are departments that easily support the access of people from community to the hospital environment and vice versa. This is in correlation with the findings of [19] where they suggested an influx of CA-MRSA within the hospital environment.

4.5. Virulence Factors

Findings for the HA-MRSA isolates revealed that 56% of the 82 HA-MRSA isolates were biofilm formers (produced biofilm), while 44% of the 82 HA-MRSA isolates are non biofilm formers. Similarly, 63% of the HA-MRSA possessed the ability to produce haemolysin, a virulent factor that damage host cell membranes and allow the spread and evasion in the host. The production of haemolysin and DNase, as well as formation of biofilms are pointers to the acquisition of some virulence determinant by these strains. The ability of most of these HA-MRSA to form biofilm is a characteristic that makes them virulent and it allows them to remain on surface for a long time before being picked up by a potential host [6]. This

is not far-fetched from this study where majority of the HA-MRSA isolates were obtained from fomites, hence, their ability to form biofilm is an adaptive feature for them to remain viable on these fomites till they are picked up by a biotic host. DNases have often been described as virulence factor in *Staphylococcus*spp because it has been shown that DNase can help bacteria to escape from neutrophil extracellular traps (NETs) which are structures secreted by neutrophils to trap and kill bacteria [2]. These structure are mainly made of DNA, Protease and Antimicrobial peptides. Hence, the possession of theseDNase enzymes from this study shows that some of these bacterial isolates are virulent in nature.

Findings from this study on the HA-MRSA isolates shows that, isolates with higher MAR index tend to possess the three genes (*pvl, blaZ* and *mecA*) in them, and also possessed majority of the virulent genes and finally, they had a mixture of SCC*mec* type of hospital and community origin. Therefore, there could be possibility of a new strain of MRSA building up due to crosslinking or influx of CA-MRSA to HA-MRSA.

5. Conclusion

In the prevalence and antibiogram studies of Hospitalacquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) in Irrua Specialist Teaching Hospital, Irrua, Edo State, a prevalence rate of 27% was recorded. From these HA-MRSA isolated from ISTH, Irrua, in descending order were 32% from fomites, 23% from cleaners, while nurse had 18%, patients had 13% and doctors had 12% of these isolates. In the distribution pattern of the HA-MRSA isolates among the wards and departments in ISTH, male surgical, female surgical and laundry departments had majority of these isolates.

From the antibiogram of these isolates, all HA-MRSA isolates were sensitive to vancomycin and linezolid, though other antibiotics such as tetracycline, gentamycin and erythromycin showed some level of efficacy to these isolates. Most (96%) of isolates were resistant to cloxacillin. The MAR index of the isolates from different wards, department and districts from ISTH and shows that Accident and Emmergency ward, female surgical, laundry, lassa institute and male surgical ward isolates in ISTH had a high MAR index of 0.4 and 0.3 which connotes an environment of high risk source of antibiotic usage.

The occurrence of virulent factors among the HA-MRSA isolates revealed that some of the isolates possessed some of these virulent determinants including haemolysin, DNase and the ability to form biofilm.

Abbreviations

MRSA	Methicillin Resistant *Staphylococcus aureus*
MAR	Multiple Antibiotic Resistance
ISTH	Irrua Specialist Teaching Hospital

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Data Availability Statement

The data used in this article are sourced from materials mentioned in the references.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Xia, J., Xin, L., Li, J., Tian, L., Wu, K., Zhang, S., Yan, W., Li, H., Zhao, Q., and Liang, C. Discovery of Quaternized Pyridine-Thiazole-Pleuromutilin Derivatives with Broad-Spectrum Antibacterial and Potent Anti-MRSA Activity. Journal of Medicine and Chemical Pathology. 2023, 66(7), 5061–5078. https://doi.org/10.1021/acs.jmedchem.2c02135

[2] Nikolic, P. and Mudgil, P. The Cell Wall, Cell Membrane and Virulence Factors of *Staphylococcus aureus* and Their Role in Antibiotic Resistance. Microorganisms. 2023, 11, 253-259. https://doi.org/10.3390/microorganisms11020259

[3] Liu, S., She, P., Li, Z., Li, Y., Li, L., Yang, Y., Zhou, L., and Wu, Y. Antibacterial and antibiofilm efficacy of repurposing drug hexestrol against methicillin-resistant Staphylococcusaureus. International Journal of Medical Microbiology. 2024, 313(2), 151-158. https://doi.org/10.1016/j.ijmm.2023.151578

[4] Thierer, L. M., Petersen, A. A., Michaud, M. E., Sanchez, C. A., Brayton, S. R., Wuest, W. M., and Minbiole, K. P. C. (2023). Atom Economical QPCs: Phenyl-Free Biscationic Quaternary Phosphonium Compounds as Potent Disinfectants. ACS Infectious Diseases. 2023, 9(3), 609– 616. https://doi.org/10.1021/acsinfecdis.2c00575

[5] Ephraim, E. I., Idahosa, O. E. and Muinah, F. Prevalence of mecA gene among Staphylococci from clinical samples of a tertiary hospital in Benin City, Nigeria. African Health Sciences. 2017, 17(4), 1000-1010. https://doi.org/10.4314/ahs.v17i4.7

[6] André, C., Schrank, C. L., Cheng Jaramillo, A. V., Mylonakis, E., Wuest, W. M., Gilmore, M. S., Kim, W., and Bispo, P. J. M. Antimicrobial activity of a new class of synthetic retinoid antibiotics and comparator agents against ocular staphylococci. Frontiers in Antibiotics. 2023, 2, 110-119. https://doi.org/10.3389/frabi.2023.1101450

[7] Isibor, J. O. and Otabor, E. Asymptomatic carriage of plasmid mediated multidrug resistant *Staphylococcus aureus* in nasal tract of persons in a semi-urban area of Nigeria. American Journal of Life Sciences. 2014, 2(4), 200-204. https://doi.org/10.11648/j.ajls.20140204.12

[8] Cheng, W., Xu, T., Cui, L., Xue, Z., Liu, J., Yang, R., Qin, S., and Guo, Y. Discovery of AmphiphilicXanthohumol Derivatives as Membrane-Targeting Antimicrobials against Methicillin-Resistant *Staphylococcus aureus*. Journalof Medicine andChempathology. 2023, 66(1), 962– 975. https://doi.org/10.1021/acs.jmedchem.2c01793

[9] Clinical and Laboratory Standard Institutes. Performance Standards for Antimicrobial SusceptibilityTesting. Clinical and Laboratory standard institute; Wayne, PA, USA. 2014.

[10] Shittu, A. O., Okon, K., Adesina, S., Oyedera, O., Witte, W. and Strommenger, B. Antibiotics resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. British MedicalCouncil of Microbiology. 2011, 2, 92-95. https://doi.org/10.1186/1471-2180-11-92

[11] Okon, K., Uba, A. B. P., Oyawoye, O. M., Yusuf, I. Z., Shittu, A. O. and Blanc, D. T. Epidemiology and characteristic pattern of methicillin resistant *Staphylococcus aureus* recovered from tertiary hospitals in Northern Nigeria. International Journal of TropicalMedicine. 2011, 6(5), 106 – 112.

[12] Igbinosa, E. O., Beshiru, A., Akporehe, L. U. and Ogofure, A. G. Detection of methicillin resistant *Staphylococcus aureus* isolated from food producing animals: A Public health implication. Vertinary Science. 2016, 3, 1 – 11.

[13] Brayton, S. R., Toles, Z. E. A., Sanchez, C. A., Michaud, M. E., Thierer, L. M., Keller, T. M., Risener, C. J., Quave, C. L., Wuest, W. M., and Minbiole, K. P. C. Soft QPCs: Biscationic Quaternary Phosphonium Compounds as Soft Antimicrobial Agents. ACS InfectiousDiseases. 2023, 9(4), 943– 951. https://doi.org/10.1021/acsinfecdis.2c00624

[14] Ayepola, O. O., Taiwo, O. S. and Anifowose, A. Nasal carriage of *Staphylococcus aureus* and associated risk factors among students of Nigeria University. Acta Science ofMicrobiology. 2018, 1, 6-8.

[15] Wei, M. Z., Zhu, Y. Y., Zu, W. B., Wang, H., Bai, L. Y., Zhou, Z. S., Zhao, Y. L., Wang, Z. J., and Luo, X. D. Structure optimizing of flavonoids against both MRSA and VRE. European Journal ofMedicine and Chemical Pathology. 2024, 271, 116-123. https://doi.org/10.1016/j.ejmech.2024.116401

[16] Zeczycki, T. N., Milton, M. E., Jung, D., Thompson, R. J., Jaimes, F. E., Hondros, A. D., Palethorpe, S., Melander, C. and Cavanagh, J. Aminoimidazole Analogs Target PhoP Altering DNA Binding Activity and Affect Outer Membrane Stability in Gram-Negative Bacteria. Biochemistry. 2024, 61(24), 2948–2960. https://doi.org/10.1021/acs.biochem.2c00560

[17] Zhang, F., Fang, H., Zhao, Y., Zhao, B., Qin, S., Wang, Y., Guo, Y., Liu, J., and Xu, T. A membrane-targeting magnolol derivative for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Frontiers in Microbiology. 2024, 15, 1385-1392. https://doi.org/10.3389/fmicb.2024.1385585

[18] Yang, R., Cheng, W., Huang, M., Xu, T., Zhang, M., Liu, J., Qin, S., and Guo, Y. Novel membrane-targeting isoxanthohumol-amine conjugates for combating methicillin-resistant *Staphylococcus aureus* (MRSA) infections. European Journal ofMedicine and Chemical Pathology. 2024, 268, 113-121. https://doi.org/10.1016/j.ejmech.2024.116274

[19] Ogefere, H. O. and Lawrence, A. O. Molecular characterization of methicillin resistant *Staphylococcus aureus* among apparently healthy students. Universal Medicina. 2019, 38, 25-32.