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Molecular Detection of Methicillin Resistant *Staphylococcus Aureus* Isolated From Hospital Patients and Food Handlers in FCT, North Central, Nigeria

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Abstract: ***Antimicrobial susceptibility profile and molecular detection of methicillin resistant genes isolated from hospital patients and food handlers in Abuja, Nigeria was evaluated. Specimen collection, identification, isolation and characterization were done following standard microbiological procedures. Polymerase chain reaction (PCR) was employed for the detection of the mecA gene associated with methicillin resistance and other virulent marker genes associated with pathogenicity of S. aureus such as Panton-Valentine Leukocidin (PVL), BlaZ and Nuc genes. The result showed a 27.34% prevalence rate of S. aureus isolates in Abuja metropolis; 38.02% prevalence rate was observed in clinical samples while food handlers had 16.67%. Isolates from hospital patients were more resistant to the antibiotics tested than those from the food handlers. Vancomycin was the most effective antibiotic for MRSA isolates from both clinical patients and food handlers while linezolid could be used as an alternative. This study also observed that all the MRSA isolates were resistant to more than three antibiotics (multidrug resistant). High percentage of the isolates (83.33%) that had cefoxitin MIC of ≥ 6µg/ml encoded mecA gene. Nuc gene was observed in 45.83% of the isolates; 4.17% encoded PVL gene while none of the isolates harbored the blaZ gene. The finds from this study showed a high prevalence of methicillin-resistance S. aureus from clinical patients and food handlers sampled and these sources could serve as source of MRSA dissemination in the community.***

Keywords: *Staphylococcus aureus*, antibiotics resistance, *mecA* gene, hospital patients, food handlers, Abuja

1. Introduction

Methicillin resistance *Staphylococcus aureus* (MRSA) causes a number of hard-to-treat infections due to the development of drug-resistant strain of *Staphylococcus aureus* (Kluytmans, 2009). Approximately, 126,000 hospitalizations are due to MRSA yearly. Recent data suggest that MRSA causes a large percentage of all skin and soft-tissue infections.

In US, invasive MRSA infections occur in approximately 94,000 people each year and are associated with approximately 19,000 deaths, reportedly more deaths than HIV per year (Charles and Melissa, 2017). Of these MRSA infections that cause death, about 86% are Hospital associated MRSA and 14% are community associated MRSA (Charles and Melissa, 2017). In a study conducted by Rupak and Huang (2008), infections associated with MRSA are predominantly pneumonia (39%), soft-tissue infection (14%), and central venous catheter infection (14%); of which 24% of these infections involved bacteremia and 38% occurred during a new hospitalization. They also observed that MRSA contributed to 14 deaths, with 6 of these deaths deemed to be attributable to MRSA, and concluded that asymptomatic individuals who are known to have harbored MRSA for >1 year are at high risk for subsequent MRSA morbidity and mortality and should be considered to be targets for intervention, in addition to individuals who have newly acquired this pathogen.

This mortality incidence might be possible due to the presence of virulence factors (extracellular enzymes and toxins) implicated in the pathogenesis of *S. aureus* such as coagulase, which clots plasma and interferes with phagocytosis and also facilitate its spread in the tissues; haemolysins, that lyses red cells, (Normanno *et al.*, 2007); leucodin that kills leucocytes, staphylokinase that causes fibrinolysis; lipase that breaks down fat, (Sandel and Mckillip, 2004); hyaluronidase that facilitate spread in tissues by destroying hyaluronic acid; Protein A that prevents complement activation of cells and staphylococcal enterotoxins (SEs) (also known as toxic shock syndrome toxin TSST), that causes shocks, rashes and desquamation of skin. This enterotoxin produced by *S. aureus* especially toxic shock-like syndromes, have been implicated in food poisoning. This study was design to isolate *S. aureus* from the community (food handlers) and hospital samples in Abuja, Nigeria and molecular detection of MRSA genes among the multidrug resistant isolates.

2. Methodology

Ethical Clearance

Ethical clearance with assertion number FHREC/2014/01/65/05-11-14 was obtained from Health Research Ethics Committee of Federal Capital Territory, Health and Human Services Secretariat, Area 11, Garki Abuja. Individual consent was also obtained using the information leaflet and consent form during sampling survey.

Study Area

The study area was carried out in the six Area Councils in Abuja, Federal Capital Territory (FCT). The six area councils are Abuja Municipal, Gwagwalada, Abaji, Kuje, Kwali, and Bwari area councils.

Sample Size Determination
This was achieved using 50% prevalence obtained by Udoh *et al.,* (2000) in a similar comparative study and using the method describe by Kadam and Bhalerao (2010).

$n_o = \frac{z^2pq}{L^2}$. Where, n_o= the sample size, Z^2 = is the standard normal distribution at 95% confidence interval -1.96, L = allowable error- 0.05, p = prevalence rate, q = 1-p.
$\frac{(1.96)^2(0.5)(0.5)}{(0.05)^2}$ = 384 samples

Sample Collection
A total of 384 samples comprising, 192 samples from the skin and soft tissue samples (Nasal, wound and hand swabs) of food handlers, and 192 from clinical samples (pus, wound sites, ear, vagina,cervix,urethra) were randomly collected for the period of 10 months from February 2015 to November 2015 by qualified medical laboratory scientists.

Isolation, Identification and Biochemical Characterization of *Staphylococcus aureus*
The samples collected were cultured and screened for *S. aureus* on mannitol salt agar (MSA) and incubated at 37°C for 24h. Isolates that showed golden yellow colouration on MSA were further examined using Gram's staining for morphological characteristics; and biochemical properties were determined using coagulase, catalase, Dnase and serology tests according to Cheesbrough (2002).

Phenotypic Detection of MRSA using ETEST Strip for Cefoxitin
Cefoxitin Liofilchem® MIC Test Strips were used for this study according to the manufacturer's instruction.

Extraction of DNA
Genomic DNA of the phenotypically identified *mecA* encoded *S. aureus* were extracted from a 5ml culture, grown in Luria-Bertani (LB) broth at 30 ^{O}C for 48hours using Quick-DNA Miniprep Plus Kit. Presence of DNA was confirmed by electrophoresis on agarose gel containing ethidium bromide that reacts with the DNA and it was checked under UV light (Bio rad image reader) for DNA bands.

Detection of *nuc, blaZ, PVL* and *mecA* Genes
Polymerase chain reaction (PCR) was performed using the thermal cycling conditions as stated by Zymo Research UK (Lephoto and Gray, 2013).

Table 1: Primers for *mecA, nuc, PVL and blaZ*

PRIMER	FORWARD	REVERSE	BP	REFERENCE
MecA1	GGGATCATAGCGTCATTATTC	AACGATTGTGACACGATAGCC	500	Strommenger *et al.,* (2003)
MecA2	TCCAGATTACAACTTCACCAGG	CCACTTCATATCTTGTAACG	162	
Nuc	TCAGCAAATGCATCAAACAG	CGTAAATGCACTTGCTTCAAG	200	
blaZ	TACAACTGTAATATCGGAGGG	CATTACACTCTTGGCGGTTTC	173	Rosato *et al.,* (2003)
PVL	GCTGGACAAAACTTCTTGGAATAT	GATAGGACACCAATAAATTCTGGATTG	451	DTU national food institute (2012)

Antibiotics Susceptibility Profile, Multiple Antibiotic Resistance Index and Classification of Resistance Expressed by Methicillin Resistance *Staphylococcus aureus*
This test was conducted using 10 different antibiotics discs such as Ceftazidime (30µg), Tetracycline (30µg), Clindamycin (2µg), Erythromycin(15µg), Ciprofloxacin(5µg), Oxacillin (1µg), Cefoxitin (30µg), Amoxicillin(30µg), Linezolid (30µg) and Vancomycin (30µg) produced by Oxoid and following agar disc diffusion method modified by Kirby–bauer diffusion technique. The results were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2016). Multiple antibiotic resistance index (MARI) was calculated according to Krumperman, (1983). MAR index = x/y, where x is the number of antibiotics resistant to and y is the number of antibiotics tested.

3. Result

Table 2: Distribution of Samples collected and *Staphylococcus aureus* Isolated According to Studied Area and Sample Source

	CLINICAL SAMPLES						FOOD HANDLERS			TOTAL
STUDY AREA	Urine	Wound	HVS	Ear	ECS	Urethral	Nasal	Hand	Wound	
AMAC	10(3)	5(2)	6(2)	3(2)	5(2)	3(1)	18(3)	14(3)	0(0)	**64(17)**
Bwari	9(3)	7(4)	7(3)	4(2)	3(1)	2(1)	15(3)	16(2)	1(0)	**64(19)**
Abaji	10(3)	5(2)	7(2)	3(1)	5(2)	2(1)	16(1)	13(1)	3(2)	**64(15)**
Kuje	11(2)	5(3)	9(1)	2(1)	2(0)	3(2)	17(3)	14(1)	1(1)	**64(14)**
Kwali	16(5)	3(2)	4(2)	3(1)	3(1)	3(1)	17(4)	12(1)	3(1)	**64(17)**
GWAS	7(3)	7(4)	6(2)	4(2)	4(2)	4(2)	16(4)	13(1)	3(0)	**64(20)**
TOTAL	**63(19)**	**32(17)**	**39(12)**	**19(9)**	**22(8)**	**17(8)**	**99(18)**	**82(10)**	**11(4)**	**384(105)**
%	**30.2**	**53.1**	**30.8**	**47.4**	**36.4**	**47.1**	**18.2**	**12.2**	**36.4**	**27.3**

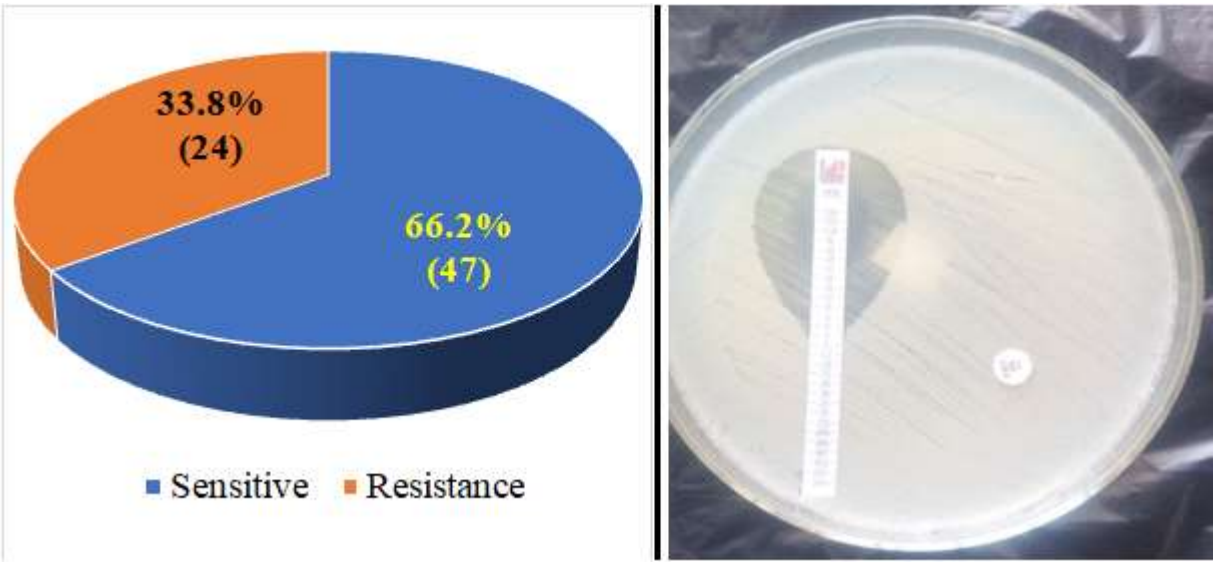

Figure 2: Phenotypic Characterization of Methicillin Resistant *S. aureus* using Cefoxitin MIC strip calibrated in µg/ml and disc

Molecular Analysis

Plate 1: Amplification of Extracted DNA using Gel Electrophoresis

Keys: L: Lane1 = Control, Lane2 = Isolate ACWS, Lane 3 = Isolate KWCUR, Lane 4 = Isolate KWCHVS, Lane 5 = Isolate KWCWS, Lane 6 = Isolate GCWS, Lane7 = Isolate GCES, Lane 8 = Isolate GCUS, Lane 9 = Isolate KJCWS, Lane 10 = Isolate GCUR, Lane 11 = Isolate AMCUR, Lane 12 = Isolate BCWS, Lane 13 = Isolate AMCES, Lane 14 = Isolate AMCUS, Lane 15 = Isolate AMCECS, Lane16 = Isolate BCWS, Lane17 = Isolate BCUR, Lane 18 = Isolate KJCUS, Lane 19 = Isolate KWFWS, Lane 20 = Isolate GFNS, Lane21 = Isolate AMFHS, Lane 22 = Isolate KWFNS, Lane23 = Isolate AFWS5, Lane24 = Isolate AFWS9

Note: The keys above apply to all plates presented in this study.

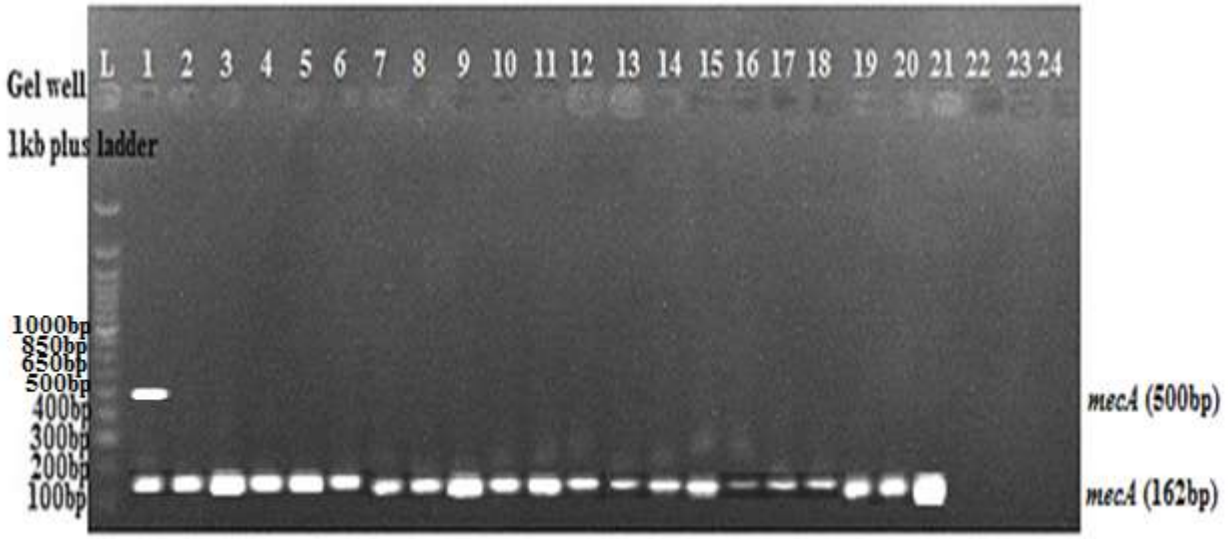

Plate 2: **Amplification of *MecA* Gene of 500bp and 162bp on Gel Electrophoresis**

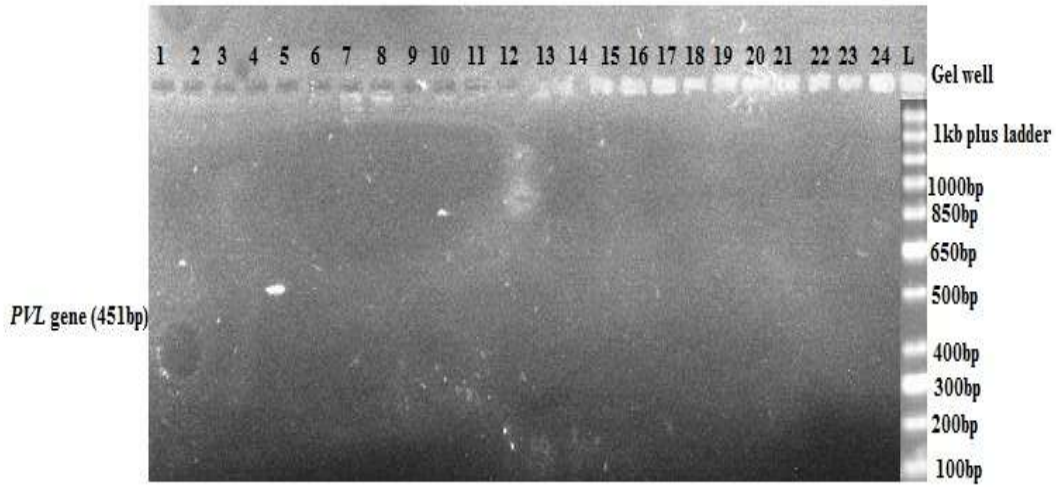

Plate 3: Amplification of PVL Gene on Gel Electrophoresis

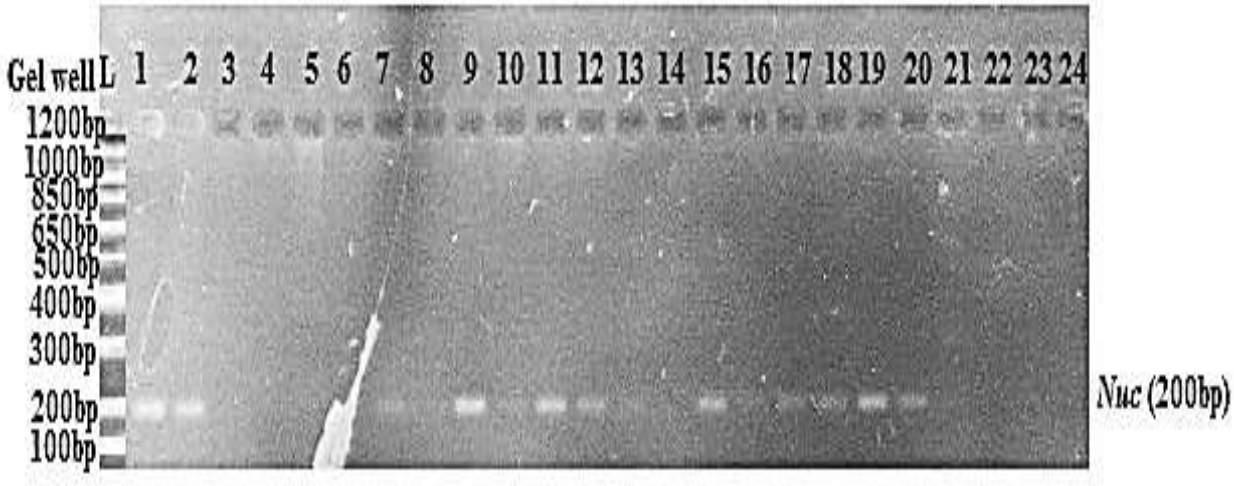

Plate 4: Amplification of *Nuc* Gene of 200bp on Gel Electrophoresis

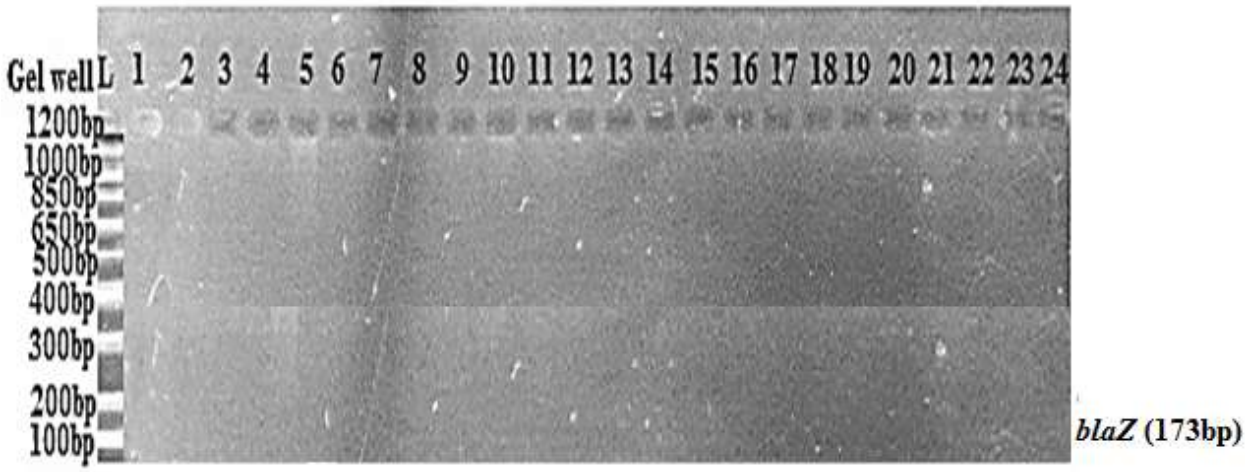

Plate 5: Amplification of *blaZ* Gene of 173bp on Gel Electrophoresis

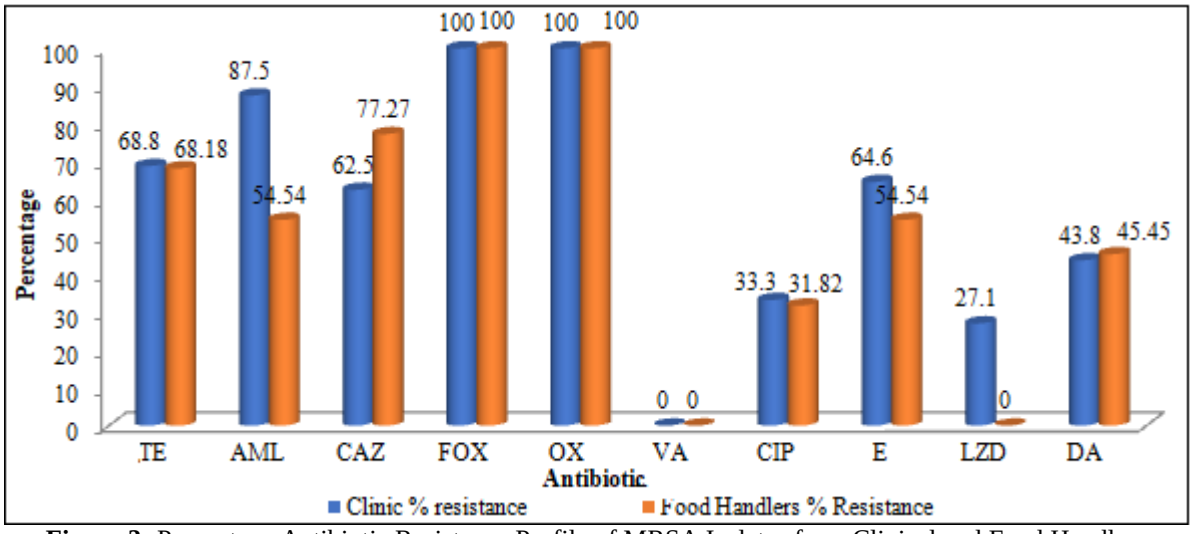

Figure 3: Percentage Antibiotic Resistance Profile of MRSA Isolates from Clinical and Food Handlers

Table 3: Antibiotics Resistant Pattern, MARI and Classification of Resistance Expressed by MRSA Isolates from Clinical Patients and Food Handlers

S/N	IC	Resistant Pattern	NART	MARI	MIC	CR
1	ACWS	FOX,OX,AML,CAZ,DA.LZD,CIP	7	0.7	8	MDR
2	KWCUR	FOX,OX, AML,CAZ,TE,LZD	7	0.6	8	MDR
3	KWCHVS	FOX,OX,AML,CAZ,DA.LZD	6	0.6	8	MDR
4	KWCWS	FOX,OX,AML,CAZ,AML,E,DA	7	0.6	6	MDR
5	GCWS	FOX,OX,AML,CAZ,E,DA	6	0.6	8	MDR
6	GCECS	FOX,OX, TE,E, DA, LZD,CIP	7	0.7	64	MDR
7	GCES	FOX, OX, AML, CAZ,TE,E,DA	7	0.7	34	MDR
8	GCUS	FOX,OX,AML,CAZ,TE,E ,DA,CIP	8	0.8	8	MDR
9	KJCWS(3F)	FOX,OX,AML,TE ,E,CIP	6	0.6	8	MDR
10	GCUR	FOX,OX,AML,TE,E	5	0.5	64	MDR
11	AMCUR	FOX,OX,CAZ,TE,DA,LZD	6	0.6	24	MDR
12	BCWS (30 F)	FOX,OX,AML,TE,CIP	5	0.5	8	MDR
13	AMCES	FOX,OX,AML,CAZ,TE,E	6	0.6	24	MDR
14	AMCUS	FOX,OX,E,DA,LZD,CIP	6	0.6	6	MDR
15	AMCECS	FOX,OX,AML,CAZ,TE	5	0.5	12	NMDR
16	BCWS	FOX,OX,AML,CAZ,E,CIP	6	0.6	24	MDR
17	BCUR	FOX,OX,AML,TE,DA,CIP	6	0.6	6	MDR
18	KJCUS	FOX,OX,AML,TE,DA,LZD	6	0.6	24	MDR
19	KWFWS	FOX,OX,AML,CAZ,TE,E,DA,LZD	8	0.8	6	MDR
20	GFNS	FOX,OX,AML,CAZ,TE,E,DA,LZD	8	0.8	12	MDR
21	AMFHS	FOX,OX,AML,TE,DA,CIP	6	0.6	8	MDR
22	KWFNS	FOX,OX,AML,CAZ,TE,E,CIP	7	0.7	12	MDR
23	AFWS	FOX,OX,CAZ,TE,DA	5	0.5	24	MDR
24	AFWS	FOX, OX,AML,CAZ,TE,E,DA	7	0.7	8	MDR
25	Control (*mecA*)				32	

Keys: IC = isolates code, NORP = number of occurrence of resistance pattern, NART = number of antibiotics resistant to, MARI = multiple antibiotics resistance index, MIC = cefoxitin minimum inhibitory concentration in µg/ml, CR = classification of resistance

4. Discussion

This study observed 27.3% prevalence of *S. aureus* from samples evaluated in FCT, Nigeria. 16.7% (32) prevalence was obtained from food handlers while clinical samples yielded 38.0% (73) prevalence. The findings in this study are similar to the work of Raghabendra *et al*, (2017) who reported 35.50% prevalence of MRSA among clinical samples. Santos *et al.* (2008) and Burkitt *et al.* (2010) who reported 22.4% and 24% prevalent rate in food handlers. The report of Onanuga *et al.* (2006), who observed 71.7% prevalence of MRSA among healthy women volunteers in Abuja, supported the possibility of isolating MRSA in the studied area. Based on studied area, the highest number of *S. aureus* was isolated from University of Abuja Teaching Hospital (15), followed by Kubwa General Hospital for Clinical specimens. While AMAC (6) and Kwali (6) had the highest isolation rate followed by Bwari (5) and Kuje (5) for food handlers sampled. Among the samples evaluated from the food handlers, the wound swab of the food handlers (36.4%) had the highest isolation rate of *S. aureus* followed by the nasal carriage (18.2). In clinical samples, the highest isolation rate was wound (53.1%), Ear (47.4%), and urethral (47.1%) while the least was isolated from urine samples (30.2%) (Table 2).

The MRSA isolates were totally resistant (100%) to cefoxitin and oxacillin. The isolates were also observed to be highly resistant to CAZ, TE, AML and E; mildly resistant to DA, CIP but sensitive to VA and LZD. High percentages of the isolates from the clinical samples were observed to express more resistant profile than those from the food handlers. The most common resistant pattern among the MRSA isolates are FOX,OX,AML,CAZ,TE,E,DA (8.3%), FOX,OX,AML,CAZ,TE,E,DA,LZD (8.3%) and FOX,OX,AML,TE,DA,CIP (8.3%). indicating that some of the isolates have been exposed to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms (Orozova *et al.*,2008). All the MRSA isolates had MAR index ≥ 0.3 and showed resistance to more than 3 antibiotics tested while 95.4% of the MRSA isolates are MDR.

Significant percentage of the total *S. aureus* isolated (22.9%) harbored MRSA properties phenotypically and had MIC value ≥ 6 harbored (Figure 2). Further susceptibility study using Vancomycin showed that the isolates were 100% susceptible to Vancomycin with MIC value ranging from 0.38 to 1.5μg/ml. This result is also in line with the finding of Raghabendra *et al.* (2017) who reported that the MIC value of vancomycin for *S. aureus* ranged from 0.016 μg/ml to 1 μg/ml. Twenty-four isolates that had cefoxitin MIC of ≥ 6µg/ml and a known *mecA* positive isolate (lane 1) were selected for DNA extraction. This study is in agreement with the report of Wielders *et al.,* (2002), who reported the carriage of *mecA* among 95% isolates that display a phenotype of methicillin resistance *S. aureus*. According to Marwa *et al.,* (2015), all the strains (100%) which appeared oxacillin and cefoxitin resistant phenotypically by the disk diffusion technique were positive for the presence of mecA gene and were therefore confirmed as MRSA strains with base pair of 162.

The DNA of the isolates that were extracted is shown on plate 1. Plate 2 showed that 83.3 % (20) of the *S. aureus* with cefoxitin MIC of ≥ 8µg/ml harbor *mecA* gene that amplified at 162 base pair while none of the isolates harbor *mecA* gene with 500bp except the control (Lane 1). Plate 3 showed that 4.2% (1) of the isolates harbor the PVL gene among the *S. aureus* evaluated. These genes have been reported in several studies to be responsible for the recent outbreaks of severe invasive disease in previously healthy children and adults in the United States of America and Europe (Holmes *et al.,* 2005). Correlating the findings of this study with other reports, the percentage expression of PVL in this study is higher than the findings reported in the study of Holmes *et al.,* (2005), who reported an incidence of 1.6% but lower than Huang *et al.* (2006) that reported that PVL genes were detected in 79% of the CA-MRSA isolates but in only 20% of the HA-MRSA isolates. The presence of PVL among the isolates in this study is indeed a case for reflection as PVL genes have been reported as pore-forming cytotoxins that targets human and rabbit mononuclear and polymorphonuclear cells (Prevost *et al.,* 2001). PVL genes have also been detected in isolates responsible for community-acquired pneumonia, burn infections, bacteremia, and scalded skin syndrome (Holmes *et al.,* 2005).

Plate 4 showed that 45.8% (11) of the isolates encode the *Nuc* gene while 54.2% do not. *S. aureus* produces an extracellular thermostable nuclease, encoded by *nuc* gene, which is one of the most distinguishing and successful characteristics that might be used for distinguishing *S. aureus* from other *Staphylococcus* spp. This suggests that *nuc* gene is a specific marker gene and PCR is a useful method for identifying this gene in *S. aureus* (Zhang *et al.,* 2004). The study conducted by Sahebnasagh *et al.,* (2014) in Iran which showed that isolates with *mecA* gene has high propensity (80.2%) to encode nuc and other virulent genes of medical importance correlate with the finding of this study. In this study the MRSA isolates did not harbor the *blaZ* gene see Plate 5 and this result is similar to the finding of Robles *et al.* (2014) and Tende (2017), who reported that some *S. aureus* isolates showed phenotypic activity for beta-lactamase production in their studies but did not harbor the *blaZ* gene. Beta-lactamase phenotype can be as a result of the expression of more than one gene, and there is more than one mechanism that grants Staphylococci beta-lactam resistance other than the expression of *blaZ* gene (Malik *et al.,* 2007).

5. Conclusion

The finds of this study showed a significant prevalence of methicillin-resistance *S. aureus* from clinical and food handlers sampled; and these sources could serve as source of

MRSA dissemination in the community. Urban regions with populations of lower socioeconomic status and evidence of overcrowding appear to be at high risk for the emergence of MRSA in Abuja. Drugs such as beta lactames, tetracyclines, erythromycin, gentamicin and quinolones commonly prescribed in various Hospitals and Pharmaceutical outlets were observed to have reduced therapeutic effects against MRSA isolates but drugs such as linezolid had high therapeutic activity and could be used as a substitute to vancomycin during MRSA outbreak in Abuja.

6. Recommendations

1) These findings indicate the need for intensive training/retraining and health education of all food service vendors and employees.
2) The finds of this study also call for the development of models that could assist in limiting the spread of drug resistant bacteria. Some of which include the use of better treatment strategies; better immunization programmes; antibiotic resistance surveillance programmes; development of new antibiotics; improved hygiene and nutrition; and initiatives targeting the poor populations.
3) A high standard of personal hygiene among individuals engaged in the handling, preparation and cooking of food is of great importance as they could serve as rout of dissemination of diarrhoea, dysentery, throat infection etc. among the populace.

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