



MOLECULAR IDENTIFICATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN STREET VENDED FOODS AND FOOD HANDLERS IN PORT HARCOURT METROPOLIS, NIGERIA

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Article Received on 22/07/2019

Article Revised on 12/08/2019

Article Accepted on 02/09/2019

ABSTRACT

The emergence of a genetically different MRSA strain more virulent than the hospital strains in the community termed community-associated (CA-MRSA) has raised pertinent questions about the possibility of its spread in the food chain and within the community through contaminated food and food handlers. In this study, the presence of methicillin resistant *S. aureus* (MRSA) in 135 street vended foods and their handlers respectively was determined using polymerase chain reaction. A high proportion of *S. aureus* isolates in this study were resistant to Augmentin (81.5%), Zinnacef (67.0%) and Oxacillin (49.7%) while high sensitivity was observed in Gentamicin, Perfloroxacin and Erythromycin, (86.2%) suggesting that these drugs may serve as treatment options in developing countries. MRSA was detected in isolates from food samples (6%) of Beef burger and food handlers (26%) of Beef burger, Meat pie, Doughnut and Cupcake by positive amplification of the *mecA* gene, representing an overall prevalence of 33%. The presence of MRSA in street foods and their handlers increases the risk of their transmission through the food chain and poses a public health threat to consumers because of the narrow range of therapeutic options available to clinicians for their treatment. This calls for stricter enforcement of public health laws and a focused educational and awareness campaign targeting sectors involved with street vended foods in Port Harcourt metropolis.

KEYWORDS: Methicillin Resistant *S. aureus* (MRSA), Street vended foods, Food handlers, Polymerase Chain Reaction (PCR), Community Acquired Methicillin Resistant *S. aureus* (CA-MRSA).

INTRODUCTION

Staphylococcus aureus is a normal flora of the skin and mucous membranes of apparently healthy humans. This organism possesses several virulence factors with which it causes a wide range of clinical infections from minor skin and soft tissue infections to life threatening conditions such as septic arthritis, endocarditis, osteomyelitis etc.^[1,2] *S. aureus* infections are difficult to treat due to its ability to acquire and develop resistance to multiple antibiotics representing a significant cause of morbidity and mortality throughout the world.^[3] Benzyl penicillin was the initial therapeutic option used to combat *S. aureus* infections but it became ineffective not long after it was introduced because of the acquisition of a plasmid-encoded beta-lactamase that conferred resistance in the organism. As a result, methicillin was developed with the sole aim of tackling strains resistant to penicillin but unfortunately encountered resistance as well two years after it was introduced in 1961.^[4] MRSA which are strains of the organism that exhibit resistance to methicillin and other antibiotics in the beta lactam

class have recently been listed as one of the high-priority antibiotic-resistant pathogens by the World Health Organization.^[5] A majority of MRSA associated with disease in hospitalized patients is known as hospital-associated (HA-MRSA) but in the early 1990s, a new type of genetically different MRSA strain more virulent than the hospital strains evolved in the community and were termed community-associated (CA-MRSA). Furthermore, MRSA infections in the community caused by strains primarily associated with livestock are known as livestock-associated (LA-MRSA).^[6,7] Food microbiologists consider MRSA a food borne hazard and that food items serve as vehicles of spread of these strains within the food chain, in hospital settings as well as in the community.^[8] It is therefore imperative that food sampling and testing to detect the presence of the *mecA* gene which is the current gold standard for MRSA detection should be focused on foods of animal origin especially street vended foods that require frequent human handling and food handlers who frequently contaminate food items with their colonizing strains.

This study was carried out to identify MRSA in street vended foods and their respective handlers in Port Harcourt, Nigeria, using PCR.

MATERIALS AND METHODS

Sample Collection

135 street vended food samples and 135 hand swab samples from food handlers making a total of 270 samples were collected with informed consents from the food handlers. The food samples were from different food categories as follows: Meat pie, Fried meat, Roasted meat ('Suya'), African salad ('Abacha'), Salad dressing, Cupcake, Beef burger, Doughnut and Fish pie all purchased in then containers used by the food vendors to sell them and immediately transferred to sterile containers with ice packs. The hand swab samples were collected by thorough swabbing of the palms and in between fingers of food handlers using a sterile cotton wool swab stick moistened in 0.1% peptone water. All samples were immediately transported to the laboratory for analysis.

Isolation and Identification of *S. aureus*

Samples were processed using standard methods as described by El-Jakee et al.^[9] Ten (10g) of a representative portion of each food sample was homogenized in 90ml of sterile peptone water (0.1%) and a 10 fold serial dilution done. Samples from food and hand swabs of food handlers were inoculated onto mannitol salt agar for the presumptive identification of *S. aureus* by the streak plate method and incubated aerobically at 37°C for 24 hours. Gram's staining and other confirmatory tests such as catalase and coagulase tests were done according to the methods described by Cheesbrough.^[10]

Antibiotic Susceptibility Testing

Susceptibility to antibiotics was carried out according to methods described by Bauer & Kirby^[11] using commercially available antibiotic disks (Oxoid, UK) The antibiotics used were Vancomycin (30µg), Oxacillin (1µg), Cefoxitin (30µg), Ampiclox (30µg), Zinnacef (20µg), Amoxicillin (30µg), Rocephin (25µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septrin (30µg), Erythromycin (10µg), Perfloxacin (10µg), Gentamicin (10µg), Sparfloxacin (10µg), Augmentin (25µg), Tarivid (30µg) and Chloramphenicol (30µg). A bacterial suspension was prepared to match a 0.5

Mcfarland turbidity standard and introduced into the agar plate. The antibiotic discs were then placed on the surface of the agar and incubated aerobically for 24 hours. The zones of inhibition around the discs were measured with a metre rule to the nearest whole millimeter (mm) and interpreted as sensitive, moderately sensitive (intermediate) and resistant according to the standards set by the clinical and laboratory standards institute (CLSI).^[12] Multiple drug resistance was defined as resistance to at least three classes of antibiotics.

Phenotypic Detection of MRSA

Phenotypic detection of MRSA was done using Oxacillin and Cefoxitin disc diffusion method Isolates that showed resistance to oxacillin in disc diffusion were tested for the minimum inhibitory concentration (MIC) for oxacillin by broth dilution method as prescribed by the CLSI.^[12]

Molecular Characterization

Fifteen (15) isolates that exhibited resistance phenotypically to cefoxitin and suspected to be MRSA were selected and subjected to molecular analysis for identification of the bacterial 16s rRNA gene, thermonuclease (nuc) and methicillin resistance genes (mecA) in accordance with the methods and protocols described by Klindworth et al, Brakstad et al and Nahaei & Milani^[13-15] using the primers: 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' for the 16srRNA gene with an expected amplicon size of 1500 base pairs and F: 5'-GCG ATT GAT GGT GAT AGG GTT-3' and R: 5'-AGC CAA GCC TTG ACG AAC TAA AGC- 3' for the nuc gene with an expected amplicon size of 270 base pairs and F: 5'-AAAATCGATGGTAAAGGTTGGC-3' and R: 5'-AGTTCTGCAGTACCGGATTTTGC-3' for the mecA gene with an expected amplicon size of 530bp. This was carried out in a thermal cycler (ABI 9700, Applied Biosystems) with thermal cycling conditions as described in Table 1. The PCR mixture included: the 2X Dream Taq Mastermix (Taq polymerase, buffer, DNTPs, MgCl₂, 0.4M Primers) supplied by Inqaba, South Africa and the extracted DNA. PCR conditions are described by Rall et al., 2002 (Table 1). The PCR products were resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue light transilluminator.

Table 1: PCR Thermal Cycling Conditions.

Gene	Initial Denaturation		Denaturation		Annealing		Extension		Cycles	Final Extension	
	Temp (°C)	Time (min)	Temp (°C)	Time (sec)	Temp (°C)	Time (sec)	Temp (°C)	Time (sec)		Temp (°C)	Time (min)
16s rRNA	95	5	95	30	52	30	72	30	35	72	5
nuc	95	5	95	30	52	30	72	30		72	30s
mecA	95	5	95	30	56	30	72	30		72	5

Source: Klindworth et al, Brakstad et al and Nahaei & Milani.^[13-15]

RESULTS

Antibiotic susceptibility of *S. aureus*

Antibiotic susceptibility showed high percentage of resistance against Augmentin (81%) followed by Zinnacef (67%), Chloramphenicol (58.7%), Oxacillin (49.7%), Vancomycin (40.7%), Septrin (31.7%), Cefoxitin (22.3%), Amoxicillin, Streptomycin, Sparfloxacin (18%), Ampiclox and Rocephin (13.3%), Tarivid, Ciprofloxacin (9.0%) respectively, Gentamicin, Perfloxacin and Erythromycin (4.2%) (Table 2). 158 out of 233 isolates (68%) were found to exhibit resistance

phenotypically to a minimum of three different classes of antibiotics and were classified as multi drug resistant.

Phenotypic detection of MRSA using Oxacillin and Cefoxitin

A total of 50 (21%) of the 233 *S. aureus* isolates were resistant to cefoxitin 30µg (zone $\leq$ 21mm) and 111 (47%) of the 233 *S. aureus* isolates were resistant to oxacillin 30µg (zone $\leq$ 10mm). MIC for oxacillin was found to be ≥ 8 µg/ml for isolates that were identified as resistant in disc diffusion method.

Table 2: Overall Antibiotic Susceptibility Profile from Food and Food Handlers.

Antibiotics	Std disc conc (µg)	Total number of isolates n = 233		
		Susceptible (%)	Resistant (%)	Intermediate (%)
Vancomycin	30	78 (36.0)	88 (40.7)	48 (22.3)
Oxacillin	1	68(31.7)	108(49.7)	39(18.0)
Cefoxitin	30	137(63.5)	49(22.3)	29(13.3)
Ampiclox	30	88(40.7)	29(13.3)	98(45.0)
Zinnacef	20	59(27.0)	46(67.0)	10(4.2)
Amoxacillin	30	117(54.5)	39(18.0)	59(27.0)
Rocephin	25	186(86.2)	10(4.2)	20(9.0)
Ciprofloxacin	10	166(77.2)	10(4.2)	39(18.0)
Streptomycin	30	127(58.7)	39(18.0)	49(22.3)
Septrin	30	108(49.7)	68(31.7)	39(18.0)
Erythromycin	10	185(86.2)	29(13.3)	0
Perfloxacin	10	166(77.2)	20(9.0)	29(13.3)
Gentamicin	10	185(86.2)	20(9.0)	10(4.2)
Sparfloxacin	10	136(63.5)	39(18.0)	39(18.0)
Augmentin	25	29(13.3)	175(81.5)	10(4.2)
	30	20(9.0)	10(4.2)	0
Chloramphenicol	30	49(22.3)	127(58.7)	39(18.0)

Table 3: Detection of MRSA in Food and Food Handlers.

Isolates	Source	Phenotypic Disc Diffusion Tests		Polymerase Chain Reaction (PCR)
		Oxacillin	Cefoxitin	mecA
1	Roasted meat ('Suya') ^F	R	S	-
2	Roasted meat ('Suya') ^H	I	S	-
3	Fried meat ^F	R	R	-
4	Doughnut ^H	I	R	+
5	Beef Burger ^F	R	R	+
6	Beef Burger ^H	R	R	+
7	Meat pie ^F	I	S	+
8	Meat pie ^H	I	S	-
9	Cupcake ^F	I	S	-
10	Cupcake ^H	R	R	+
11	African salad ('Abacha') ^F	R	S	-
12	African salad ('Abacha') ^H	R	S	-
13	Doughnut ^F	S	S	-
14	Fried meat ^H	S	S	-
15	Meat pie ^H	S	S	-

Key: S: sensitive, R: resistant, +: Presence, -: Absence, mecA: methicillin resistance gene, F: Isolate from food, H: Isolate from food handler.

Detection of the 16s rRNA and Thermonuclease Genes

The 16s rRNA and thermonuclease (nuc) gene amplification produced amplicons of the expected sizes for all *S. aureus* isolates tested (Figure 1).

Identification of Methicillin Resistant *S. aureus* (MRSA)

Out of the fifteen (15) selected multi drug resistant (MDR) isolates subjected to PCR 1(6%) from food

sample of Beef burger and 4(26%) from food handlers of Beef burger, Doughnut, Meat Pie and Cupcake harboured the *mecA* gene with amplicons of the expected sizes (Figure 1; Table 3). This result represents an overall percentage detection of MRSA in this study to be 33%.

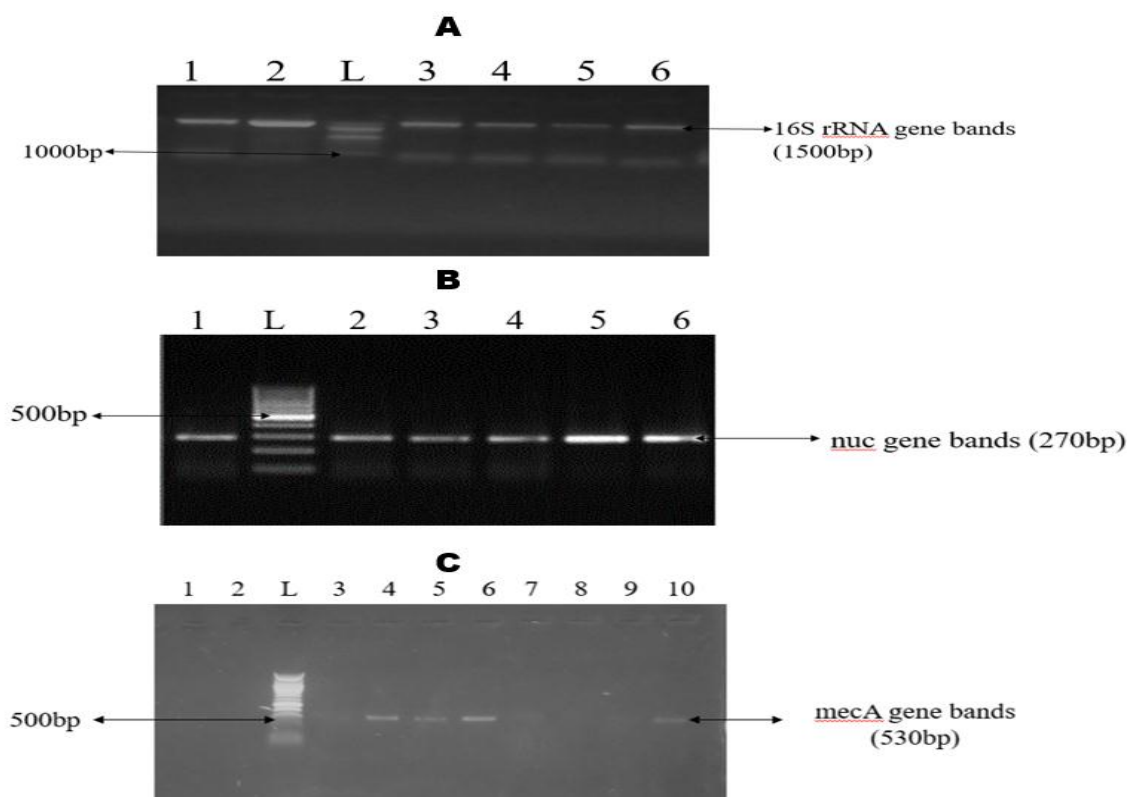


Fig. 1: Agarose gel picture showing the amplified genes. A: 16s rRNA gene of 1500bp in all isolates, Lane L represents the DNA ladder at 1000bp. B: nuc gene of 270bp in all isolates, Lane L represents the DNA ladder at 500bp. C: *mecA* gene of 530bp in isolates 4, 5, 6, 7 and 10, Lane L represents the DNA ladder at 500bp.

DISCUSSION

Comparative DNA sequence analysis of microbial genes has provided evidence that the 16S rRNA gene is a good molecular marker for detecting bacteria but limited in distinguishing closely related species thus the thermonuclease gene unique to *S. aureus* was amplified to molecularly confirm the identity of the organism. Molecular amplification of the *mecA* gene is recognized as a benchmark and major evidence to diagnose MRSA in the community because these genes are highly conserved among staphylococcal species.^[16] The presence of *S. aureus* in street vended foods could most likely be due to frequent handling by the colonized food handlers during food preparation activities as reported by several studies stating that processed foods requiring frequent handling during preparation are more vulnerable to *S. aureus* contamination^[17] evident in the fact that most street vended foods in Port Harcourt are often processed and served with bare hands. This study

provides evidence of the occurrence of MRSA in street vended foods and food handlers in Port Harcourt by polymerase chain reaction with a prevalence of 6% in food and 26% in food handlers. This rate is relatively higher than the reports from other countries, for example, the prevalence of MRSA in dairy products from Italy was 0.5% and 1.3% in retail foods from China^[18] and lower than rates of MRSA prevalence in India of 36% from hand rinse samples from food handlers responsible for food preparation, serving and cleaning.^[19] A similar study in Zimbabwe showed that 32% of food handlers carried *S. aureus* on their hands.^[20] Food handlers have been reported to be the major culprits in cross contamination by contaminating the food items with their colonizing strains which may have been exposed to antibiotic stress by the misuse and over use of antibiotics directly responsible for the resistance phenotypes in isolated strains.^[21] The emergence of food-borne outbreak caused by MRSA has already been reported. In

Japan, Hammad et al.^[22] examined 200 samples of retail ready-to-eat raw fish (sashimi) of which they recovered 10 MRSA from 10 different samples (5%, 10/200). These reports further emphasize the threat of MRSA in foods. Clinical management of staphylococcal infections rely on antibiotic treatment which often fails due to aggressive resistance of the organism to antibiotics. A high proportion of isolates in this study were resistant to Augmentin (81.5%), Zinnacef (67.0%) and Oxacillin (49.7%) while high sensitivity was observed in Erythromycin, Gentamicin (86.2%) and ciprofloxacin (77.2%) suggesting that these drugs may serve as an alternative in the treatment of these infections in developing countries as opposed to the newer more expensive options. In previous studies, all MRSA strains carried the *mecA* gene. But in this study not all phenotypically detected MRSA harboured the *mecA* gene and a possible reason for this phenomenon could be the hyperproduction of β -lactamase. This is consistent with several studies including a previous study in Nigeria that demonstrated the complete absence of five major SCCmec types and *mecA* genes and the gene product of PBP2a in *S. aureus* isolates which were phenotypically MRSA thereby confirming the high sensitivity of PCR in detecting enterotoxin genes as reported by other authors.^[22,23]

CONCLUSION

The presence of MRSA in street foods and their handlers in Port Harcourt, Nigeria increases the risk of their transmission through the food chain and poses a public health threat to consumers. This calls for stricter enforcement of public health laws and a focused educational and awareness campaign targeting sectors involved with street vended foods. Furthermore, the findings described herein could also be generally relevant to developing countries of Asia, Africa and all other places where street vended foods are frequently sold and consumed.

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