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Detection of Methicillin-Resistant *Staphylococcus aureus* in Ready-to-Eat Shellfish (*Corbiculid heterodont*) in Bayelsa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author SRE managed the analyses of the study, wrote the protocol and wrote the first draft of the manuscript. Author NPA designed, supervised and performed the statistical analysis of the study. Author CEIN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims/Objective: Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a dominant cause of severe healthcare-associated (HA) infections but has recently emerged as Community-Associated (CA-MRSA) and Livestock-Associated MRSA (LA-MRSA). This study was carried out to detect the presence of Methicillin-Resistant *S. aureus* in Ready-to-Eat (RTE) *Corbiculid heterodont* in Bayelsa state, Nigeria.
Place of Study: This study was conducted in Yenagoa, Southern Ijaw and Sagbama Local Government Areas, Bayelsa State, Nigeria.
Methodology: Fifty-three samples (dried, fried and stewed) of the RTE *C. heterodont* were collected and subjected to standard microbiological procedures.
Results: Seventy *Staphylococcus* spp. were isolated, consisting of 65 (92.86%) *S. aureus* and 5 (7.14%) coagulase negative *Staphylococcus* (CONS). Out of the 65 *S. aureus* isolates, 53.8% were

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MRSA. Susceptibility pattern of MRSA showed a decreasing trend of resistance in the order: Amoxacillin, Ampicillin-Cloxacillin, Cefoxitin, and Oxacillin (100%) > Streptomycin (82.9%) >Ceftriaxone (40%) >Cefuroxime (31.4%), > Ciprofloxacin and Trimethoprim-Sulfamethoxazole (17.1%) > Gentamycin and Pefloxacin (8.6%) > Erythromycin (2.9%).Out of the 65 *S. aureus* isolates, 62 (95.4%) had Multidrug Resistance Index > 0.2 while 3 (4.6%) had 0.2 as their Multidrug Resistance Index. The MRSA isolates were 100% Multidrug Resistant (MDR) while the Methicillin-Sensitive *S. aureus* (MSSA) were 90% MDR. Molecular characterization of ten *S. aureus* isolates confirmed 80% *S. aureus* isolates and 20% CONS (*S. sciuri* and *S. warneri*). Polymerase chain reaction (PCR) and Agarose gel-electrophoresis were used to detect the presence of *mecA* gene, a gene that confers methicillin resistance. The *mecA* gene with size 500bp was identified in 50% of MRSA. This study reported presence of MRSA and MDR *S. aureus* in *C. heterodont* sold in Bayelsa state, Nigeria. This could be the avenue for the spread of MRSA. Public health awareness campaigns should be advocated to enhance hygienic practices in the handling and distribution of food.

Keywords: Methicillin Resistant Staphylococcus aureus (MRSA); ready-to-eat food; Corbiculid heterodont; Multi Drug Resistance (MDR); Staphylococcus sciuri; Staphylococcus warneri.

1. INTRODUCTION

Ready-to-eat (R.T.E) food is food that is in a form that is edible without further preparation to achieve food safety and is sold as perishable meals for immediate consumption [1]. They are foods that are not cooked or re-cooked prior to serving. These foods include; salads, cooked meats, smoked fish, desserts, sandwiches, cheese and food that have been cooked in advance to serve cold. They could also be shelf-stable products, refrigerated or frozen foods [2]. Since primordial times Ready-to-eat –foods have been an important constituent of human diet with examples like bread, yoghurt, butter, ice cream, smoked fish, and so on [3].

Every country has varieties of R.T.E foods which are unique to them and may not be acceptable in other countries [1]. Ready-to-eat foods that are widely consumed in Nigeria include snacks such as; doughnut, sausage, egg roll, meat pie, chin-chin, puff-puff, fried meat, moi-moi and Akara [4]. They also include foods such as Suya, Bole, Abacha, Edible worms, Plantain Chips, and so on. Examples of RTE foods in other countries include; dairy products and delicatessen meat from Malaysia, sashimi (fish slivers or raw meat) and sushi (rice seasoned with Vinegar and combined with a variety of toppings) from Japan.

Corbiculid heterodont, a popular shellfish bivalve (Plate 1) seen in the southern part of Nigeria especially in Bayelsa state is a delicacy and serves as a rich source of protein [5]. It is locally called 'gbou' or "Okpoku" by the Ijaws and it is used to generate income for the people. It is usually sold by food vendors in the market in either fresh, fried, stewed as kebab or smoke-dried forms. Therefore, it can be called Ready-to-eat-food (RTE) or a street-vended food [6].

Plate 1. Fresh *Corbiculid heterodont*

Seafood such as Shellfish has traditionally been an important source of proteins in low-income countries, whereas it is increasingly appreciated as a major constituent in the distinctive and nourishing foods of high-income countries [7,8]. Nevertheless, the consumption of seafood is not without risk, and a wide range of microbiological and chemical hazards can occasionally be present [9]. Diseases due to infection or intoxication associated with the consumption of fish/shellfish have been specifically associated with pathogens, some of which are resistant to antibiotics [10].

Consumers' health in many parts of the world has been at risk of the contamination from street vended foods [11]. Foodborne pathogens can cause serious health hazards including food poisoning and other foodborne diseases [12,13]. The proliferation of foodborne pathogens and the resultant outbreak of foodborne illness in Ready-to-eat food is a consequence of the mode of preparation and handling. Food handlers can contaminate RTE food through poor hygiene or by cross-contamination while handling contaminated food [14].

S. aureus, an opportunistic and ubiquitous pathogen of the nose, skin and hair of humans and animals is a predominant cause of foodborne diseases in humans [15]. After initial contact with *S. aureus* by the food vendors, it can survive on the hands and environmental surfaces for a prolonged period. *S. aureus* causes food poisoning by the production of several enterotoxins which are vastly stable and extremely resistant to heat and other environmental conditions such as drying and freezing. These characteristics enhance the proliferation of *S. aureus* in foodstuffs; temperature range of 48.5ºC to 70°C with an optimum temperature of 30ºC to 37ºC, pH of 4.2 to 9.3 having optimum of 7 to 7.5 and a 15% Sodium Chloride concentration [16,17].

The opportunistic nature of *S. aureus* and the fact that it is a normal skin flora contributes to its prevalence as a common pathogen variously implicated in cases of food poisoning alongside its heat stability. The hazards associated with RTE foods cannot be overemphasized; *Staphylococcus aureus*, *Klebsiella sp., Salmonella sp., Escherichia coli* amongst other bacterial pathogens have been isolated from RTE foods such as moi-moi [18], meat-pie [4], roasted plantain [19] and vegetable burger [20].

Patients with Staphylococcal infections had a better prognosis with the introduction of Penicillin. However, after decades of clinical usage, resistance developed due to the production of the β-lactam enzyme. The antibiotic resistance of *S. aureus* has become a major concern following the emergence of MRSA (methicillin-resistant *S. aureus*) and CA-MRSA (community-acquired MRSA) [15].

The incidence of MRSA was first grappled with in the 1960s, and in 2005 about 100,000 critical MRSA infections occurred including approximately 19,000 deaths directly related to MRSA as compared with the 17,000 deaths from HIV/AIDS. Overall, there was a two-fold increase in hospitalizations due to MRSA between 1999 and 2005 [21].

Up to this present time, several studies have reported the isolation (with variable occurrences) of MRSA from wild animals,livestock and derived foods, both ready-to-eat and raw, in addition to professionals working in the animal husbandry or the food production chain settings [22,23].

Methicillin-resistant *S. aureus* indicates a key public health challenge as effective therapeutic options are becoming limited [24]. Outbreaks caused by drug-resistant food-borne microorganisms are now not considered a rising main issue; instead, they constitute a founded difficulty. Consequently, antimicrobial resistance renders the corresponding antimicrobials ineffective in treating such infections, leading to serious public health problems [25]. The presence of bacteria that is resistant to antibiotics in sea-food is not only a threat to human health but can also result in the transfer of resistant genes to other clinically important bacteria [26].

The available data on foodborne outbreaks due to antimicrobial-resistant bacteria are most of the time insufficient. Quite often the isolated microorganisms are not subjected to antimicrobial susceptibility testing and even when tested, reporting the outcome is not mandatory to the Public health authorities [27].

There is a dearth of information on the prevalence and susceptibility pattern of Methicillin-Resistant *S. aureus* in Ready to Eat *Corbiculid heterodont* in Bayelsa State.Accurate susceptibility testing of *S. aureus* isolates and screening of ready-to-eat foods for colonisation with MRSA are important tools to limit the spread

of this organism. The aim of this research was to investigate the Prevalence of Methicillin-Resistant *S. aureus* in ready-to-eat *C. heterodont,* detection of *mecA* genes and their susceptibility pattern to commonly used antibiotics to expose the public health hazards associated with its consumption.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was performed in Yenagoa (4º55'44.909"N, 6º18'31.705"E), Southern Ijaw (4º30'41.524"N, 5º42'35.056"E) and Sagbama (5º04'45.572"N, 6º06'07.364"E) Local Government Areas, Bayelsa State, Nigeria.

2.2 Sample Collection

A total of 53 commercially available ready-to-eat *C. heterodont* (smoke-dried, fried and stewed as seen in Plates 2 and 3) were bought from marketers in the three local government areas, Bayelsa State. All samples were collected and wrapped in sterile sampling bottles and transported in ice chests to the laboratory for analysis.

Plate 2. Stewed (Right) and fried (left) RTE *C. heterodont*

Plate 3. *Smoked Dried C. heterodont*

2.3 Isolation, Preservation and Biochemical Identification of *S. aureus*

Staphylococcus aureus was isolated with Mannitol Salt agar using the spread plate method by [28]. The isolates were preserved in 10% v/v glycerol at -4^{o}C until required for further tests. Biochemical tests such as Gram staining, catalase, coagulase and sugar utilization tests were carried out to confirm *S. aureus* [29].The preserved isolates were used for molecular identification of the methicillin-resistance gene (*mecA*).

2.4 Detection of MRSA by Disk Diffusion Method

The *S. aureus* isolates were screened for methicillin resistance by testing them with 30µg Cefoxitin and 1µg oxacillin disks according to [30] Guidelines. The results were reported as Methicillin-resistant or susceptible based on the cefoxitin results. Methicillin is no longer commercially available in many countries due to its toxicity and it does not maintain its activity during storage. Cefoxitin is a more reliable inducer of the *mecA* gene, and disk diffusion tests using cefoxitin are easier to read as they give clearer endpoints. The isolates were considered methicillin-resistant if the diameters of the zones of inhibition for cefoxitin were ≤21 mm and susceptible if ≥22 mm.

2.5 Molecular Identification

2.5.1 Extraction of DNA

The extraction of DNA was carried out using the Boiling method. Twenty-four hours old pure culture of the *S. aureus* Isolates were put in Luria-Bertani (LB) Broth and allowed to incubate at 37^{o}C. After 24hours, the cells were washed by pipetting 0.5 ml of the cultured broth into properly labeled Eppendorf tubes and filling to mark with normal saline. This was centrifuged for 3minutes at 14,000rpm after which the supernatant was decanted leaving the DNA at the base. The DNA was washed with 1 ml of normal saline and vortexed to properly mix and then centrifuged again. This process was repeated 3times. The bacterial suspension in 500 µl of normal saline was heat blocked at 95ºC for 20minutes after which cooled on ice, centrifuged for 3 mins at 14000rpm. Using a 1.5 ml Eppendorf tube the supernatant containing the DNA was transferred and stored at 20ºC for other downstream processes [31].

2.5.2 DNA quantification

The quantification of the extracted DNA was carried out using the Nanodrop 1000 Spectrophotometer which not only evaluated the quantity of Genomic DNA but the quality using the Beer Lambert's principle. The Nanodrop spectrophotometer was connected to a computer which had the Nanodrop software installed. The sample pedestals were properly cleaned. The instrument was initialized using a 2µl of sterile distilled water and blanked using 2µl of Normal Saline. To measure the concentration of the sample, the extracted DNA was dropped into the lower pedestal, then the upper pedestal was brought down to make contact with the DNA on the lower pedestal. Finally, the DNA concentration was measured by clicking the measure button on the computer screen.

2.5.3 16S rRNA amplification

The 16S rRNA Amplification was done using an ABI 9700 Applied Biosystems Thermal Cycler. The 16S rRNA region of the rRNA genes of *S. aureus* isolates were amplified using the forward primer; 27F: 5' AGAGTTTGATCMTGGCTCAG-3' and Reverse Primer 1492R: 5'-CGGTTACCTTGTTACGACTT-3' at a final Volume of 30µl for 35cycles. The PCR cocktail was prepared using the primers at 0.6µM concentration, the Template (the extracted DNA), Buffer 1X, water, PCR Mix (15M) which consists of; dNTPs, MgCl, and Taq Polymerase. The conditions for PCR were as follows; Initial denaturation, 95ºC for 5minutes; Denaturation, 95ºC for 30 Seconds; Annealing 52ºC for 30 seconds; Extension, 72ºC for 5minutes. The product was fixed in a 1% agarose gel at 120V for 15minutes and visualized on a UV transilluminator [32].

2.5.4 DNA sequencing

Sequencing was performed using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria, South Africa. The sequencing was done at a final volume of 10µl; the components included 0.25µl BigDye® terminator v1.1/v3.1, 2.25µl of 5x BigDye sequencing buffer, 10µM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10seconds, 55°C for 5 seconds and 60°C for 4 minutes.

2.6 Phylogenetic Analysis

The bioinformatics algorithm Trace edit was used to edit the obtained sequences; BLASTN was used to download sequences that were alike from the National Center for Biotechnology Information (NCBI) database.These sequences were aligned using MAFFT. The evolutionary history was deduced using the Neighbor-Joining method in MEGA 6.0 [33]. The bootstrap consensus tree inferred from 500 replicates [34] is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [35].

2.7 Antibiotic Susceptibility Testing

Twelve commonly used antibiotic discs (µg/disc) which include: Amoxacillin (AM)- 30µg, Ampicillin-Cloxacillin (APX) 30µg, Cefoxitin (CEF) 30µg, Ciprofloxacin (CPX) 10 µg, Erythromycin (E) 10 µg, Gentamycin (CN) 10 µg, Oxacillin (OX) 1µg, Pefloxacin (PEF) 10 µg, Ceftriaxone (Rocephine) (R) 25 µg, Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (SXT) 30µg, Streptomycin (S) 30 µg, and (Zinnacet) Cefuroxime (Z) 20 µg were tested. The antimicrobial susceptibility profiles of the isolates were determined by the Kirby-Bauer disk diffusion method [36] on sterile Mueller-Hinton agar. Inoculum which was taken from fresh over-night cultures corresponding to 0.5 McFarland standard was swabbed evenly across the plates in 3 directions with sterile swab sticks. The antibiotic discs were aseptically placed using a sterile forceps after 3-5minutes. The plates were inverted within 30 minutes of applying and incubated for 16-18hours at 35°C. The experiments were done in duplicates, the zones of inhibition were measured and interpreted with [30] standards.

2.8 Amplification of *mecA* Genes

MecA genes from the isolates were amplified using the *mecA*Forward: 5' AAAATCGATG-GTAAAGGTTGGC-3' and *mecA* Reverse: 5'-AGTTCTGCAGTACCGGATTTTGC-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: The X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, dNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 55ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel prepared with EZ vision dye at 120V for 25 minutes and visualized on a blue light trans-illuminator for a 500bp product size [31].

2.9 Determination of Multiple Antibiotic Resistance Index (MAR)

Multiple antibiotic resistance for this study is defined as resistance of *S. aureus* isolate to three or more antibiotics [37]. Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula MAR = a/b, where a stands for the number of antibiotics to which the test isolate depicted resistance and b stands for the total number of antibiotics to which the test isolate has been evaluated for susceptibility [38].

2.10 Data Analysis

Statistical Package for the Social Sciences (SPSS) version 22 was used to analyze the data obtained. The summary of the data was done using descriptive statistics and graphical representation.

3. RESULTS

3.1 Molecular Identification of *Staphylococcus aureus*

Results of Agarose gel electrophoresis on Plate 4 shows the amplified 16S rRNA gene bands at 1500bp. Lane L represents the 100 bp molecular ladder. The obtained 16S rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of isolates 1, 2, 3, 4, 5, 6 and 8 showed a 100% similarity to *S. aureus*. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16SrRNA of the isolates within the *Staphylococcus spp.* and revealed a close relatedness to *S. aureus.*

The 16S rRNA of the isolate 7 & 9 showed a percentage similarity to other species at 91.8% and 99% respectively. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Staphylococcus spp.* and revealed a close relatedness to *S. warneri* and *S. sciuri* (MG813731.1) respectively than other *Staphylococcus spp.* (Fig. 1).

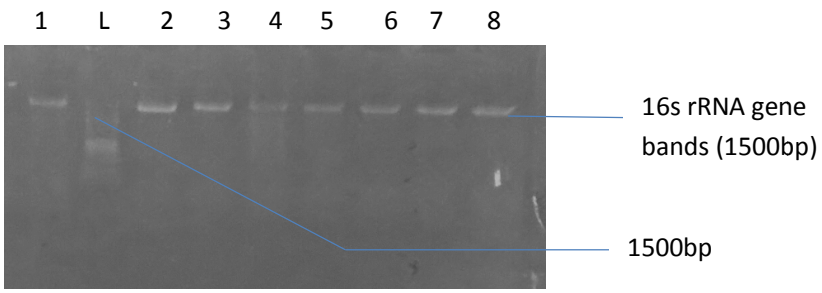

Plate 4. Agarose gel electrophoresis showing the amplified 16S rRNA gene bands at 1500bp

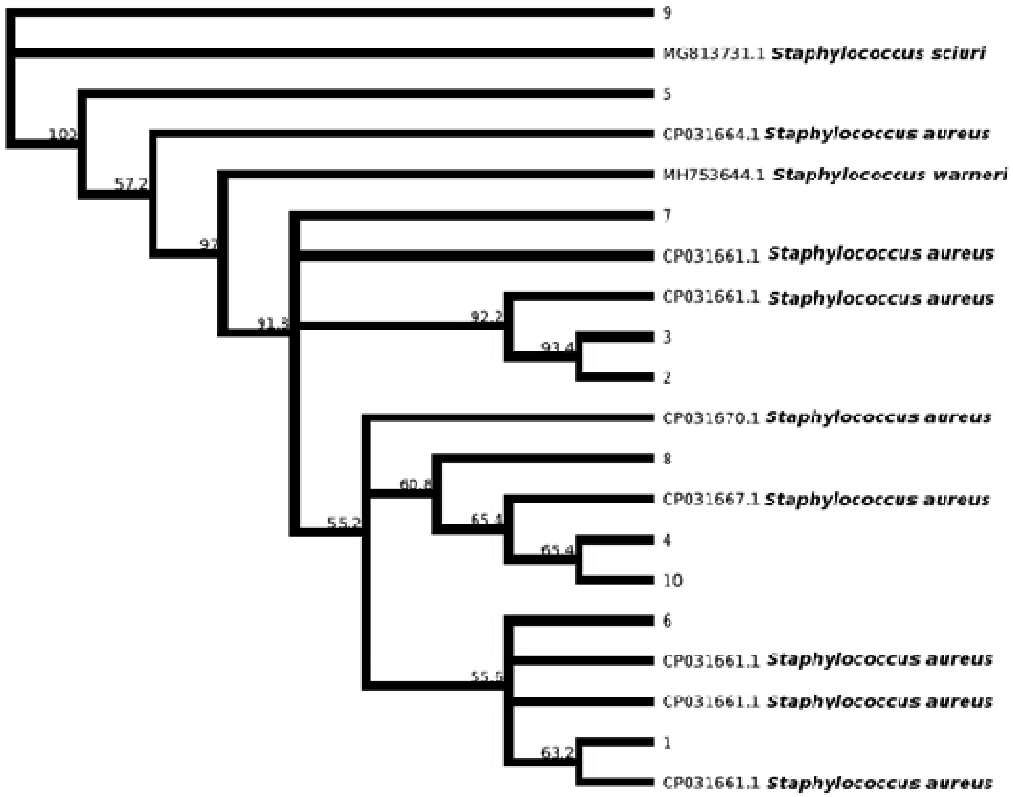

Fig. 1. Phylogenetic tree showing evolutionary relatedness between isolates

3.2 Prevalence of *S. aureus* from RTE *C. heterodont*

Results of prevalence of *S. aureus* from RTE *C. heterodont* revealed that out of a total of 70 *Staphylococcus* spp. isolates, recovered from the Local Government Areas, 92.85% were *S. aureus* and 7.15% were Coagulase Negative Staphylococcus (CONS) of these, Yenagoa Local Government had the predominant 45.71% and Sagbama L.G.A had the least occurrence 24.62% (Table 1).

3.3 Identification of Methicillin-Resistance *Staphylococcus aureus*

The isolates that persisted in the presence of oxacillin and cefoxitin with zones of inhibition ≤21 mm for cefoxitin discs were considered as Methicillin-Resistant. The Methicillin-Resistant *S. aureus* were 35 isolates out of the 65 *S. aureus* isolates with the majority from Yenagoa 15(23.08%) and Sagbama 14 (21.54%) Local Government Areas (Table 2).

Table 1. Frequency distribution of *Staphylococcus* spp., *Staphylococcus aureus* and coagulase negative staphylococcus from RTE *C. heterodont* from different LGAs in Bayelsa State Nigeria

Source L.G.A	*Staphylococcus* spp. n (%)	*S. aureus* n (%)	CONS n (%)
Sagbama	17(24.29)	16(22.85)	1(1.43)
Southern- Ijaw	18(25.71)	17(24.29)	1(1.43)
Yenagoa	35(50.00)	32(45.71)	3(4.29)
Total	70(100)	65(92.85)	5(7.15)

Key: CONS: Coagulase Negative Staphylococcus

Table 2. Frequency distribution of MRSA from RTE *C. heterodont* from different LGAs in Bayelsa State Nigeria

L.G.A	Number	% Occurrence
Sagbama	14	21.54
Southern Ijaw	6	9.23
Yenagoa	15	23.08
Total	35	53.85

3.4 Susceptibility Pattern of MRSA Isolated from RTE *C. heterodont*

Results of susceptibility pattern of MRSA isolates as presented in Table 3 revealed that the isolates showed 100% resistance to Ampicillin-Cloxacillin, Amoxacillin, Cefoxitin and oxacillin with 82.9% resistance to Streptomycin. The susceptibility was highest with Gentamycin (80%), followed by Trimethoprim-Sulfamethoxazole (77.1%) Ceftriaxone (60%).

3.5 Detection of *mecA* Genes

Ten Methicillin-Resistant *Staphylococcus aureus* isolates were further characterized and examined for *mecA* gene using Polymerase Chain Reaction. Fifty percent of the isolates showed positive for the *mecA* gene as seen on Plate 5. The bands at 500bp; Lanes 1, 4, 5, 6, 8 showed *mecA* bands while Lane L represents the 100bp molecular ladder.

3.6 Multi-Antibiotics Resistant Indices of *S. aureus*

Out of the 65 *S. aureus* isolates, 62 (95.4%) had multidrug resistance index of > 0.2 while 3 isolates (4.6%) had 0.2 as their Multidrug-resistance index (Table 4).

3.7 Multidrug-Resistant Isolates among Methicillin-Resistant *S. aureus* and Methicillin Sensitive *S. aureus*

Methicilin-Resistant isolates were 100% Multi-Drug Resistant while 27 (90%) isolates amongst the Methicillin-Sensitive *Staphylococcus aureus were* Multidrug-resistant giving a 90% MDR prevalence for MSSA (Table 5).

Table 3. Antibiotics susceptibility pattern for methicillin-resistant *S. aureus* isolated from RTE *C. heterodont*

Antibiotics	Concentration (µg)	Resistance n (%)	Intermediate n (%)	Susceptible n(%)
Ampicillin-Cloxacillin	30	35100)	0 (0.00)	0 (0.00)
Amoxacillin	30	35(100)	0 (0.00)	0 (0.00)
Gentamycin	10	3(8.6)	4(11.4)	28(80)
Ciprofloxacin	10	6(17.1)	29 (82.9)	0 (0.00)
Erythromycin	10	1 (2.9)	34 (97.1)	0 (0.00)
Cefoxitin	30	35100)	0 (0.00)	0 (0.00)
Oxacillin	10	35(100)	0 (0.00)	0 (0.00)
Pefloxacin	10	3(8.6)	31(88.6)	1(2.8)
Rocephin (Ceftriaxone)	25	14(40)	0 (0.00)	21(60)
Streptomycin	30	29(82.9)	0 (0.00)	6(17.3)
Trimethoprim-sulfamethoxazole	30	6 (17.3)	2(5.7)	27(77.1)
Zinnacef (Cefuroxime)	20	11(31.4)	20 (57.2)	4(11.4)

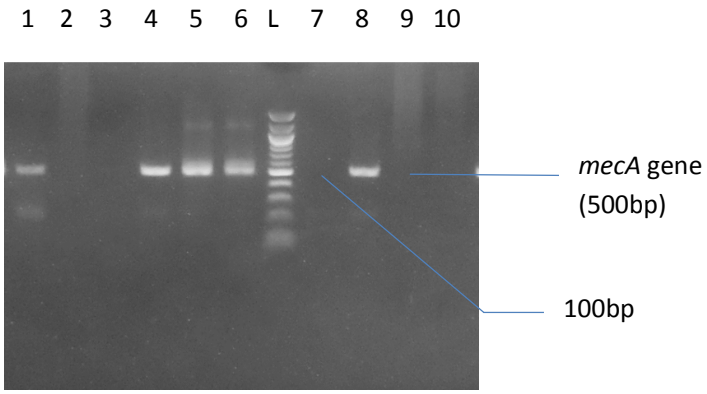

Plate 5. Agarose gel electrophoresis showing the amplified *mecA* Gene bands at 500bp

Table 4. MAR indices of *Staphylococcus aureus* (N=65)

MAR index	Number (%)
0.2	3 (4.6)
0.3	10(15.4)
0.4	23(35.4)
0.5	15 (23.0)
0.6	12 (18.5)
0.7	2 (3.1)

Table 5. Multidrug-resistant isolates among methicillin-resistant *Staphylococcus aureus* and methicillin–susceptible S*taphylococcus aureus*

Multi-Drug resistant isolates	MRSA n(%)	MSSA n(%)
MDR	35(100)	27(90)
NON-MDR	0(0.00)	3(10)
Total	35(100)	30(100)

4. DISCUSSION

The presence of *Staphylococcus* spp. was established in RTE *C. heterodont* sold in Bayelsa state, Nigeria. Seventy isolates consisting of *S. aureus* and coagulase negative *Staphylococcus* spp. were detected in this important delicacy. Other studies have also isolated *Staphylococcus* spp. from ready-to-eat foods [39,40,41]. RTE foods in Nigeria have also been associated with staphylococci contamination [42]. Moreso, *Staphylococcus* spp. have been detected in RTE food in Bayelsa State [4]. The presence of *Staphylococcus* spp. has been blamed on a myriad of factors, including handling [39]. Apart from some detrimental consequences in food, staphylococci has been shown to be beneficial in food production [41] suggesting that staphylococci thrive in food.

The presence about 50% of the isolated *S. aureus* in Yenagoa L.G.A presents a great worry. Yenagoa is the capital city of Bayelsa state and routes of contamination in relation to the display methods could explain the higher incidence of *S. aureus* as well as CONS in this location. The result of this study is logical considering the population of these LGAs assuming the contaminations routes are the same. Yenagoa has a population of about 385,000 Southern Ijaw 267,000 while Sagbama is about 187,000 [43]. These figures support the results obtained from this study based on the contamination routes including handling and hygiene conditions. The RTE foods are displayed in trays and could be handled in attempts to purchase this product. Price bargain usually precedes purchase attempts with the possible oral aerosol also dropping on the exposed foods. Staphylococci are normal flora in man and its presence has been established in the mouth as well as other body parts including the skin [44]. This level of contamination is worrisome considering the role of *S. aureus* in public health issues, especially antibiotic resistance [39,45]. CONS were once

neglected but recently considered relevant reservoirs of antibiotic resistance genes and proven pathogenic [41]. The presence of both *S. aureus* and CONS in RTE *C. heterodont* widely consumed in Bayelsa State therefore poses an appreciable risk to consumers in terms of infection and difficulty in treating same.

The high prevalence of *S. aureus* in Ready-to-eat *C. heterodont* shows that *S. aureus* could serve as potential vehicles for transmission of resistant strains of pathogens as clearly demonstrated by the results of this study showing *S. aureus* having a 100% resistance to the Penicillins (Amoxacilin and Ampicillin-Cloxacillin), 80% resistance to Streptomycin, 53.8% to cefoxitin and 52.3% to Ceftriaxone.

The Results of Susceptibility patterns of MRSA to antibiotics showed a decreasing trend of resistance in the order: Amoxicillin, Ampicillin-Cloxacillin, Cefoxitin, and Oxacillin (100%)> Streptomycin (82.9%) >Ceftriaxone (40%)>Cefuroxime (Zinnacef) (31.4%), > Ciprofloxacin and Trimethoprim-Sulfamethoxazole (17.1%) > Gentamycin and Pefloxacin (8.6%), >Erythromycin (1%). A high percentage of the *MRSA* isolates were susceptible to Gentamycin (80%), Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (77.1%) and Ceftriaxone (Rocephine) (60%) (Table 3). This study showed that commonly used antibiotics such as Ampicillin-Cloxacillin (Ampiclox) and Amoxicillin are no longer reliable in treating staphylococcal infections in this region as clearly seen in the 100% resistance of the isolates to these antibiotics. Further, the high resistance to the beta-lactam antibiotics can be explained by the extensive and uncontrolled use of these antibiotics as well as affordability. This result agrees with the results of [46] which showed high resistance to Ampicillin-Cloxacillin (Ampiclox) and Penicillin and high susceptibility of *S. aureus* to gentamycin as observed in this study. In another study by [47], some street vended foods in Ogun State Nigeria also showed the highest susceptibility of *S. aureus* to gentamycin. Results from [48] was also similar showing 100% susceptibility of *S. aureus* from dairy milk to gentamycin, and 64.29% to Trimethoprim/ Sulfamethoxazole. Previous works on *S. aureus* isolates from stool samples of 120 subjects from Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State showed a high susceptibility of *S. aureus* to gentamycin which is similar to findings of this study [49]. They also observed a 34.2% Methicillin-Resistance in their isolates which is also similar to findings in this study. In a similar study, [50] observed that out of a total of 200(29%) *S. aureus* isolated from retailed ground meats, their antibiogram to commonly used antibiotics revealed that 69% of the isolates were resistant to tetracycline, 26% to penicillin, 17% to ampicillin, 13% to methicillin, 8% to erythromycin, 4.5% to clindamycin, 1.5% to Gentamycinand 0.5% to chloramphenicol, oxacillin and cefoxitin. while 27% were susceptible to all tested antimicrobials.

This study further confirmed the presence of *mecA* gene in up to 50% of the *S. aureus* isolates. The inability of the procedure to show the *mecA* gene in all may be blamed on an array of factors. Some important factors that may have led to the absence of *mecA* gene include storage and subculturing of the isolates [51]. According to [51], inability to detect the *mecA* gene by Agarose Gel electrophoresis may also be due to the presence of other variants of this gene making it impossible for the chosen primer to detect same. It is noteworthy that antibiotic resistance has thrived due to ability of resistant organisms to mutate at any opportunity [52]. This mutation makes it impossible for antimicrobials to locate target sites/processes of action for the desired effect. An instance is the reported Vancomycin intermediate-resistant *Staphylococcus aureus* (VISA) [52]. Vancomycin was discovered a drug of choice in the 1990s with cell wall synthesis targeted. However, [52] report resistance to this drug of choice based on mutation. Further, the absence of *mecA* genes in up to 50% of *S. aureus* isolated from this study is not a total relief. Loss of *mecA* gene could be compensated for in these organisms and other mechanisms deployed for antibiotic resistance. For example, β lactamase production has been used for antibiotic resistance on organisms lacking the *mecA* gene [53].

Appreciable Multiple Antibiotic Resistance (MAR) indices were recorded in the present study. A MAR index greater than 0.2 was recorded for 95.4% of all isolates tested [38]. This is indicative of a high risk source of contamination and an environment where antibiotic is often used. Previous studies from Goldberg et al. [54] made similar observation. The antibiotic resistance gene which accounts for MRSA's resistance to several classes of antibiotics is found on insertion sites for plasmids and transposons in *mecA* complex of MRSA [55]. The presence of Multi-drug resistance strains also shows how *S. aureus* is developing different

methods to resist antibiotics effectiveness. This is making therapeutic options more limited and expensive. The cost of treating MRSA infections is no doubt of great economic importance and hence more care should be taken to curb the transmission of resistant strains. However, MRSA showed highest susceptibility to Gentamycin and Trimethoprim-Sulfamethoxazole indicating that they can serve as alternative treatment for foodborne staphylococcal infection instead of the Penicillins. This result is similar to Goldberg et al. [56] who showed a high efficacy of Co-trimoxazole similar to that of Vancomycin for cases of Multidrug-Resistant *S. aureus*.

5. CONCLUSION AND RECOMMENDATIONS

RTE *C. heterodont* sold in Bayelsa state is appreciably contaminated with Methicillin-Resistant *S. aureus.* The presence of these organisms in the food is a public health hazard with enormous effects. Some life threatening diseases have been associated with MRSA and its resistance to most known antimicrobials makes the consequences of *S. aureus* infection/infestation worse. Improved hygiene, better preparation methods and awareness could improve the quality of this important snack and reduce public health risks. Finally, since this is the first time *Staphylococcus scuiri* and *Staphylococcus warneri* have been isolated from ready-to-eat *C. heterodont* in Bayelsa state, Nigeria further research should be done on their presence and role as foodborne pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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