

**PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF
STAPHYLOCOCCUS AUREUS ISOLATED FROM READY-TO-EAT FRESHWATER
BIVALVE (*CORBICULID HETERODONT*) IN BAYELSA STATE, NIGERIA.**

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ABSTRACT

There has been a significant increase in antimicrobial resistance amongst foodborne pathogens over the past few decades. The increase in antibiotic resistance is associated with foodborne diseases resulting from the consumption of contaminated ready-to-eat foods. This study was carried out to investigate the prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* in ready-to-eat *Corbiculid heterodont* in Bayelsa State. A total of 53 samples were collected from 3 Local Government areas; Sagbama, Southern Ijaw, and Yenagoa and analyzed using standard microbiological methods. There was no difference ($p \geq 0.05$) in the mean Staphylococcal count with Yenagoa having the lowest count ($6.08 \pm 2.07 \log_{10} \text{cfug}^{-1}$); Sagbama ($6.37 \pm 1.9 \log_{10} \text{cfug}^{-1}$) and Southern Ijaw, the highest ($6.71 \pm 1.76 \log_{10} \text{cfug}^{-1}$). A total of 65 isolates of *S. aureus* were obtained with incidence ranging from 24.62% in Sagbama; 26.15% in Southern Ijaw and 49.23% in Yenagoa. Although isolates showed varied susceptibility pattern with a decreasing trend of resistance in this order; Amoxicillin and Ampiclox (100%) > Streptomycin (80.0%) > Rocephin (53.8%) > Zinnacef (33.8%) > Erythromycin (16.9%) > Ciprofloxacin and Trimethoprim-Sulfamethoxazole (15.4%) > Gentamycin (10.8%), no difference ($p \geq 0.05$) was observed in the various local government areas sampled except for Ciprofloxacin ($p \leq 0.05$). The extent of antibiotic resistance reflects the heterogeneous use and abuse of antibiotics in this region hence to adequately know the pattern of multidrug resistance, regular surveillance is required.

KEYWORDS: Ready-to-eat food, *Corbiculid heterodont*, *Staphylococcus aureus*, Foodborne Disease and Antimicrobial Resistance.

INTRODUCTION

Consumers' health in many parts of the world have been at risk of the contamination of street vended foods (FAO, 2009; INFOSAN, 2010). Foodborne pathogens can cause serious health hazards including food poisoning and other foodborne diseases (FAO, 2009). 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 hand hygiene or by cross-contamination while handling contaminated food (Verhaelen *et al.*, 2013).

Staphylococcus aureus, an opportunistic and ubiquitous pathogen of the nose, skin and hair of humans and animals is one of the predominant causes of foodborne illnesses in humans. (Lekshmi *et al.*, 2016). After initial contact with *S. aureus* by the food vendors, it can survive on the hands and environmental surfaces for a prolonged period. *Staphylococcus 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.

The growth of *S. aureus* in food products is enhanced by these characteristics; temperature range of 70°C to 48.5°C with an optimum temperature of 30°C to 37°C, pH of 4.2 to 9.3 having optimum of 7 to 7.5 and a 15% Sodium Chloride concentration (Chaibenjawong and Foster, 2011).

The opportunistic nature of *Staphylococcus aureus* and the fact that it is a normal skin flora contributes to its prevalence as a common pathogen for the outbreak 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 (Akani and Vareba, 2017), meat-pie (kigigha *et al.* 2017), roasted plantain (Opara and Elijah, 2017), Vegetable Burger (kaur and Kahlon 2017).

Patients with Staphylococcal infections had a better prognosis with the introduction of Penicillin. However, after several years of clinical use resistance developed owing to the production of β -lactamases. 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) (Kumar *et al.*, 2016). Methicillin-resistant *Staphylococcus aureus* represents a major public health challenge as effective therapeutic options are becoming limited (Sandrine *et al.*, 2008). Outbreaks caused by drug-resistant foodborne 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 (EFSA and ECDC 2017).

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 tests are implemented, the reporting of the outcome is not mandatory to the Public health authorities (WHO 2014).

Corbiculid heterodont, a popular shellfish bivalve seen in the southern part of Nigeria especially in Bayelsa state, it is a delicacy and serves as a rich source of protein (Kigigha, *et al.*, 2013). 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 and can hence be referred to as a Ready-to-eat-food (RTE) or a street-vended food.

This study was carried out to investigate the prevalence of foodborne *Staphylococcus aureus* in ready-to-eat *Corbiculid heterodont* and their susceptibility pattern to commonly used antibiotics with a view to expose the public health hazards associated with its consumption and recommend possible drug of choice.

MATERIALS AND METHODS

Study Area

The study was carried out in three Local government areas in Bayelsa state; Yenagoa (4°55'44.909"N, 6°18'31.705"E), Southern Ijaw (4°30'41.524"N, 5°42'35.056"E) and Sagbama (5°04'45.572"N, 6°06'07.364"E) local government areas.

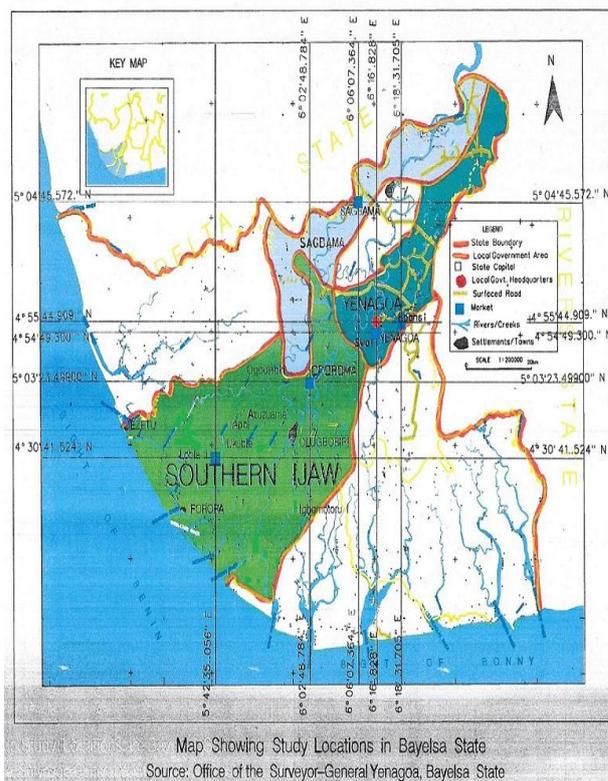


Figure 1: Map Showing Study Locations In Bayelsa State.

Source: Office of the Surveyor-General, Yenagoa, Bayelsa State.

Sample Collection

Samples of ready-to-eat shellfish (smoke-dried, fried and stewed) were collected with sterile sampling bags from the marketers in three different local government areas in Bayelsa state. A total of 53 samples were taken randomly (17 from Sagbama L.G.A, 13 from Southern Ijaw L.G.A, and 23 from Yenagoa L.G.A). The samples were put in an ice-chest and transported to the Microbiology laboratory of Rivers State University for analysis.

Microbiological Analysis Enumeration of Bacteria

The enumeration of Total Viable Bacteria Count was carried out on Nutrient Agar, Coliform count on MacConkey agar and Staphylococcal count on Mannitol salt agar according to the method of the American Public Health Association (APHA, 2001). The preparation of the stock analytical unit was done by weighing ten (10) grams of the shellfish and homogenizing in 90ml of sterile normal saline. Subsequent serial dilution was done by pipetting 1ml of the sample into 9ml of sterile normal saline for up to 6 dilutions. 0.1ml aliquot of the appropriate dilutions (10^{-5} and 10^{-6}) was inoculated in duplicates into already prepared sterile plates of Mannitol salt agar, Nutrient agar and MacConkey agar using the spread plate technique and finally incubated at 37°C for 24hours (Midura and Bryant, 2001).

Isolation of *Staphylococcus aureus*

Staphylococcus aureus was isolated with Mannitol Salt agar using the spread plate method by APHA 2001 (Lancette and Bennett, 2001). Biochemical tests such as Gram staining, catalase, coagulase and sugar utilization tests were carried out to confirm *S. aureus* (Cheesbrough, 2006).

Antibiotic Susceptibility Testing

Ten commonly used antibiotic discs ($\mu\text{g}/\text{disc}$) which include: Amoxicillin (AM)- 30 μg , Ampiclox (APX) 30 μg , ciprofloxacin (CPX) 10 μg , Erythromycin (E) 10 μg , Gentamycin (CN) 10 μg , Pefloxacin (PEF) 10 μg , Rocephin (R) 25 μg , Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (SXT) 30 μg , Streptomycin (S) 30 μg , and Zinnacef (Z) 20 μg were tested. The antimicrobial susceptibility profiles of the isolates were determined by the Kirby-Bauer disk diffusion method (Bauer et al., 1996) 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 3-5 minutes. The plates were inverted within 30 minutes of applying and incubated for 16-18h at 35 $^{\circ}\text{C}$. (Clinical and Laboratory Standard Institute, 2017). The experiments were done in duplicates, the zones of inhibition were measured and compared with CLSI 2017 standards.

Data Analysis

Statistical Package for the Social Sciences (SPSS) version 22 was used to analyze the data obtained from

Table 1: Distribution of Microbial Types in *C. heterodont* in the Various Local Government Areas Sampled in Bayelsa State Nigeria.

LGA sampled	Microbial Types		
	THB ($\log_{10}\text{cfug}^{-1}$)	TSC ($\log_{10}\text{cfug}^{-1}$)	TCC ($\log_{10}\text{cfug}^{-1}$)
Sagbama	7.17 \pm 1.74 ^a	6.37 \pm 1.90 ^a	6.25 \pm 2.13 ^a
Southern IJAW	7.22 \pm 1.83 ^a	6.71 \pm 1.76 ^a	5.83 \pm 2.13 ^a
Yenagoa	7.13 \pm 1.81 ^a	6.08 \pm 2.07 ^a	5.58 \pm 2.17 ^a

Key: THB (Total Heterotrophic Bacteria), TSC (Total Staphylococcal count), TCC (Total Coliform Count). *Mean with the same superscript along the columns is not significantly different ($p \leq 0.05$)

The mean Staphylococcal count showed Yenagoa with the lowest count (6.08 \pm 2.07 $\log_{10}\text{cfug}^{-1}$) then Sagbama (6.37 \pm 1.9 $\log_{10}\text{cfug}^{-1}$) and finally Southern Ijaw with the highest count (6.71 \pm 1.76 $\log_{10}\text{cfug}^{-1}$). This range of

the aerobic plate count and measurement of zones of inhibition on isolates from each RTE *C. heterodont* sample. The data were summarized using descriptive statistics for tabulation and graphical representation. Analysis of Variance (ANOVA) was used to test for significant difference ($p \geq 0.05$) between Local Government Areas. Where differences existed, Tukey method was used to separate the means.

RESULTS AND DISCUSSION

The present study showed that out of the 53 samples of *C. heterodont*, the mean of total heterotrophic bacterial count was highest in Southern Ijaw local government area followed by Sagbama then Yenagoa Local government area ranging from 7.13 \pm 1.81 $\log_{10}\text{cfug}^{-1}$ to 7.22 \pm 1.83 $\log_{10}\text{cfug}^{-1}$. The total Coliform count was in this order Sagbama (6.25 \pm 2.13 $\log_{10}\text{cfug}^{-1}$) > Southern Ijaw (5.83 \pm 2.13 $\log_{10}\text{cfug}^{-1}$) > Yenagoa (5.58 \pm 2.17 $\log_{10}\text{cfug}^{-1}$). The high Total bacterial counts could be a result of the movement of the food by the vendors throughout the market thereby exposing the *C. heterodont* to not only the microorganisms in the air but to also microbes on the hands of various customers that handle it before purchase. There was no significant difference ($p \leq 0.05$) in the microbial types amongst the various local Government areas due to their similar cultural practices and the proximity of these areas.

values is similar to work done by Opara and Elijah (2017) on Street-Vended Roasted plantain in Bayelsa state.

Table 2: Distribution of Microbial Types associated with different preparation methods of RTE *C. heterodont*.

Method of Preparation	Microbial Types		
	THB ($\log_{10}\text{cfug}^{-1}$)	TSC ($\log_{10}\text{cfug}^{-1}$)	TCC ($\log_{10}\text{cfug}^{-1}$)
Drying	7.29 \pm 1.57 ^a	6.04 \pm 2.03 ^a	4.83 \pm 1.93 ^a
Frying	8.22 \pm 0.59 ^a	7.06 \pm 1.48 ^a	7.24 \pm 1.70 ^b
Stewing	7.82 \pm 1.16 ^a	6.67 \pm 1.79 ^a	7.86 \pm 0.32 ^b

Key: THB (Total Heterotrophic Bacteria), TSC (Total Staphylococcal count), TCC (Total Coliform Count). *Mean with the same superscript along the columns is not significantly different ($p \geq 0.05$).

Corbiculid heterodont is usually prepared as fried or stewed kebab and sold in trays by street Vendors. It is also smoke-dried and sold in buckets. The results showed that the stewed and fried ones had the same pattern in their total Coliform counts different from that of the dried ones. The count of the dried shellfish was lowest for both staphylococcal ($6.04 \pm 2.03 \log_{10} \text{cfug}^{-1}$) and the coliform count ($4.83 \pm 1.93 \log_{10} \text{cfug}^{-1}$) indicating that the fried and stewed were more predisposed to contamination as their mode of preparation should be brought into question as to how hygienic the utensils and the source of water used for preparation. The activity of heat in the process of drying the shellfish reduces the water content and also destroys bacteria. However, post-processing contamination can occur especially during handling and transportation of RTE foods to their points of sale (Odu *et al.*, 2012)

However, *Staphylococcus aureus* has the ability to remain alive in potentially dry and stressful environment, hence it is a desiccation tolerant organism with a wide range of temperature (7°C to 48.5°C ; optimum 30°C to 37°C), pH (4.2 to 9.3; optimum 7, to 7.5) and NaCl concentration up to 15% (Chaibenjawong *et al.*, 2011).

The results of this study showed that the staphylococcal counts in all L.G.A were higher than the microbiological limits as stated by International Commission for Microbiological Standards of food and the Centre for food safety guidelines for ready to eat foods which stated a count of $\geq 10^4 \text{cfug}^{-1}$ as unsatisfactory and potentially injurious to human health. The results were also similar with Odu *et al.*, 2012.

The distribution of microbial types amongst the different methods of preparing the shellfish; drying, frying and stewing showed that the dried *C. heterodont* had the least count of microorganisms, as well as the least number of *Staphylococcus aureus* and coliforms. The mean count of *S. aureus* was highest in the fried *C. heterodont*

($7.06 \pm 1.48 \log_{10} \text{cfug}^{-1}$) followed by the stewed ones ($6.67 \pm 1.79 \log_{10} \text{cfug}^{-1}$) then the dried *C. heterodont* had the least count ($6.04 \pm 2.03 \log_{10} \text{cfug}^{-1}$) of *Staphylococcus aureus*. The Coliform count showed a significant difference ($p \leq 0.05$) between the different method of preparation; the stewed *C. heterodont* had the highest mean coliform count of $7.86 \pm 0.32 \log_{10} \text{cfug}^{-1}$ then the fried *C. heterodont* with a mean count of $7.24 \pm 1.70 \log_{10} \text{cfug}^{-1}$ and finally the dried *C. heterodont* which had the least mean coliform count of $4.83 \pm 1.93 \log_{10} \text{cfug}^{-1}$.

Table 3: Frequency Distribution of *S. aureus* from RTE *C. heterodont* from Different LGA in Bayelsa State Nigeria.

L.G.A	Occurrence (n=65)	% Occurrence
Sagbama	16	24.62
Southern Ijaw	17	26.15
Yenagoa	32	49.23

The highest occurrence of *S. aureus* was seen in Yenagoa local government area having 49.23% followed by Sothern Ijaw with 26.15% then Yenagoa with 24.62%. This result is similar to work done by Kigigha *et al.*, 2017 on RTE food (meat pie) sold in Yenagoa metropolis which showed 48.8% occurrence of *Staphylococcus aureus*. This could be accounted for by the population Density in Yenagoa which has a population of 352,285 as at 2006 Census (Wikipedia, 2018) and the fact that *C. heterodont* was easily seen in the markets in Yenagoa and probably the handling and hygiene practices of the Street vendors. The high occurrence of

Staphylococcus aureus in R.T.E *Corbiculid heterodont* could also be a consequence of several sanitary factors such as; poor cleaning and hand hygiene, undercooking, poor quality of raw materials, cross-contamination and poor temperature and time control.

Table 4: Susceptibility pattern (using zones of inhibition) of *S. aureus* isolates to various antibiotics.

Sample Location	Antibiotics									
	APX (30µg)	AM (30µg)	CN (10µg)	CPX (10µg)	E (10µg)	PEF (10µg)	R (25µg)	S (30µg)	SXT (30µg)	Z (20µg)
Sagbama	14.9±4.2 ^a	0.0±0.00	14.9±1.2 ^a	17.9±2.3 ^a	17.8±2.5 ^a	18.1±2.1 ^a	13.6±3.1 ^a	15.4±5.1 ^a	18.4±5.3 ^a	12.9±5.3 ^a
Southern IJAW	16.4±3.4 ^a	0.0±0.00	15.0±1.5 ^a	0.5±2.9 ^{ab}	16.4±3.2 ^a	19.5±3.0 ^a	10.9±4.0 ^a	16.9±3.0 ^a	18.9±5.7 ^a	14.7±4.7 ^a
Yenagoa	15.5±3.2 ^a	0.0±0.00	14.9±2.3 ^a	18.6±2.5 ^c	17.4±3.3 ^a	18.8±1.9 ^a	13.4±3.7 ^a	16.2±4.2 ^a	18.5±3.1 ^a	16.3±4.5 ^a

*Mean with the same superscript along the columns is not significantly different ($p \geq 0.05$).

Key: AMX(Amoxicillin), APX(Ampiclox), CN (Gentamycin), CPX (Ciprofloxacin), E (Erythromycin), PEF (Pefloxacin), R (Rocephin), S (Streptomycin), SXT (Trimethoprim-Sulfamethoxazole), Z(Zinnacet).

Results of Susceptibility patterns of *S. aureus* isolates from the three local government areas as indicated by their Zones of inhibition showed no significant difference ($p \geq 0.05$) except for ciprofloxacin ($p \leq 0.05$) (Table4) and also had a reduced susceptibility of 15.4% with more in the intermediate zone 69.2%. This result is

in contrast with Okpo *et al.*, (2016) who showed 92.86% of *S. aureus* to Ciprofloxacin.

Table 5: Susceptibility pattern of *S. aureus* isolated from RTE *C. heterodont*.

Antibiotics (µg)	Resistance N (%)	Inter Mediate N (%)	Susceptible N (%)
APX (30)	65(100)	0(0.00)	0(0.00)
AM (30)	65(100)	0(0.00)	0(0.00)
CN (10)	7(10.8)	7(10.8)	51(78.4)
CPX (10)	10(15.4)	45(69.2)	10(15.4)
E (10)	11(16.9)	54(83.1)	0(0.00)
PEF (10)	12 (18.5)	42 (64.6)	11(16.9)
R (25)	35(53.8)	0(0.00)	30(46.2)
S (30)	52(80.0)	0(0.00)	13(20.0)
SXT (30)	10(15.4)	4(6.2)	51(78.4)
Z (20)	22(33.8)	36(55.4)	7(10.8)

Key: AMX (Amoxicillin), APX (Ampiclox), CN (Gentamycin), CPX (Ciprofloxacin), E (Erythromycin), PEF (Pefloxacin), R (Rocephin), S (Streptomycin), SXT (Trimethoprim-Sulfamethoxazole), Z(Zinnacet)

The Results of Susceptibility patterns of *S. aureus* to antibiotics showed a decreasing trend of resistance in the order: Amoxicillin and Ampiclox > Streptomycin > Rocephin > Zinnacef > Erythromycin > Ciprofloxacin > Septrin > Gentamycin. A high percentage of the *S. aureus* isolates were susceptible to Gentamycin and Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (78.4%) followed by Rocephin (ceftriaxone) 46.2% as seen in Table5 above. This study showed that commonly used antibiotics such as 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 the antibiotics.

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 Adebola and Tarilate (2011) which showed high resistance to Ampiclox and Penicillin and high susceptibility of *S. aureus* to Gentamycin similar to results from this study. In another study by Bello and Bankole (2013) some street vended foods in Ogun state Nigeria also showed the highest susceptibility of *S. aureus* to Gentamycin. Results from Okpo *et al.*, (2016) was also similar showing 100% Susceptibility of *S. aureus* from dairy milk to Gentamycin, and 64.29% to Trimethoprim/Sulfamethoxazole (Co-trimoxazole).

From this study, Gentamycin, Co-trimoxazole, and Ciprofloxacin has been shown to be an alternative treatment for foodborne staphylococcal infection instead of the Penicillins. This result is similar to Goldberg *et al.*, (2010) who showed a high efficacy of Co-trimoxazole similar to that of Vancomycin for cases of Multidrug-Resistant *S. aureus*.

In conclusion, this study showed a high bacterial count in ready to eat *C. heterodont* sold in Bayelsa state and unacceptable level of *Staphylococcus aureus* signaling a

threat to the health of the consumers. No matter the method of preparation used to prepare street vended or Ready-to-eat foods, utmost care should be taken to ensure they are safe for consumer consumption.

The drugs of choice for staphylococcal foodborne illnesses associated with the consumption of *Corbiculid heterodont* should be Co-trimoxazole and Gentamycin.

RECOMMENDATIONS

Street Vendors of Ready-to-eat food especially *Corbiculid heterodont* should be sensitized about the implication of poor hygienic practices in the preparation of this delicacy as well as employ food safety measures at every level of production of the food down to packaging, storage and transportation. Continuous monitoring of this food should be carried out due to the growing rate of antibiotic resistance.

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