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Assessing the Hygienic Status of Processed Fresh Water Clam (*Galatea paradoxa*) in Yenagoa Metropolis, Bayelsa State, Niger Delta, Nigeria

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Abstract This study was carried out to assess the sanitary quality of processed (fried) freshwater clam *(Galatea paradoxa*), sold in Yenagoa metropolis, Bayelsa State, Nigeria. "Water snail" as it is popularly called is vended by women and children. This delicacy is widely consumed in Yenagoa and its environs. Sixteen (16) Samples were collected randomly from four different hawkers within the Swali market in Yenagoa. Analysis included cultural techniques and bacterial quality assessment by enumeration of viable bacterial colonies using dilution techniques. The microbial analysis revealed Total Coliform Count of $7.33 \times 10^6 \pm 1.18 \times 10^6$ cfu\ml, Total Thermotolerant Count of $3.3 \times 10^5 \pm 0.05 \times 10^5$ cfu\ml, Total Heterotrophic Count (22°C) of $5.6 \times 10^6 \pm 2.02 \times 10^6$ cfu\ml and Total Heterotrophic Count (37°C) of $5.13 \times 10^6 \pm 0.55 \times 10^6$ cfu\ml. Thus, the Total Viable Count ranged from $3.3 \times 10^5 \pm 0.5 \times 10^5$cfu\ ml to $7.33 \times 10^6 \pm 1.18 \times 10^6$. A routine biochemical test was carried out to confirm the presence of potential pathogenic bacteria. The pathogens isolated were *Escherichia coli*, *Staphylococcus epidermis*, *Enterobacter spp*, *Shigella spp*, *Proteus spp* and Salmonella *Paratyphi A*. The presence of indicator organisms from faecal sources, environmental contamination and relatively potential bacteria could be attributed to poor hygienic practices during handling and processing of the clams and waste management practices within the point of sales. A good health education package needs to be given to vendors on good handling practice of vended foods.

Keywords: *bacteria load, clams, pathogens, viable counts*

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1. Introduction

The increasing demand for fish and fishery products is greatly challenged globally by microbial infection of fish and contamination of fish products. They are prone to contamination at various stages of handling, processing, and quality is a major concern to food processor and public health authorities [1]. Food borne diseases continued to be a major public health problem on a global scale, especially in developing countries due to difficulties in safeguarding food from cross contamination [2].

However, street foods have been in transmission of foodborne diseases [3,4,5,6]. The problem of food safety in developing countries is usually attributed to poor hygiene practices during foods processing and packaging [7,8,9].

Processed freshwater Clam (*Galatea paradoxa*) is a well-known delicacy in towns and communities of the Niger Delta, including the city of Yenagoa, in Byaelsa State. It is commonly called "water snail" and "Gbou" by the Izon-speaking people of Bayelsa State. The processed Clams are prepared either smoked, fried or cooked and sold in the streets and markets of Yenagoa. They are readily available for purchase and consumption. Several pathogenic microorganisms have been associated with many street vended foods [10].

Published literatures on the microbial load in the processed clams, even though, a popular delicacy in Bayelsa State in particular and Niger Delta as a whole, is absent. Therefore, this study was therefore undertaken to examine the bacterial quality of processed freshwater Clam that is a highly patronised delicacy within the state and its environs.

2. Materials and Methods

2.1. Study Area

This study was carried out in Yenagoa Metropolis, capital of Bayelsa state, while facilities at the Federal Medical Centre was used for laboratory analysis.

2.2. Collection of Samples

Sixteen (16) samples used for this study were fried Clam (*Galatea paradoxa*). The samples were bought from

four different hawkers around Yenagoa metropolis. Samples were put in zipper sterile polythene bags, properly labelled and carried to the Microbiology laboratory of the Federal Medical Center (FMC), Yenagoa for analysis within two hours of collection.

2.3. Total Coliform Count and Thermotolerant Coliform Count

Using the spread plate technique as described by [11], 1 ml of the 10^5 dilution for each sample was seeded on already prepared McConkey agar plates for the enumeration of total colony forming units of thermotolerant coliform (*E. coli*) and total coliform colony forming units. The plates were incubated at 37°C for total coliform count and 45°C for thermotolerant coliform at 24 hours. The samples were analyzed in triplicates and enumeration for total colony forming units (CFU) was based on mean values [12].

2.4. Total Heterotrophic Count

Total heterotrophic plate counts were also carried out using the spread plate technique, but using Nutrient agar plates. Incubation was carried out at 37°C for 24 hours to enable the growth of bacteria of mammalian origin, and 22°C for 72 hours for bacteria derived principally from the environment. Enumeration of bacteria was also done in triplicates.

2.5. Isolation and Identification of Bacteria

The method adopted by [2] was used with slight modification for the isolation of food borne bacteria. Ten (5g each) of each food samples were homogenised with sterile mortar and pestle, the resulting homogenate were aseptically added to 9 ml prepared nutrient broth. Streaking on the media directly from the overnight broth culture was done aseptically on EMB, SSA, chocolate agar, nutrient agar and Macconkey agar and incubated at 37°C for 24-48 hours. The streaked plates after incubation were examined for colonies which showed dissimilar cultural characteristics and subcultured on respective media. Pure colonies where obtained by subculturing on nutrient agar. All presumptive isolates were further identified using conventional biochemical methods [13]. These characteristics of differentiation for the isolated strains were read as described by [11] and [14].

2.6. Antibiotic Susceptibility

The Sensitivity of the bacterial isolates to different antibiotics was determined using the Kirby- Bauer disc diffusion technique. Discs used contained the following antibacterial agents: Ofloxacin (5ìg), Ciprofloxacin (5ìg), Gentamicin (10ìg), Ceftazidine (30ìg), Nitrofuratoin (300ìg), Augmentin (30ìg), Cefixime (5ìg) and Cefuroxime (30ìg). Oxoid sensitivity test agar plates were swabbed with cells from the bacteria stock solution, pre-adjusted to 0.5 McFarland's turbidity standard. The discs were thereafter, carefully placed on the agar with a sterile forceps and incubated at 37°C for 24 hours. Zones of sensitivity was measured with a meter rule.

3. Results

Enumeration of colony forming units for bacteria with respective temperature and media gave total coliform count (TCC) of $7.33x\ 10^6 \pm 1.18x10^6$ cfu/ml, total thermotolerant count (*E coli*) of $0.33x10^6 \pm 0.05x\ 10^6$ cfu/ml, total heterotrophic count (THC 22°C) of $5.60\ x\ 10^6 \pm 2.02\ x\ 10^6$ cfu/ml and total heterotrophic count (THC 37°C) of $5.13x10^6 \pm 0.55x\ 10^6$ cfu/ml from this result obtained total coliform forming unit had the highest colony counts per ml of samples, while total thermotolerant colony showed the least count/ml of samples. However colony forming unit of bacteria from food sample rangeed from $0.33x10^6 \pm 0.05\ x\ 10^6$ cfu/ml to $7.33\ x\ 10^6 \pm 1.18\ x\ 10^6$ cfu/ml.

Table 1 shows isolation and identification of foodborne bacteria from samples. It reveals eighteen (18) bacteria isolate based on cultural characteristics, morphological and biochemical characterizations. Out of the 18 bacteria isolated, 6 isolates were *Escherichia coli*, 3 *Enterobacter spp*., 1 *Salmonella spp.*, 1 *Proteus*, 1 *Shigella spp.* 2 *Streptococcus spp.* and 4 *Staphylococcus spp*.

Table 2 shows the result of antibiotic susceptibility of pathogens isolated. The result indicate complete resistance for Augumentin and complete susceptibility for Ofloxacin. However other antibiotics used in this study showed variability in their susceptibility pattern.

Table 1. Morphological and biochemical characterization of food borne bacteria isolated.

Bacteria Isolated	Gram reaction	Morphology	Oxidase	Motility	Indole	Urea	Citrate utilization	H_2S production	Catalase	coagulase	Total isolates
Escherichia coli	-	Rod-shaped	_	+	+	+	+	_	+	_	6
Enterobacter spp	-	Rod-shaped	_	+	_	_	+	_	+	_	3
Salmonella paratyphi A	-	Rod-shaped	_	+	_	_	_	+	+	_	1
Proteus spp	-	Rod-shaped	_	+	_	++	+	++	+	_	1
Shigella spp	-	Rod-shaped	_	_	+	_	_	_	+	_	1
Enterococcus spp	+	coccus	_	_	_	_	_	_	_	_	2
Staphylococcus epidermis	+	coccus	_	_	_	_	_	_	+	_	4
Total											**18**

Key: += positive, - = negative.

Table 2. Antibiotic susceptibility pattern of bacteria isolated.

Bacterial isolates	Antibiotics							
	Augmentin (30ug)	Ofloxacin (5ug)	Gentamicin (10ug)	Nalidixic (30ug)	Nitrofuratoin (200ug)	Cotrimoxazole (25ug)	Amoxillin (25ug)	Tetracycline (25ug)
E. coli	R	S	R	R	S	S	S	S
S. paratyphi A	R	S	S	R	S	R	R	R
Proteus spp	R	S	S	S	S	R	R	R
Shigella spp	R	S	S	S	S	S	R	S
Enterococcus spp	R	S	S	R	S	R	S	R
Enterobacter spp	R	S	R	S	S	R	R	R
S. epidermidis	S	S	S	R	R	R	S	R

KEY: R-Resistant S-Susceptible.

4. Discussion

This study has showed that processed clams commonly sold by food vendors in Yenagoa metropolis is a potential threat to public health, as it contains viable colonies of potential pathogens. Several reports have shown that street vended foods have been implicated in transmission of foodborne disease [3,4,5,6].

Total Viable Count (TVC) observed in this study was higher than the range of 9.6×10^2 to 2.8×10^3cfu\ml of fried fish in Owerri, Imo State of Nigeria, reported by [15]. [1] also reported a lower TVC count of 6.5×10^3Cfu\ml from the skin of freshwater catfish in Akungba-Akoko, Ondo State, Nigeria. Coliforms are considered indicators for assessing general hygienic status of food contact surfaces [17]. The most prevalent bacteria in fried clams sold in Swali market was *E. coli* (33%). This was similar to the findings of [15] who reported *E. coli* (57%) as the most prevalent bacteria isolated from fried fish in Owerri metropolis. The least prevalent bacteria was Salmonella *paratyphi* A (5%). *Enterobacter* spp, *Proteus mirabilis*, *Shigella* spp, *Enterococccus* spp and *Staphylococcus epidermis* had prevalences of 17%, 6%, 6%, 11% and 22% respectively. *Shigella* spp, and some strains of *E. coli* cause diarrhea. Salmonella is associated with enteric fever. Staphylococcus is associated with food poisoning while Enterobacter, *Enterococcus* and *Proteus spp* cause various infections in the human body. The high bacteria count in fried clams could be attributed to socio-cultural factors strongly influencing sanitation practices. Several pathogens of public health importance, especially those implicated in food borne diseases had been isolated and similar to finding by [5,6,17]. Therefore, surveillance and monitoring should be of great concern in Yenagoa and early responses on the outbreaks of faeco-oral infections.

Finally, this study also reveals the extent of antibiotic resistance and susceptibility of food-borne bacteria pathogens isolated.

5. Conclusion/Recommendation

Food safety is best ensured by the shared responsibility of everybody involved with food from the professional to the consumer. The best way to practice food safety is to be well-informed about the basics of food and the consequences involved in unhygienic practices in handling and processing of food. Therefore, the study suggests that there should be high level of awareness campaign and health education on human hygiene behaviours and the role of food in diseases transmission. Finally the results also indicated that some of the food-borne pathogens are resistant to some of the common antibiotics, which if neglected become a very serious public health problem.

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Statement of Competing Interest

The authors have no competing interests.

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