

Evaluation of the population of *Salmonella* **isolated from prawn (***Penaeus monodon)* **and clam (***Mercenaria mercenaria)* **sold in some markets in Port Harcourt, Nigeria**

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ABSTRACT

The microbial contamination of seafood is primarily due to poor hygienic conditions in markets and the improper disposal of sewage and septic waste into the habitats of seafood. This study aimed to evaluate the population of *Salmonella* species isolated from prawns (*Penaeus monodon*) and clams (*Mercenaria mercenaria*) sold in several markets in Port Harcourt, Nigeria. A total of seventy-two (72) samples (both raw and processed) were purchased from three markets: Rumuokoro, Creek Road, and Mile 1. *Salmonella* species were isolated using Salmonella-Shigella agar and subjected to standard bacteriological procedures. The isolates were identified based on their morphological and biochemical characteristics. Data revealed that samples from Rumuokoro Market had the highest Salmonella count, at 5.69±4.49 x 10³ cfu/g, followed by Creek Road Market with 2.18 \pm 2.00 x 10³ cfu/g, and Mile 1 Market with the lowest count at 1.51 \pm 1.59 x 10^3 cfu/g. A significant difference (p < 0.05) was observed between the markets sampled. The results also showed that prawn samples had a higher *Salmonella* count (3.36±3.83 x 10³ cfu/g) compared to the clam samples (2.33±2.44 x 10³ cfu/g), though there was no significant difference (p > 0.05) between the two. The prevalence of *Salmonella* was higher in clam samples (88.9%) compared to prawn samples (83.3%). Overall, the prevalence of *Salmonella* species in this study was 86.1%. The high prevalence observed underscores the need for seafood handlers to adopt strict measures to ensure safe handling and processing, thus preventing contamination and reducing the risk of foodborne illnesses such as Salmonellosis.

Keywords: Population, *Salmonella*, seafood, prawn (*Penaeus monodon*), clam (*Mercenaria mercenaria*).

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INTRODUCTION

Prawns and clams are popular sources of animal protein worldwide, commonly featured in traditional meals. They are widely consumed, especially in developing countries, not only for their taste but also due to their high nutritional value (Heinitz et al., 2000). These seafood items are major sources of animal protein and are typically found in marine aquaculture. They contain several vital nutrients that contribute to improving human health in various ways (Stokstad, 2010; Adhikari et al., 2014; Venupogal and

Gopakumar, 2017). However, consumers often overlook the potential health risks associated with consuming inadequately processed seafood (Hibbeln et al., 2007; Oliver, 2005).

A significant bacterial population, such as *Salmonella* species, can be present in seafood due to its natural habitat in water bodies, where contamination may occur through improper waste disposal and untreated sewage. Additionally, contamination can result from the use of

unsanitary utensils, poor storage conditions, improper handling by vendors, and inadequate hygiene practices among processors (Barika et al., 2023; Okonko et al., 2009).

Salmonella is a widely prevalent pathogen in both humans and animals, causing salmonellosis, a disease that affects over one million Americans annually. A 2019 Global Burden of Disease study estimated that Nigeria experiences at least 291,909 cases, 3,584 deaths, and 273,473 disability-adjusted life years (DALYs) lost to *Salmonella* infections each year (Akinyemi et al., 2018). Salmonellosis is a common infection worldwide, typically presenting as self-limiting food poisoning (gastroenteritis) in humans. However, it can sometimes develop into a serious systemic infection (enteric fever), which requires prompt antibiotic treatment. In livestock, salmonellosis may lead to significant economic losses (Stanaway et al., 2019).

The global prevalence of *Salmonella* in seafood and other food products has prompted increased research interest in ensuring the safety of clams and prawns, which are major transmission vehicles of *Salmonella* to humans. This study aims to evaluate the presence of *Salmonella* in prawns (*Penaeus monodon*) and clams (*Mercenaria mercenaria*) sold in markets in Port Harcourt, Nigeria. The findings of this research will help raise public awareness of the health risks associated with consuming contaminated seafood.

MATERIALS AND METHODS

Description of Study Area

This study was conducted at three different locations in the Port Harcourt metropolis: Creek Road Market (4.7588° N, 7.0261° E), Mile 1 Market (4.7921° N, 6.9984° E), and Rumuokoro Market (4.8676° N, 7.0011° E) (Figure 1). These markets were selected due to the high population density in the area and the availability of seafood. Additionally, these markets experience high patronage of seafood, and the unhygienic handling practices by vendors pose a risk for rapid bacterial growth. Prawns and clams were chosen for the study because they are widely available, economically valuable, and nutritionally important.

Figure 1. Map of Rivers State displaying sampling locations. **Source:** (Quantum GIS 3.4, 2019).

Collection of samples

A total of 72 samples, including both raw and processed seafood, were collected, consisting of prawns and clams. There were 36 samples of each seafood type, with 18

dried and 18 fresh samples. From each market, 24 samples were collected, with all samples properly labeled using alphabetical codes. The samples were placed in sterile containers and transported in ice-packed coolers to Rivers State University's Microbiology Laboratory for

bacteriological analysis (Rahman et al., 2012).

Sample preparation

Edible portions of the seafood prawns and clams weighing ten grams were used to prepare the analytical stock unit. The samples were homogenized using a sterile blender, with 10 grams of each sample blended in 90 ml of normal saline. This process followed standard procedures for sample homogenization and dilution as recommended by the International Organization for Standardization (ISO 6887-1:2017) for the microbiological examination of food products.

Bacterial enumeration

Ten grams of each seafood sample were weighed and homogenized in 90 ml of distilled water. A ten-fold serial dilution was then performed, and aliquots from the appropriate dilutions $(10^{-1}$ and $10^{-2})$ were inoculated in duplicate and spread-plated on solidified Salmonella-Shigella agar (commercial). The plates were incubated at 37°C for 24 hours, after which typical *Salmonella* colonies were counted and recorded to estimate the total *Salmonella* count. Discrete colonies were purified by subculturing on freshly prepared sterile nutrient and SSA plates and incubated at 37°C for 24 hours to obtain pure cultures, which were then preserved in 10% (v/v) glycerol suspension and refrigerated at 4°C for subsequent identification (Cheesbrough, 2005).

Characterization and identification of *Salmonella* **isolates**

The colonial morphological characteristics and biochemical tests were conducted on pure isolates for *Salmonella* species identification. Growth on SSA plates was observed, where *Salmonella* strains producing hydrogen sulfide formed black-centered colonies, characteristic of non-lactose fermenters (Kukulska and Zimmer, 1989). The pure culture was further characterized based on biochemical properties.

Gram staining and biochemical tests including citrate utilization, oxidase, catalase, methyl red, Voges-Proskauer, indole, glucose fermentation, lactose fermentation, hemolysis, and sugar fermentation were performed on the isolates (Cheesbrough, 2005). The pure Salmonella cultures were stored in bijou bottles containing 10% (v/v) glycerol suspension and refrigerated at 4°C (Cheesbrough, 2005).

Statistical analysis

All statistical analyses were performed using SPSS

version 22, with ANOVA tests to assess significance and percentages of bacterial counts from the two seafood types (prawns and clams). The Tukey-Kramer HSD test was used to separate the means (Sampson et al., 2022).

RESULTS

Comparison of the level of *Salmonella* **contamination in seafood from the markets sampled**

Samples of prawns and clams collected from Rumuokoro Market recorded the highest *Salmonella* contamination levels, with counts of 5.69 ± 4.49 x 10^3 cfu/g. This was followed by Creek Road Market, which had contamination levels of $2.18\pm2.00 \times 10^3$ cfu/g, while Mile 1 Market recorded the lowest counts at 1.51 ± 1.59 x 10^3 cfu/g (as shown in Table 1). A statistically significant difference (p < 0.05) was observed in the total *Salmonella* counts between the various markets sampled.

The results in Table 2 show that prawn samples recorded higher *Salmonella* counts, at 3.36±3.8 x 10³ cfu/g, compared to the clam samples, which had lower counts of $2.33\pm2.44 \times 10^3$ cfu/g. However, the difference between prawn and clam samples was not statistically significant ($p > 0.05$).

The study revealed that Rumuokoro Market had the highest *Salmonella* counts for fresh prawns (9.33±3.41 x $10³$ cfu/g), while Mile 1 Market had the lowest counts $(1.1\pm0.41 \times 10^3 \text{ cftu/g})$, as shown in Figure 2. For dried prawns, Creek Road Market recorded the highest contamination levels $(1.85\pm0.87 \times 10^3 \text{ cftu/g})$, followed by Rumuokoro Market (0.4 \pm 0.1 x 10³ cfu/g), with Mile 1 Market again showing the lowest counts $(0.13\pm0.05 \times 10^3)$ cfu/g) (Figure 2).

Rumuokoro Market also recorded the highest *Salmonella* counts for fresh clams (5.4±3.42 x 10³ cfu/g), followed by Mile 1 Market $(3.9\pm1.25 \times 10^3 \text{ cfta/g})$, while Creek Road Market had the lowest counts (1.03±0.27 x $10³$ cfu/g). The study further showed that Mile 1 Market recorded the highest *Salmonella* counts for dried clams $(0.9\pm0.39 \times 10^3 \text{ cfta/g})$, whereas Rumuokoro Market had the lowest counts $(0.7\pm0.1 \times 10^3 \text{ cftu/q})$, as depicted in Figure 2.

Prevalence of *Salmonella* **species in this study**

The prevalence of *Salmonella* species in the samples examined is shown in Figure 3. The study found that clam samples had a higher prevalence (88.9%) compared to prawn samples (83.3%). The overall prevalence of *Salmonella* species in this study was 86.1% (Figure 3).

Table 3 lists the various *Salmonella* species identified from the samples, with a total of 62 isolates identified.

Table 1. Mean *Salmonella* counts from the markets sampled.

Market ID	$x 103$ cfu/g
Rumuokoro Market	$5.69{\pm}4.49^a$
Creek Road Market	2.18 ± 2.00^b
Mile 1 Market	1.51 ± 1.59^b
P-value	$< 0.0001*$

Table 2. Mean *Salmonella* counts for prawn and clam samples from the various markets.

Figure 2. *Salmonella* counts of fresh and dry seafoods in the markets sampled.

Figure 3. Theoverall prevalence of *Salmonella* contamination in the study.

DISCUSSION

The possible sources of *Salmonella* infection in seafood include inappropriate handling or processing, as well as contamination that takes place in the natural aquatic environment (Martinez-Urtaza et al., 2015; Robertson,

2007).

Salmonella contamination in seafood samples (prawn and clam) from different markets was evaluated and found to be very high. The study showed that samples from Rumuokoro Market recorded the highest *Salmonella* counts of 5.69 \pm 4.99 x 10³ cfu/g followed by Creek Road

Table 3. Morphological and biochemical characteristics of the isolates.

Table 3. Continues.

Market 2.18 \pm 2.0 x 10³ cfu/g while Mile 1 Market recorded the lowest *Salmonella* counts of $1.51±1.59 \times 10^3$ cfu/g. A statistically significant difference ($p < 0.05$) was observed between the markets sampled. The high load associated with Rumuokoro Market is attributable to the high population density of bacteria due to environmental contamination. In overcrowded urban markets, waste management and general hygiene may be insufficient, leading to the contamination of seafood with bacteria from the surrounding environment (Sinclair et al., 2012). Similar studies by previous researchers had reported higher counts associated with raw seafood with higher population density; $6.7\pm0.2 \times 10^4$ cfu/g as recorded by Barika et al., (2023).

A comparison of the *Salmonella* counts between prawn and clam samples revealed higher counts in the prawn samples than in the clam. However, there was no significant difference ($p > 0.05$) between the prawn and clam samples. The observed difference between prawn and clam samples could be due to handling practices by traders, use of contaminated containers, and lack of

proper hand hygiene by vendors (Martinez-Urtaza et al., 2015).

On the other hand, there was a statistically significant difference at ($p <$ 0.05) between the fresh and dry samples, as the fresh samples recorded higher counts than the dry samples. This suggests that fresh samples may pose a higher bacteriological risk than dry samples. The higher counts in fresh samples compared to dry samples could be due to water activity and other nutritional components of the seafood (Venupogal and Gopakumar, 2017). It may also be because the environment where the seafood was harvested was exposed to waste dumps and fecal matter of animal origin (Kostyla et al., 2015; Olalemi et al., 2020). Bacterial growth was limited in dry samples as most bacterial activities depend on water availability in the environment. The total *Salmonella* counts in the seafood samples were above the specified limits proposed by the Health Products and Food Branch (HPFB, 2012), the World Health Organization (WHO, 2009), and the International Commission on Microbiological Specifications for Foods (ICMSF, 2002). The

HPFB's Guidelines specified that seafood products should be free of any detectable *Salmonella* per 25 grams of the seafood sample. This means that, in 25 grams of a tested seafood product, *Salmonella* should not be detected at all, as even small numbers of *Salmonella* can cause food-borne illnesses. The ICMSF global microbiological criteria for various foods apply a zero-tolerance policy for *Salmonella* in sea foods. The ICMSF limit for *Salmonella* in seafood is the absence of *Salmonella* in 25 grams of the sample. Drying on the other hand, has been widely used as a food preservation method in rural and urban communities. The reduced bacterial load in the dry samples may be due to the reduced water content that makes the seafood less susceptible to spoilage and exposure to contamination (Venupogal and Gopakumar, 2017). Microorganisms in the seafood samples could not be completely removed by drying because they might be heat resistant, meaning they could produce heat-resistant toxins or contain spores (Odu et al., 2012). This should be a subject of interest to people from the Riverine area where these sea foods are predominant and who prefer to eat dry prawns without proper washing.

The obtained prevalence of *Salmonella* species from the various markets was relatively high. The results showed that 62 Salmonella species were isolated from the three different markets, with an overall prevalence of 86.1%. Salmonella species in the clam samples recorded higher prevalence 88.9% than the prawn samples 83.3% which could be due to the filter-feeding behaviour of clams (Takeda, 2011; Akani et al., 2018), as they are in constant contact with large volumes of potentially contaminated water, which increases the likelihood of bacterial contamination (Silva et al, 2011).

Prawns, on the other hand, are bottom feeders that primarily consume organic matter, which reduces their direct intake of contaminated water when compared to clams (Takeda, 2011). The prevalence may also be due to poor hygiene during handling and processing (Bose et al., 2014; McFarland et al., 2019). *Salmonella* contamination via surface runoffs from poultry feaces used as manure in agricultural farms near rivers may also be the cause of the high occurrence of the organism (Majowicz et al., 2010).

CONCLUSION

This study has demonstrated high *Salmonella* counts in seafood (prawn and clam) sold in Port Harcourt, Rivers State, with notable differences in *Salmonella* counts across the three markets sampled. The high prevalence of *Salmonella* found in this study, compared to suggested guidelines for seafood, raises public health concerns. Since humans frequently consume these seafoods, the presence of *Salmonella* raises significant concerns for food safety and public health. This study suggests a higher probability of *Salmonella* infection in fresh samples compared to dry (processed) ones.

An overall high prevalence was documented in this study, likely influenced by factors such as the environment where the seafood was harvested or the methods used in processing and handling the samples. It is recommended that seafood vendors be educated to maintain clean and hygienic facilities and properly refrigerate seafood to slow bacterial growth; maintaining a temperature of 4°C is critical.

REFERENCES

- **Adhikari** S, Sahu BC, Mahapatra AS, Dey L, **2014**. Nutrient budget and effluent characteristics in giant fresh water prawn culture ponds. Bull Environl Contam Toxicol, 92: 509-513.
- **Akani** NP, Opukiri SR, Douglas SI, **2018**. Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Ready-To-Eat Freshwater Bivalve (*Corbiculid heterodont*) in Bayelsa State, Nigeria. World J Pharm Life Sci, 4(7): 56-61.
- **Akinyemi** KO, Oyefolu AOB, Mutiu WB, **2018**. Typhoid fever: Tracking the trend in Nigeria. Am J Trop Med Hyg, 99(3): 41-47.
- **Barika** PN, Akani LO, Amadi LO, Sampson T, **2023**. Prevalence and Antibiogram of *Salmonella enterica* isolated from seafood sold in Rivers State, Nigeria*.* Int J Microbiol Appl Sci, 2: 83-92.
- **Bose** R, Jana HH, Zaman S, Mitra A, **2014**. Study of the microbial health in and around the lower stretch of Hooghly Estuary. J Marine Sci Res Dev, 11(4): 1-13.
- **Cheesbrough** M, **2005**. District Laboratory Practice in Tropical Countries. 2nd ed, University press, University of Cambridge, Edinburgh, Cambridge, United Kingdom, 38(39): 194 – 201.
- Health Products and Food Branch (**HPFB**), **2012**. Standards and Guidelines for the Microbiological Safety of Foods: An Interpretive Summary. Government of Canada. P. 12.
- **Heinitz** ML, Ruble RD, Wagner DE, Tatini SR**, 2000**. Incidence of *Salmonella* in fish and seafood. J Food Prot, 63: 579-592.
- **Hibbeln** JR, Davis JM, Steer C, Emmett P, Rogers I, Williams C, Golding J, **2007**. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet, 369: 578-585.
- International Commission on Microbiological Specifications for Food (**ICMSF**), **2002**. Microorganisms in food 1. Their Significance and Methods of Enumeration. 2nd edition, Toronto: University of Toronto Press.
- **Kostyla** C, Bain R, Cronk R, Bartram J, **2015**. Seasonal Variation of fecal contamination in drinking water sources in developing countries: A systematic review. Sci Total Environ, 514: 333-343.
- **Kukulska** D, **Zimmer** A, **1989**. Selected Properties of lactosefermenting and non-fermenting *Salmonella agona*strains isolated from specimens from hospitalized infants. J Clin Microbiol, 41: 115- 120.
- **Majowicz** SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM, **2010**. The Global Burden of Nontyphoidal *Salmonella* Gastroenteritis. Clin Infect Dis J, 50(6): 882-889.
- **Martinez-Urtaza** J, Saco M, De Nova J, Perez-Pieiro P, Peiteado J, Lozano-Leon A, **2015**. Influence of environmental factors and human activity on the presence of *Salmonella* serovars in a marine environment. J Appl Environ Microbiol, 70: 2089-2097.
- **McFarland** P, Checinska-Sielaff A, Rasco B, Smith S, **2019**. Efficacy of food safety training in commercial food service. J Food Sci, 84: 1239- 1246.
- **Odu** NN, Njoku HO, Mepba HD, **2012**. Microbiological Quality of Smoked-Dried Mangrove Oysters (*Crassostrea Gasar*) sold in Port Harcourt, Nigeria. Agric Biol J North Am, 3(9): 340-360.
- **Okonko** IO, Adejoye OD, Ogunjobi AA, Nkang AO, Adebayo DC, **2009**. Hazard Analysis Critical Analysis and Control Points (HACCP) and Microbiology Quality of Sea-foods as Affected by Handlers Hygiene in Ibadan and Lagos, Nigeria. Afr J Food Sci, 3(2): 35-50.
- **Olalemi** AO, Ige OM, Oladejo OT, Yusuf OR, Akinmolayan B, **2020**. Comparative hazard evaluation of enteric bacteria in two surface

 water sources in Akure, Nigeria. Water Pract Technol, 15(3): 826- 839.

- **Oliver** JD, **2005**. Wound infections caused by *Vibrio vulnificus* and other marine bacteria. Epidemiol Infect, 133: 383-391.
- **Rahman** MM, Rahman F, Afroz F, Yesmin F, Fatema KK, Das KK, Noor R, **2012**. Prevalence of pathogenic bacteria in shrimp amples collected hatchery, local markets and the shrimp processing plant for export quality frozen shrimp. Bangladesh J Microbiol, 29(1): 7-10.
- **Robertson** LJ, **2007**. The potential for marine bivalve shellfish to act as a transmission vehicle for outbreaks of protozoan infections in humans: A review. Int J Food Microbiol, 120: 201-216.
- **Sampson** T, Moro K, Peekate LP, **2022**. Conceptual Profile of Vibrio species and the Associated Physicochemical Dynamics of Some Aquatic Reservoirs in Andoni Local Government Area of Rivers State, Nigeria. Acad J Life Sci, 8(1): 1-7.
- **Silva** J, Leite D, Fernandes M, Mena C, Gibbs PA, Teixeira P, **2011**. Campylobacter as a foodborne pathogen: A review. Front Microbiol, 2: 1-12.
- **Sinclair** RG, Rose JB, Hashsham SA, Gerba CP, Haas CN, **2012**. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Appl Environ Microbiol, 78(6): 1969- 1977.
- **Stanaway** JD, Parisi A, Sarkar K, Blacker BF, Reiner RC, Hay SI, Nixon MR, Dolecek C, James SL, Mokdad AH, 2019. The global burden of non-typhoidal *Salmonella* invasive disease: A systematic analysis for the Global Burden of Disease Study. Lancet Infect Dis, 19: 1312- 1324.

Stokstad E, **2010**. Down on the shrimp farm. Science, 323: 1504-1505.

- **Takeda**, Y. (**2011**). *Vibrio parahaemolyticus*, enterotoxigenic *Escherichia coli*, enterohemorrhagic *Escherichia coli* and *Vibrio cholerae.* Proceedings of the Japan Academy, Series B. Phys Biol Sci, 30(3): 210-219.
- **Venupogal** V, **Gopakumar** K, **2017**. Shellfish: Nutritive value, health benefits, and consumer safety. Compr Rev Food Sci Food Saf, 16(6): 1219-1242.
- World Health Organization (**WHO**), **2009**. Database of Microbiological Specifications for Selected Countries. Available online at [http://www.who.gk/foodsafety/Microbiological/200204188.](http://www.who.gk/foodsafety/Microbiological/200204188) Accessed March, 2012.

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