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Prevalence and Molecular Characterization of *Escherichia coli* Isolated from Periwinkles (*Tympanotonos fuscatus* and *Pachymelania aurita*)

Deeyah, F. E.*, Williams, J. O. and Sampson, T.
Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
***Corresponding Author:** *faithezzy.deeyah@gmail.com*

ABSTRACT

Seafood like periwinkles is reported to contain high bacterial load and serve as a route of transmitting pathogenic *Escherichia coli* into the human body. This study determines the prevalence and genomic characteristics of *Escherichia coli* isolated from periwinkles sold in Port Harcourt, Rivers State, Nigeria. Fifty-four (54) samples (raw and parboiled) were purchased from three different markets and subjected to standard bacteriological and PCR based genomic screening procedures. Results showed Oil mill market recorded the highest Total Coliform Count (TCC) of $12.5\pm10.11\times10^4$CFU/g while the least count of $5.72\pm2.02\times10^4$CFU/g was from Creek Road Market. There was no significant difference ($P>0.05$) in the TCC between the markets sampled. Oil mill market also had the highest count ($6.98\pm3.33\times10^3$Cfu/g) of Fecal Coliform (FCC), while the least ($2.12\pm0.82\times10^3$CFU/g) was from Mile 3 Market. There was a significant difference ($P<0.05$) in the FCC between the markets sampled. Data obtained based on the type of samples showed the highest TCC ($13.5\pm8.64\times10^4$CFU/g) was associated with the raw samples, while the least ($4.92\pm3.16\times10^4$CFU/g) was obtained from parboiled-with-no-shell. Data obtained based on the type of species of periwinkles showed that *Pachymelania aurita* recorded the highest TCC ($9.24\pm5.34\times10^4$CFU/g), while the least was $9.1\pm8.27\times10^4$CFU/g, obtained from *Tympanotonus fuscatus*. The prevalence of *E. coli* based on the markets sampled was in the order; Creek road (66.7%), Mile 3 (50%) and Oil mill (33.3%), which was however higher in *P. aurita* (55.6%) than *T. fuscatus* (44.4%). Molecular screening of the isolates indicated the presence of STX2 gene markers. The study thus revealed the pattern of *E. coli* contamination of periwinkles, with potentials to cause foodborne infection due to the presence of toxigenic genes. Safe handling and proper processing of periwinkles is therefore recommended to prevent any outbreak.

Keywords: *Escherichia coli*, molecular characterization, periwinkles, prevalence, seafood**.**

Introduction

Escherichia coli is characterized as a gram-negative, rod-shaped non-spore forming facultative anaerobic bacteria belonging to the family, *Enterobacteriaceae* with virulence groups including Enteroaggressive *E.coli* (EAEC), Enteroinvasive *E.coli* (EIEC), Enterohaemorrhagic *E.coli* (EHEC), Enteropathogenic *E.coli* (EPEC),Enterotoxigenic *E.coli* (ETEC), and Diffusely Adherent *E.coli* (DAEC) (Assefa *et al.*, 2018).

Escherichia coli normally inhabits the gastrointestinal tract of warm blooded animal as well as humans, and has been globally accepted as a fecal indicator organism linked with the contamination of water, food and food products (Sampson *et al*., 2020a).

E. coli are often non-pathogenic although different strains are virulent and may cause diseases such as gastroenteritis, urinary tract infection (UTI), bacteremia, pneumonia and neonatal meningitis (Williams *et al*., 2018).

Escherichia coli have been associated with many infections and the risk groups include infants, young children under the age of five, adults older than sixty five years and people with weakened immune systems are more prone to develop severe illnesses.

The potential cause of *E.coli* contamination of seafood include: poor handling, improper processing, poor hygiene of handlers, lack of good storage facility for preservation (Fahim *et al*., 2017).

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Escherichia coli has been associated with human infections through ingestion of edible parts of periwinkles which are deposited in this seafood during filter feeding, and the testing for the presence of *E.coli* in seafood products is a valid standard used to assess the fecal contamination (Okabe and Savichtcheva, 2006).

There are three genera of periwinkles but in Nigeria especially Niger Delta regions, *Tympanotonos fuscatus* and *Pachymelania aurita* are the predominant genera and mostly consumed because it is a cheap source of protein, mineral and vitamins (Jimmy, *et al*,. 2016). The two predominant genera are different morphologically. *Tympanotonus fuscatus* is characterized by turreted granular and spiny shell with tapering end (Onwuteaka *et al*., 2017). *Pachymelania aurita* is characterized by a sharp spine, a broader aperture and its sharpness is dependent on the age of the species (Aigbenia *et al*., 2018). Seafoods like periwinkle concentrate in water bodies and industrial wastes. It has been reported that they can get contaminated in the water bodies used for dumping of refuse and sewage disposal, and they become contaminated through contact with poor sanitary condition of the market as well as improper handling by vendors in the market, lack of good storage facility and poor hygiene of processors (Akani, *et al*., 2019) hence the presence of high bacterial load and toxic contaminant which pose health risk to consumers.

In Nigeria, especially Niger Delta region, periwinkle has been known for its versatility in preparing several delicacies such as in soup preparations like Afang soup, edikaikong soup and some native foods like Ekpang Nkukwo. The method used to process periwinkles before consumption differs among the populace. Some believe that the sharp end should be cut off and then cooked with its shell because of its nutritive value. Food poisoning due to pathogens has remained a major issue of public health concern worldwide, with countries expending a lot of resources to overcome it (WHO, 2008). Periwinkle is a good example of shellfish found in the estuary and it is common seafood consumed in Nigeria, especially in the south (Bradley *et al*., 2020).

Phenotypic characterization of *E. coli* and other bacterial species has been used by several researchers to detect the presence of contaminants in sea food (Sampson *et al*., 2020b), including periwinkle (Akani *et a*l., 2019).

However, several molecular techniques have emerged as very valuable methods for the detection of bacteria, including toxigenic gene markers in the organisms. This study therefore, was targeted to determine the prevalence and molecular characteristics of *Escherichia coli* Isolated from Periwinkles (*Tympanotonus fuscatus* and *Pachymelania aurita*) sold in Port Harcourt, Rivers State, Nigeria.

Materials and Methods

Description of Study Area

The study was carried out in three different locations in Rivers State Nigeria, such as Creek Road and Mile 3 markets in Port Harcourt City Local Government Area (4.758^{o}N, 7.023^{o}E and 4.8042^{o}N, 6.9924^{o}E) and as well as Oilmill market in ObioAkpor Local Government Area (4.8585^{o}N, 7.068^{o}E).

Sample Collection

A total of 54 samples (parboiled and raw) comprising of *T. fuscatus* and *P. aurita* was purchased monthly for duration of three months from the different locations (Creek Road, Oilmill, and Mile 3 markets) in Rivers State, Port Harcourt, Nigeria. The samples were labeled properly, put in a sterile ice bag and transported to the Department of Microbiology Laboratory Rivers State University, Port Harcourt for bacteriological analysis.

Sample Preparation

Preparation of stock analytical unit was carried out by weighing 10g of edible part of *T. fuscatus* and *P. aurita* (raw and parboiled) samples and was homogenized using sterile blender in 90ml of normal saline (diluent).

Microbiological Analysis

Enumeration and Isolation of Bacteria

Tenfold serial dilution was performed on the weighed samples of *T.fuscatus* (Raw and Parboiled) and *P.aurita* (Raw and Parboiled) after removing the shell (10g in 90ml). Aliquot (0.1ml) of appropriate dilutions was inoculated in duplicates using the spread plate method onto the already prepared sterile plates of MacConkey, and Eosine Methylene Blue (EMB) Agar. The plates were incubated at 37^{0}C for 24hours and 44.5^{0}C for EMB plates (fecal coliform count).

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The colonies formed on the plates were counted, recorded and morphologically described. The colonies formed on the Eosine methylene blue plate was used for the enumeration of the population of *E. coli* and MacConkey for total coliforms.

Discrete colonies were purified by subculturing on freshly prepared sterile nutrient agar plates and were incubated at 37^0C for 24hours to obtain pure culture.

Characterization and Identification of *E. coli* Isolates

The phenotypic characteristics and biochemical tests were conducted on the pure isolates for identification of *E. coli*. The suspected *E. coli* isolate on EMB agar were observed for the growth of a metallic green sheen coloration on the media which is the characteristics of the organism as a fecal coliform (lactose-fermenter).

Gram staining, motility test and biochemical test such as methyl red, indole, Voges Proskauer, citrate, oxidase, hemolysis test and sugar fermentation were carried out on the isolates (Aditi *et al*., 2017).

Molecular Characterization

Extraction of DNA

DNA extraction was carried out by using the boiling method described by Bell et al., 1998. A 24hour old pure culture of *E. coli* isolates were put in Luna-Bertani (LB) Broth and allowed to incubate at 37^0C. After 24hours, about 0.5ml of the cultured broth was dispensed into well labeled Eppendorf tubes and filling to mark with normal saline. It was centrifuged for 3mins at 14,000rmp after which the supernatant was decanted leaving the DNA at the base. The DNA was washed with 1ml of normal saline and spun properly to mix and then centrifuged again. This process was repeated three (3) times. The cells were re-suspended into 500µl of normal saline and heated at 95^0C for 20mins. After which, it was cooled on ice and then centrifuged for 3minutes at 1400rmp using 1.5ml microcentrifuge tube, the supernatant containing the DNA was transferred and stored at 20^0C for further reactions (Gaurabkarki, 2018; Bell *et al*., 1998).

DNA Quantification

DNA quantification was carried out to determine the average amount of DNA present in the mixture as well as its purity.

DNA quantification as described by Olsen and Marrow (2012) was adopted. The genomic DNA extracted was quantified using the Nanodrop 1000 spectrophometer. The Beer Lambert's principle was used to ascertain the quality and quantity of the genomic DNA. The nanodrop spectrophometer was connected to a computer with nanodrop software installed. The software was launched by double-clicking on the nanodrop icon. The sample pedestal were properly cleaned. The equipment was initialized using 2µl of sterile distilled water and blanked using 2µl of normal saline. To measure the concentration of the sample, 2µl of the extracted DNA was loaded onto the lower pedestal and the upper pedestal brought down to make contact with the DNA on the lower pedestal. Then, DNA concentration was measured by clicking the "measure" button displayed on the computer.

Amplification 16s rRNA

The 16srRNA amplification was performed using an ABI 9700 Applied Biosystems Thermal cycler method described by Srinivasan *et al*., 2015. The 16srRNA region of the rRNA gene of the *E.coli* isolates was amplified using the forward primer 27F; 5^i-AGAGTTTGATCMTGGCTCAG-3^i and reverse primer 1525R: 5^i-AAGGAGGTGWTCCARCCGCA-3^1 at a final volume of 40 micro-liters of 35 cycles. The PCR mix includes (12.5 µL of Taq polymerase 2X master mix from New England Biolabs,M0270), the primers at a concentration of 10µM and the extracted DNA as template, buffer IX and water. The PCR conditions were as follows: initial denaturation, 95^0C for 5 minutes, denaturation 95^0C for 30secs, annealing, 56^0 C for 30 seconds, extension, 72^0C for 45 seconds for 35 cycles and final extension, 72^0C for 5 minutes. The product was fixed in a 1% agarose gel at 150V for 30minutes and visualized on a UV transilluminator for a 1500 bp amplicons.

DNA Sequencing

Sequencing of the amplified product was performed using the Genetic Analyzer 3500xl sequencer from Applied Biosystems using manufacturers manual while the sequencing kit used was Big-Dye terminator kit on a 3510 ABI sequencer by inqaba Biotechnological, Pretoria South Africa. The sequencing was performed at a final volume of 10µl, the components included 0.25µl Big Dye terminator

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V1.1./V3.1, 2.25µl of 5X Big-Dye sequencing buffer, 10µM primer, PCR primer and 2-10ng PCR template per 100bp. The sequencing condition include the following; 32 cycles of 96^0C for 10secs, 55^0C for 5 seconds and 66^0C for 4 minutes (Srinivasan *et al*.,2015).

Phylogenetic Analysis

Sequences obtained was edited using the bioinformatics algorithm trace edit, MAFFT was used to align these sequences. The evolutionary history was inferred using the Neighbor-joining method in MEGA 6.0 (Saitou and Nei, 1985). The 500 replicates inferred from the bootstrap consensus tree was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were calculated using Jukes-cantor method.

Amplification of STX Genes

STX genes from the *E. coli* isolates were amplified using the forward; 5' TTCTTCGGTATCCTATTCCC 3' and Reverse; 5' ATGCATCTCTGGTCATTGTA 3' primers on ABI 9700 Applied Biosystems thermal cycler at final volume of 40 microlitres for 35cycles. The PCR mix includes 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward and reverse primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water.

The PCR conditions were as follows: initial denaturation, 95^0C for 5 minutes, denaturation 95^0C for 30secs, annealing, 56^0 C for 30 seconds, extension, 72^0C for 45 seconds for 35 cycles and final extension, 72^0C for 5 minutes. The product was fixed in a 1% agarose gel at 150V for 30minutes and visualized on a UV transilluminator for a 482 bp amplicons.

Statistical Analysis

All statistical analysis (ANOVA) was performed using SPSS version 22 to test for significance and percentages on the bacterial counts from (*T. fuscatus* and *P.aurita*). Tukey-Kramer HSD was used to separate the means respectively.

Results

Results of the bacterial population of *Tympanotonus fuscatus* and *Pachymelania aurita* from the various markets sampled is as presented in Table 1. The highest total coliform count of $12.5\pm10.11x10^4$CFU/g was obtained from Oil mill market while the least count of $5.72\pm2.02x10^4$CFU/g was obtained from Creek road market. There was no significant difference ($P>0.05$) in the total coliform count between the markets sampled. The least fecal coliform count ($2.12\pm0.82x10^3$CFU/g) was obtained from Mile 3 Market while the highest count ($6.98\pm3.33x10^3$CFU/g) was obtained from Oil mill Market. There was a significant difference ($P<0.05$) in the fecal coliform count between the markets sampled.

Results of the bacterial population obtained based on the type of samples are as presented in Table 2. The highest total coliform count of $13.5\pm8.64x10^4$CFU/g was obtained from the raw samples while the least count of $4.92\pm3.16x10^4$CFU/g was obtained from Parboiled with Shell (PWS) samples. There was no significant difference ($P>0.05$) in the total coliform count between the type of samples. The highest fecal coliform count ($5.13\pm4.42x10^3$CFU/g) was obtained from the Parboiled with Shell (PWS) while the least count of $2.85\pm1.52x10^3$CFU/g was obtained from the raw samples. There was no significant difference ($P>0.05$) in the fecal coliform count between the types of samples.

Results of the bacterial population obtained based on the type of species is presented in the Table 3. The highest total coliform bacterial count was $9.24\pm5.34x10^4$Cfu/g, obtained from *Pachymelania aurita* while the least count of $9.1\pm8.27x10^4$CFU/g was obtained from *Tympanotonus fuscatus*. There was no significant difference ($P>0.05$) in the total coliform count between the types of species.

The highest fecal coliform count of $4.32\pm2.77x10^3$CFU/g was obtained from *Tympanotonus fuscatus* while the least count of $3.88\pm3.57x10^3$Cfu/g was obtained from *Pachymelania aurita*. There was no significant difference ($P>0.05$) in the fecal coliform count between the types of species of periwinkles sampled.

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Table 1: Bacterial Population in the *T. fuscatus* and *P. aurita* from Various Markets Sampled

Market	Total Coliform Count (TCCx10^4 CFU/g)	Fecal Coliform Count (FCCx10^3 CFU/g)
Creek road	5.72±2.02[a]	3.2±2.32[a]
Mile 3	9.3±4.59[a]	2.12±0.82[a]
Oil mill	12.5±10.11[a]	6.98±3.33[b]
P-value	0.2291	0.0077

Table 2: Bacterial Population in the *T. fuscatus* and *P. aurita* based on the Type of Samples

Periwinkle Samples	Total Coliform Count (TCCx10^4 CFU/g)	Fecal Coliform Count (FCCx10^3 CFU/g)
Raw	13.5±8.64[a]	2.85±1.52[a]
Parboiled with Shell	4.92±3.16[a]	5.13±4.42[a]
Parboiled with no Shell	9.1±5.1[a]	4.32±2.79[a]
P-value	0.0804	0.4620

Table 3: Bacterial Population Isolated Based on the Type of Species

Type of Species	Total Coliform Count (TCCx10^4 CFU/g)	Fecal Coliform Count (FCCx10^3 CFU/g)
A (*P. aurita*)	9.24±5.34	3.88±3.57
B (*T.fuscatus*)	9.1±8.27	4.32±2.77
P-value	0.965417	0.771709

Results showing the prevalence of *E. coli* isolated from *T. fuscatus* and *P. aurita* in the different markets sampled are presented in Table 4. The result showed that a total of Twenty-seven (27) isolates of *E. coli* was obtained from the markets; out of which Creek road market had 66.7% occurrence and ranked the highest while oil mill market had 33.3% and ranked the least.

Results of the prevalence of *E. coli* based on the types of sample are as shown in Table 5.

The result showed that the Parboiled with shell (PWS) had 66.7% occurrence and ranked the highest while parboiled with no shell (PWNS) had the least occurrence (33.3%).

A result of the prevalence of *E. coli* based on the type of species is presented in Table 6. The result showed that *Pachymelania aurita* had 55.6% and ranked the highest occurrence and *Tympanotonos fuscatus* had the least occurrence of 44.4%.

Table 4: Prevalence of *E. coli* Isolated from *T. fuscatus* and *P. aurita* Based on the Markets Sampled

Markets (N)	Number of Samples Positive	Prevalence (%)
Creek road (18)	12	66.7
Mile 3 (18)	9	50
Oilmill (18)	6	33.3

Table 5: Prevalence of *E. coli* Isolated from *T. fuscatus* and *P. aurita* Based on the Types of Samples

Type of Samples	Number of Samples Positive	Prevalence (%)
Raw (18)	9	50
Parboiled with shell (18)	12	66.7
Parboiled with no shell (18)	6	33.3

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Table 6: Prevalence of *E. coli* Isolated Based on the Type of Species

Type of Species (N)	Number of Samples positive	Prevalence (%)
A (*P. aurita*) (27)	15	55.6
B (*T. fuscatus*) (27)	12	44.4

The Agarose gel electrophoresis of the amplified 16SrRNA of the *E. coli* isolates showed that Lane 1-6 represented the 16SrRNA gene band at 1500bp while Lane M represented the 1kbp ladder (Plate 1). The evolutionary distance between the *E. coli* isolates in this study, the accession numbers and their similarity index on the phylogenetic tree is revealed on Fig. 1.

The agarose gel electrophoresis of the amplified STX gene of the *E. coli* isolates showed that lane 1-6 represents the STX gene bands at 482bp while lane M represents the 50bp DNA ladder. This shows that the six (6) *E. coli* isolates screened for STX gene had the gene present in their plasmid as shown on plate 2.

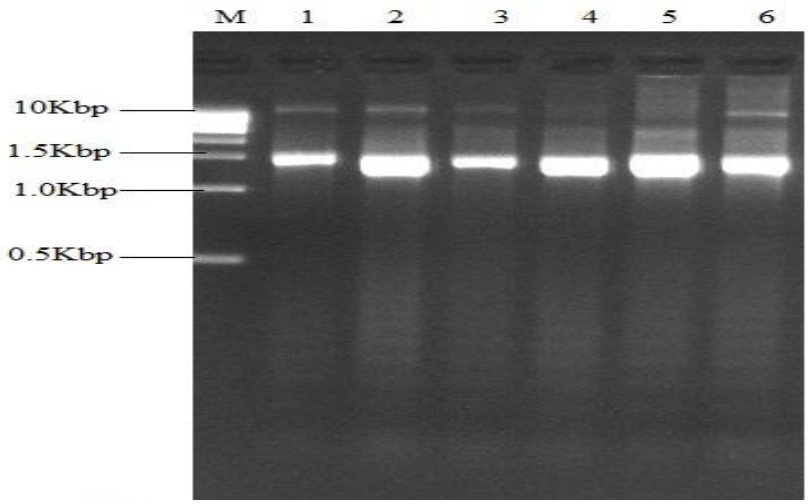

Plate 1: Amplified 16SrRNA Gene Bands of the *E. coli* Isolates at 1500bp.

(Source of *E. coli* - Lane 1= PA (*P. aurita*) parboiled periwinkle with no shell; Lane 2= PA Raw 1; Lane 3= PA Raw 2; Lane 4= TF PWS (*T. fuscatus*) parboiled with shell; Lane 5= TF PWS; Lane 6= PA Raw 2)

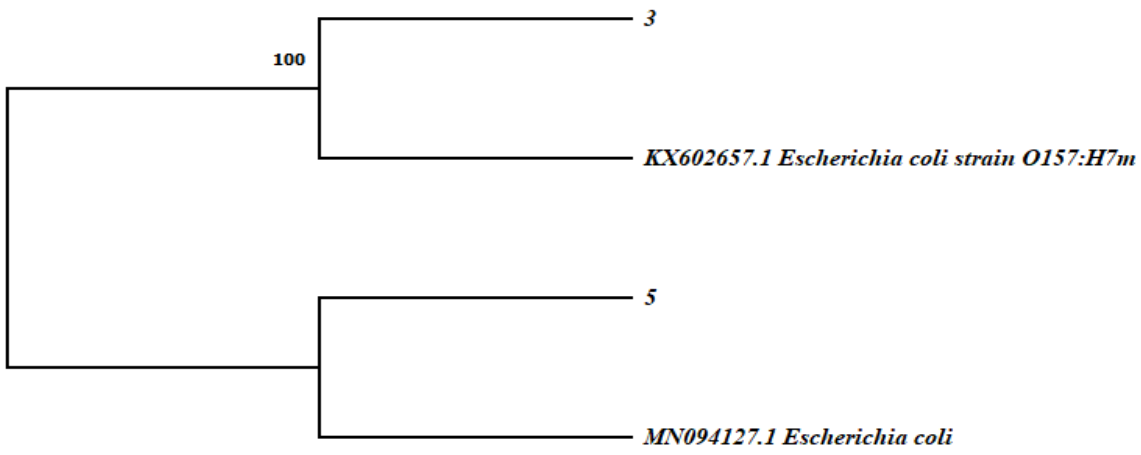

Figure 1: Phylogenetic Tree showing the Evolutionary Distance between the Bacterial Isolates

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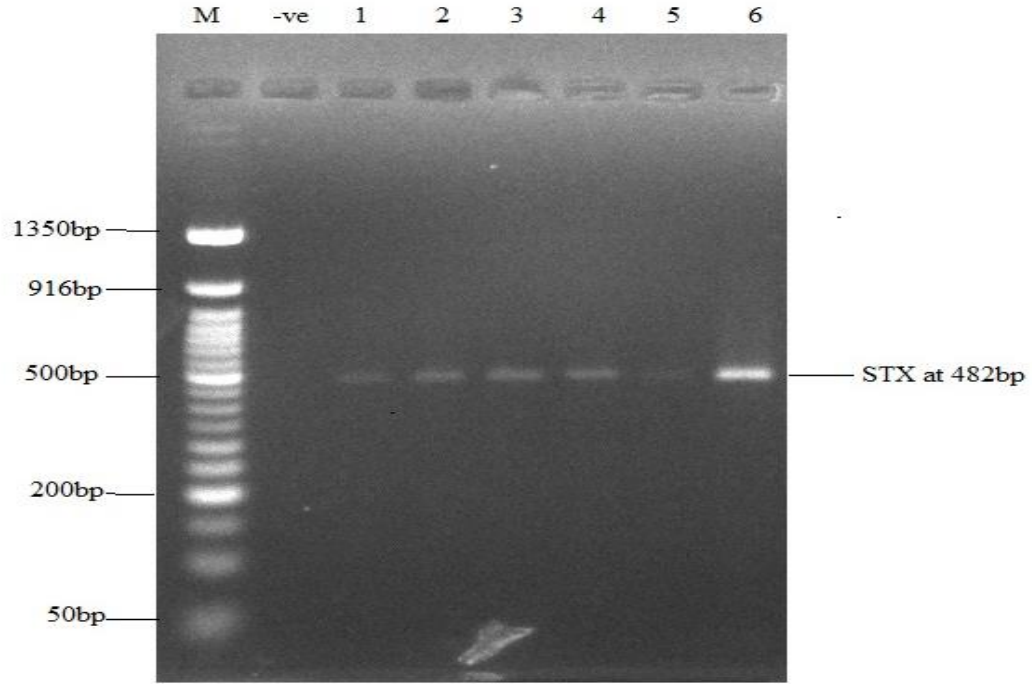

Plate 2: Amplified STX2 Gene Bands of the *E. coli* Isolates at 482bp.

(Source of *E. coli* - Lane 1= PA (*P. aurita*) parboiled periwinkle with no shell; Lane 2= PA Raw 1; Lane 3= PA Raw 2; Lane 4= TF PWS (*T. fuscatus*) parboiled with shell; Lane 5= TF PWS; Lane 6= PA Raw 2)

Discussion

The occurrence of *E. coli* in food samples including water and seafood is considered as an indicator of fecal contamination (Kumar *et al*., 2001; Gourmelon *et al*., 2006) and is usually attributed to infected handlers, storage using contaminated ice (Lateef *et al*., 2004) and on the quality of water where it is harvested (Kumar *et al*., 2009; Sampson *et al*., 2020b).

The study revealed the total coliform count was highest in samples obtained from Oilmill market followed by Mile3 market and Creek road market. The high coliform load in the oilmill sample could be attributed to the source of water used in washing, preparation and storage prior to the time of purchase (Sampson *et al*., 2020b; Takeda, 2011), and the poor sanitary condition of the market. Oilmill had the highest fecal coliform count followed by creek road and Mile 3 market had the least count. Bukola *et al*. (2006) recorded similar count Contamination of the seafood by coliform is attributed to the source of water used for preparation, washing, storage with contaminated ice and associated human activities.

The total and fecal coliform count were noted to be above the limits specified by the International commission on Microbiological specifications for food, 2002 which suggested a maximum coliform level of not greater than $1x10^2$ Cfu/g of shellfish.

The result obtained based on the types of samples revealed that for total coliform count, the raw sample had the highest count followed by the parboiled with no shell (PWNS) and parboiled with shell (PWS) had the least count. The higher count observed in the raw sample could be due to the poor water quality of the environment from which the seafood was captured and the sanitary condition of the landing centres (Kumar *et al*., 2009). Similar counts have been reported by previous researchers (Nnenna and Blessing, 2022; Sampson et al., 2020), which also reported high coliform in raw sample when compared with other sample types. The fecal coliform count in the parboiled with shell (PWS) had the highest count followed by the parboiled with no shell (PWNS) with the least count recorded in raw samples. This is in the range of study by Omenwa *et al*., 2011; Adebayo-tayo *et al*., 2008 that also reported high fecal count.

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The high count observed in the parboiled with shell could be due to the source of water used in preparation and cross contamination due to poor hand washing of the seafood processors and vendors. Also, the present result showed that parboiling could not eliminate the bacteria completely from the sample. The total coliform and fecal coliform count based on the type of samples were above the limits specified by the International commission on Microbiological specifications for food, 2002 which suggested a maximum coliform level of not greater than $1x10^2$ Cfu/g of shellfish for consumer's safety. The count obtained based on the type of periwinkle species sampled revealed that the total coliform count for *Pachymelania aurita* was the highest while *Tympanotonus fuscatus* had the least count, with no significant difference in the total coliform between the types of periwinkle species sampled. The higher count observed in the *P. aurita* could be attributed to its broader aperture or large orifice that is used in burrowing in the water bodies. Also, the high level of count in *P. aurita* could be due to the fact that it grows in fresh water which supports proliferation of a broader spectrum of bacteria as opposed to the brackish water in which *T. fuscatus* live (Bukola *et al*., 2006).

Also, there is greater pollution causing activities like bathing, washing and sewage discharge in the freshwater environment where *P. aurita* is harvested than brackish water environment. The fecal coliform count showed that *Tympanotonus fuscatus* had the highest count while *Pachymelania aurita* had the least. The high count observed in *T. fuscatus* could be attributed to the environment where it is harvested, the source of water used for preparation, washing and the release of fecal waste into the water bodies.

The total and fecal coliform count were above the limits specified by the International commission on Microbiological specifications for food, 2002 which suggested a maximum coliform level of not greater than $1x10^2$ Cfu/g of shellfish. The data obtained for prevalence showed that the prevalence of *E. coli* was high in Creek road (66.7%) followed by Mile 3 (50%) and Oilmill (33.3%). The high prevalence observed in the Creek road may be due to the proximity of the market to the rivers where anthropogenic activities take place. This report is in consonance with Sampson *et al*., 2020b which reported high prevalence of *E. coli* in cockles in the creek road market.

Based on the type of species; parboiled with shell (PWS) had the highest occurrence (66.7%) followed by the raw sample (50%) and parboiled with no shell (PWNS) had the least (33.3%). The high percentage occurrence in the parboiled with shell may be an indication of contamination of the periwinkle from the site of cultivation. This is in correlation with Adebayo-tayo *et al*., 2006; Omenwa *et al*., 2011 which stated that the level of pollution of the harvest waters determine the level of contamination of shellfish. This is because the shells of periwinkles are capable of harboring pathogenic organisms. This should be a source of interest to consumers in riverine states of Nigeria where people prefer to cook the periwinkles with shell before processing. The present results show that boiling could not eliminate the bacteria completely. Based on the type of species sampled, *Pachymelania aurita* had the highest occurrence (55.6%) and *Tympanotonus fuscatus* had the lowest (44.4%). The high occurrence observed in *P. aurita* could be attributed to its large aperture used in accumulating wastes in water bodies during filter feeding (Robertson, 2007).

The evolutionary distances computed was in agreement with the phylogenetic placement of the 16SrRNA of the isolate within the *Escherichia* species, which revealed a closely relatedness to *E. coli*. The study further detected the presence of in the isolates. The STX gene is one of the most potent toxins widely known to be responsible for the virulence found in some serogroups of *E. coli* such as O157:H7; it is a shigatoxin responsible for the hospitalization for foodborne illness and the predominat cause of post diarrheal hemolytic uremic syndrome (HUS) (Farrokh *et al*., 2013). The production of the STX2 is more correlated with the severity of the disease Hemolytic uremic syndrome (Farrokh *et al*., 2013). The detection of this toxigenic gene marker in most of the isolates is of serious public health concern as consumers may end with gastroenteritis and post diarrheal hemolytic uremic syndrome.

In conclusion, this study showed a high coliform load in seafood (*P. aurita* and *T.fuscatus*) sold in Port Harcourt, Rivers State, Nigeria, with difference in the fecal coliform count of the three sampling markets. High prevalence of *E. coli* in this study will further engender interest of public health when compared with the recommended standards for shellfishes.

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It can be inferred from this study that the risk of *E. coli* infection was higher in the parboiled with shell than raw and parboiled with no shell. Also, the risk of *E. coli* infection was higher in *Pachymelania aurita* than *Tympanotonus fuscatus.* Furthermore, it was revealed that the parboiled with no shell had least prevalence of *E. coli* infection. The presence of *E.coli* in seafood is an indication of recent contamination and is usually attributed to the sanitary condition of harvest waters, infected handlers and storage conditions. Also the presence of shigatoxin STX2 gene was observed in the isolates, which could serve as a virulence factor gene responsible for its pathogenicity and severity of this disease associated with consumption of contaminated seafood from this source. The presence of shigatoxin 2 gene (STX2) is believed to be the major pathogenicity of this organism and it's over production is the leading cause of the severity of disease associated with seafood. Proper processing and storage is therefore recommended. Good sanitary hygienic practices amongst vendors are required to control bacterial contamination of this product.

References

Adebayo-tayo, B, C., Ogunjobi, A, A. (2008). Comparative effects of oven drying and sun drying on the microbiological, proximate nutrient and mineral composition of *Trypanotonus* spp (periwinkle) and *Crassostrea spp* (Oyster). *Electronic Journal of Environmental, Agricultural and Food Chemistry, 7(4),* 2856-2862.

Aditi, F.Y., Rahman, S.S. & Hosain, M.M. (2017). A study on the microbiological status of mineral drinking water. *Open Microbiology Journal*, *11*, 31- 44.

Aigbenia, A.O. & Izah. S.C. (2018). Evaluation of heavy metals in tissue of *Tympanotonos fuscatus* sold in some markets in Port Harcourt, metropolis. *Nigeria MOJ Toxicology*, *4(5),* 334-338.

Akani, N.P., Hakam, I.O. & Sampson, T. (2019). Prevalence and antibigram of *Pseudomonas aeruginosa* isolated from West African mud creeper (*tympanotonos fuscatus*). *South Asian J. Res. Microbiol., 5(2),* 1-8.

Assefa, A. & Bihon, A.A. (2018). Systematic review and meta-analysis of prevalence of *Escherichia coli* in foods of animal origin in Ethiopia. *PubMed Central, 4(8),* 1-22.

Bell, J. M., Paton, J. C. & Turnidge, J. (1998). Emergence of vancomycin resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. *Journal of Advances in Biology, 1(1),* 23-44.

Bradley, B., Byrd,K.A., Atkins, M., Isa, S.I., Akintola, S.I., Fakoya, K.A., Ene-obong, H. & Thilsted S.H. (2020). Fish in food systems in Nigeria. A review. *Penang Malaysia; Worldfish Program Report.* (06).

Bukola, C., Adebayo-Tayo, A., A. Onilude, A., Adeniyi, A. Ogunjobi, D., O. & Adejoye, O. (2006). Bacteriological and proximate analysis of periwinkles from two diiferent creeks in Nigeria. *World Applied Science Journal, 1(2),* 87-91.

Fahim, A. E.S., Edris, A.A.I., Fatin, S. H., Azza, H.E. & Nairoz, M.A. (2017). Microbiological evaluation of some heat-treated fish products in Egyptian markets. *Journal of European Commission Nutrition, 12(3),* 123-132.

Farrokh, C. Jordarck, Auvray, F, Glass. K. (2013). Review of shiga-toxin producing *E. coli* (STEC) and their significance in dairy production. *Int. J. Food Microbiol., 162,* 190-212.

Gourmelon, M., Montet, M.P., Lozach, S. L. E., Mennec, C., Pommepuy, M., Beutin, L. & Vernozy-Rozand, C. (2006). First isolation of shiga toxin producing *Escherichia coli* variant strains in shellfish from coastal areas in France. *Journal of Applied Microbiology, 100,* 85-97

ICMSF -International Commission on Microbiological Specifications for Foods. (2002). *Microorganisms in foods. 7ed.* New York, Premium Publishers.

Jimmy E.O. & Okonkwo, M.A. (2016). Periwinkle (*Pachymelania aurit*a) consumption in vivo electrolyte disorders. *International Research Journal of Medicine and Medical Sciences*, *4(2),* 17-23.

Kumar, H.S., Otta, S.K., Karunasagar,I. & Karunasagar, I. (2009). Detection of shiga toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Letters Applied Microbiology, 33(5),* 334-338.

Lateef, A., Oloke, J.K., Gueguim, K. & Pacheco, E. (2004). Microbiological quality of ice used to cool drinks and foods in Ogbomoso Metropoli Southwest Nigeria. *Int Journal of Food safety, 8,* 39-43.

Citation: Deeyah *et al*. (2024). Prevalence and molecular characterization of *Escherichia coli* isolated from periwinkles (*Tympanotonos fuscatus* and *Pachymelania aurita*). *International Journal of Microbiology and Applied Sciences*. *3(1)*: 248 - 257.

Okabe, S. & Savichtcheva, O. (2006). Alternative indicators of fecal pollution: Relations with pathogens and Conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Journal of Water Resources, 40,* 2463-2476.

Olsen, N.D. & Morrow J.B. (2012). DNA extract characterization process for microbial detection methods development and validation. *BMC Research Notes, 5,* 668.

Omenwa, V.C., Ansa E.J., Ayokei, O.E., Uka, A. & George, O.S. (2011). Microbiological quality of raw and processed farm reared periwinkles from brackish water earthen pond, Buguma, Nigeria. *African Journal of food, Agriculture, Nutrition and Development, 11(2),* 4623-4631.

Onwuteaka, J., Ugbomeh, A. & Oyebuchi, O.A. (2017). Aspects of the population dynamics of periwinkle (T*ympanotonos fuscatus)* along the Bonny River, Nigeria. *Annual Research and Review in Biology, 12(2),* 1-13.

Robertson, L.J. (2007). The Potential for marine bivalve shellfish to act as transmission vehicles for outbreaks of protozoan infections in humans: A review. *International Journal of Food Microbiology, 120,* 201-216.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4(4),* 406 – 425.

Sampson, T., Akani, N.P. & Hakam, I.O. (2020a). Molecular characterization and detection of antibiotic resistance genes in *Pseudomonas* species Isolated from *Tympanotonos fuscatus*. *J, Advances in Microbiol., 20 (6),* 37-45.

Sampson, T., Barika, P. N., Peekate, L. & Akani, N.P. (2020b). Prevalence and antibiogram of *Escherichia coli* isolated from edible cockles (*Senilia senilis*) in Rivers state, Nigeria. *International Research Journal of Public and Environmental health, 7(5),* 149-156.

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, L., Brodie, E. L. & Lynch, S. V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *Plos One, 10 (2),* 1 – 22.

Takeda, Y. (2011). *Vibrio parahaemolyticus*, enterotoxigenic *Escherichia coli*, enterohemorrhagic *Escherichia coli* and *Vibrio cholerae*. *Proceedings of the Japan Academy, series B. Physical and Biological sciences, 87,* 1-12.

Williams, J.O, Akani, N.P & Nnamdi, A.U. (2018). Comparative studies on effectiveness of branded and unbranded disinfectants on *Escherichia coli* and *Staphylococcus aureus*. *South Asian Journal of Research in Microbiology. 2(4),* 1-8.

World Health Organization (WHO). (2008). *Foodborne disease outbreaks, guildlines for investigation and control. World Health Organization,* ISBN 978-92 4154722.

Citation: Deeyah *et al*. (2024). Prevalence and molecular characterization of *Escherichia coli* isolated from periwinkles (*Tympanotonos fuscatus* and *Pachymelania aurita*). *International Journal of Microbiology and Applied Sciences*. *3(1)*: 248 - 257.