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Microbiological Assessment of Roasted Dried Periwinkle (*Tympanotonus Fuscatus*) Sold in Yenagoa Bayelsa State

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Abstract

The microbial load of dried periwinkle(*tympanotonus fuscatus)* sold in Opolo market, Swali market and Kpansia market in Yenagoa were assessed and the microorganisms isolated were identified. Three samples were bought from Opolo market, Swali market and Kpansia market respectively were assessed. Total bacterial populations of the samples from Opolo market range from 37×10^{-5}- 12×10^{-5} cfu/g, Swali range from 49×10^{-5} - 29×10^{-5} cfu/g, while Kpansia market range from 38×10^{-5}- 18×10^{-5} cfu/g. For fungi population, Opolo market range from 6×10^{-5} - 1×10^{-5} cfu/g, Swali market range from 9×10^{-5}- 3×10^{-5} cfu/g, while Kpansia market range from 7×10^{-5} - 2×10^{-5}cfu/g. The *Samonella/Shigella* counts from Swali market range from 13×10^{-5}- 2×10^{-5} cfu/g, kpansia market range from 12×10^{-5}- 3×10^{-5} cfu/g, while Opolo market range from 11×10^{-5}-1×10^{-5} cfu/g. The coliform counts from Swali market range from 30×10^{-5} - 12×10^{-5} cfu/g, Kpansia market range from 28×10^{-5} - 10×10^{-5} cfu/g, while Opolo market range from 25×10^{-5}- 11×10^{-5} cfu/g. For fungi population, Opolo market range from 6×10^{-5}- 1×10^{-5} cfu/g, Swali market range from 9×10^{-5}-3×10^{-5} cfu/g, while Kpansia market range from 7×10^{-5} - 2×10^{-5} cfu/g.The bacterial isolates belonged to six genera identified as: *Klebsiella, Salmonella, Escherichia coli, Shigella, Staphylococcus* and *Bacillus*. The percentage (%) occurrences of the bacterial isolates were: *Bacillus* sp. (28.5%), *Staphylococcus aureus* (25%), *Shigella* sp. (21.4%), *Escherichia coli* (17.9%), *Salmonella* sp. (3.6%) and *Klebsiella* sp. (3.6%). Four (4) fungal isolates were obtained from the dried periwinkle samples and two belonged to the genus *Aspergillus*, while the other two isolates belong to the genera *Penicillium* and *Mucor*. The percentage (%) occurrence of the fungal isolates were: *Mucor* (41.6%), *Aspergillus flavus* (25%), *Aspergillus niger* (16.7%) and *Penicillium* (16.7%). There was no significant difference in total viable count between one market and another at 5% level of significance ($p>0.05$). Also, there was no significant difference in total viable count between one seller and other sellers. The occurrence of *Bacillus, Shigella, Staphylococcus, Klebsiella, Escherichia coli, Salmonella, Aspergillus,* and *Mucor* species are pinpointing the high pathogenicity and health hazard in consuming the dried periwinkle. Due to the soaring demand of this sea food (periwinkle) and the health hazard associated with microorganisms isolated from them as revealed in this study, additional concentration should be paid to safety through proper storage and handling processes and it is important that periwinkle should be properly cooked before consumption.

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Introduction

Sea foods are vital source of food in the Niger Delta. Main seafood's consumed in the region include periwinkle which is an important sources of protein. Fish constitutes over 40 % of the animal protein consumed by an average Nigerian compared to meat and it is relatively less expensive (Adebayo-Tayo *et al.,* 2008). This accounts for the mass preference for fish products.

They are marine mollusks that are represented in mangrove swamps, lagoons and estuaries by two genera *Tympanotonus* and *Pachymelania* (Buchaan *et al.,*1954). T*ympanotonus fuscatus* is a shellfish dominantly found in brackish waters of the riverine areas of Nigeria, where they are highly prolific. This feature had made them a cheap source of protein in many homes when compared to other conventional protein sources (Bassey *et al.,* 2007). Various methods have been developed to preserve fish. These include refrigeration and drying (Ayers *et al.,* 1980). The techniques employed depend on the technological advancement of the people (Adebayo-Tayo *et al.,* 2008).

However, studies on the microbiological quality of shell fishes have shown that they harbor many pathogenic microorganisms, the most prevalent being bacteria and fungi. As a result of pollution of water bodies, pathogenic organisms may be introduced to these aquatic ecosystems from which this sea food is harvested. Sources of pollution vary and could include faecal contamination usually from untreated human waste. As a result, water bodies may contain high numbers of coliform and these organisms would also be present in sea foods harvested from such water system (Jay *et al.,* 2000).

Periwinkles are known to contain lot of microorganisms which are usually either due to untreated human wastes which are deposited into the water which the periwinkle inhabits. Such microbes as, *Vibrio sp, Bacillus sp, Escherichia coli, Micrococcus sp,* which may be indigenous flora of the water body and are responsible for diseases associated with seafood when their microbial load is high such as cholera, Camphylobacterlosis, gastroenteritis, Salmonellosis, Shigellosis, typhoid fever, Brucellosis, Amoebiasis and Poliomyelitis (Ekanem & Otti, 1997).

In Bayelsa State of Nigeria the dried periwinkle (*tympanotonus fuscatus*) are often exposed to sources of contamination like soil, dust and sand due to the fact that the dried periwinkle is not properly covered and handled during sale. The need to inform the public on the health hazard associated with the consumption of dried periwinkle sold in the market which could result in ingestion of pathogenic microorganism's lead to this study. The aim of this study is to isolate and identify the possible microbial organisms present in dried periwinkle (*tympanotonus fuscatus*) from the three sources and to evaluate their safety on consumption.

Materials and Methods

Sampling Area

The samples were bought randomly from three different markets in Yenagoa, Bayelsa State, Nigeria. Sample area 1: Opolo Market (sample OA, OB and OC), Sample area 2: Kpansia Market (sample KA, KB and KC), and Sample area 3: Swali Market (sample SA, SB and SC).

Sample Collection

The sample used for this analysis is dried periwinkle. It is a marine sea food mostly found and eaten in the Niger Delta region. The samples were bought randomly from three

different markets (three samples from three different vendors in each market) in Yenagoa and were wrapped in sterile aluminum foil, labeled and taken to the laboratory for analysis.

Preparation of sample

Microbiological analyses was carried out in triplicates on 1g of samples which were soaked and homogenized with 9 ml sterile normal saline for 3 minutes using a Kenwood blender as describe in the Bacteriological Analytical Manual (FDA, 1984). Tenfold serial dilution was prepared in 9ml normal saline and 1ml of 10-2 was pour plated in nutrient agar and salmonella shigella agar.

Enumeration of Bacterial And Fungal Colonies

Aliquot (0.1 ml) of the sample was transferred into sterile plates in duplicates agar was poured on the samples and incubated in inverted position at 37 ^{o}C for 24 hours. The isolates from nutrient agar and salmonella shigella agar were sub-cultured in a nutrient agar using streak plate method. The inoculated media were labeled and incubated for 24 hours at 37 ^{o}C. For coliform count, 1 ml of the 10^{-2} dilution for each sample was pour plated in MacConkey agar. The plates were labeled and incubated at 37 ^{o}C for 24 hours and the colonies were counted. For the enumeration of fungal colonies, 1ml of 10^{-2} was pour plated in Potato Dextrose agar and plates were incubated at 28 °C for 5 days and the fungal colonies counted.

Identification Of Bacterial And Fungal Isolates

Identification of the isolates was based on their cultural morphology, microscopic examination and biochemical tests. References were made to Bergey's manual of determinative Bacteriology (1992) for identification of bacteria. Morphological studies were carried out on different media plates used for the isolation of the organisms; pure colonies were isolated based on colony size, shape, pigmentation, elevation and texture of the individual organisms after 24 hours of growth at 37 ^{o}C. Identification of the isolated bacteria was based on cultural characteristics, cell morphology and biochemical tests which include catalase, citrate, oxidase, Gram stain, Indole tests (Holt *et al.,* 1994).

Characterization of Fungi

Identification of fungi isolate was based on the morphological and microscopic characterization such as type of mycelium, pigmentation type of sporulating structures and sexual reproduction (if present). They are examined using hand lens to determine those morphological characteristics. Fungal isolates were stained with lacto phenol cotton blue and examined microscopically. The isolates were identified based on cultural characteristics, morphology of hyphae, cells and spores and kind of fruiting bodies (Barnet and Barry, 1972).

Gram Staining And Microscopic Examination

A portion of each discrete colony was thinly smeared in a drop of peptone water on a clean grease-free glass slide with the aid of a sterile wire loop and. The slides were allowed to air dry. The fixed smear was flooded with crystal violet stain for 1 minute and washed off with water. Lugol's iodine was used in flooding it and allowed to stay for 1 minute and rinsed with water. The smear was decolourized with alcohol and rinsed immediately with water. The smear was then counterstained with safranin for 1 minute, and then it was washed off with water and allowed to air dry at room temperature. The stained smear was examined under the microscope using x100 objective with immersion oil. Gram

positive organisms retained the primary stain (blue stain, crystal violet) while the gram negative organisms ones picked up the red or pink stain of the safranin.

Statistical Analysis

The data was subjected to test of difference of means using Anova with the aid of SPSS statistical software, to determine the F statistic and probability at 5% significant level (SPSS, 2010, Version 19)

Results and Discussion

Bacterial and fungal count on the samples from different markets: Total heterotrophic bacteria and total fungi using standard plate count of the various samples from Yenagoa were shown in Table 1.

Table 1: Bacterial and fungal counts for dried periwinkle sold in markets in Yenagoa

Samples	Market	Total viable count (10^{-5}cfu/g)	Total coliform (10^{-5}cfu/g)	Total *Salmonella Shigella* count (10^{-5}cfu/g)	Total fungi Count (10^{-5}cfu/g)
OA	Opolo	22	25	1	1
OB	Opolo	37	11	11	4
OC	Opolo	12	15	5	6
SA	Swali	30	13	3	4
SB	Swali	29	12	2	5
SC	Swali	49	30	13	3
KA	Kpansia	38	17	12	4
KB	Kpansia	18	28	3	7
KC	Kpansia	20	10	8	2

Key: OA= Opolo A, OB = Opolo B, OC = Opolo C, SA = Swali A, SB = Swali B, SC = Swali C , KA = Kpansia A, KB = Kpansia B, KC = Kpansia C.

Table 1 shows the levels of microbial load in dried periwinkle samples from three different markets in Yanegoa, Bayelsa State. Total bacterial populations of the samples from Opolo market range from 37×10^{-5} - 12×10^{-5} cfu/g, Swali range from 49×10^{-5} - 29×10^{-5} cfu/g, while Kpansia market range from 38×10^{-5} - 18×10^{-5} cfu/g. For fungi population, Opolo market range from 6×10^{-5} - 1×10^{-5} cfu/g, Swali market range from 5×10^{-5} - 3×10^{-5} cfu/g, while Kpansia market range from 7×10^{-5} - 2×10^{-5}cfu/g. The *Samonella/Shigella* counts from Swali market range from 13×10^{-5} - 12×10^{-5} cfu/g, kpansia market range from 12×10^{-5} - 3×10^{-5} cfu/g, while Opolo market range from 11×10^{-5} - 1×10^{-5} cfu/g. The coliform counts from Swali market range from 30×10^{-5} - 12×10^{-5} cfu/g, Kpansia market range from 28×10^{-5} - 10×10^{-5} cfu/g, while Opolo market range from 25×10^{-5} - 11×10^{-5} cfu/g. For fungi population, Opolo market range from 6×10^{-5} - 1×10^{-5} cfu/g, Swali market range from 9×10^{-5} - 3×10^{-5} cfu/g, while Kpansia market range from 7×10^{-5} - 2×10^{-5} cfu/g, this is in accordiance with the fungal count in the work of Nrior, Iyibo and Ngerebara, (2016). Bacteriological guideline have the limit for raw molluscan shellfish contamination of not more than $5x10^5$ bacteria/g and less than 230 *Escherichia coli* /100g for sea food harvested from known unpolluted waters, using 5 sample units (Seafood Network Information Center, 2008). The microbial load varied from location (market) to another and also from samples of the same location (market), this variation may be due to the processing and handling by different people and the microbial variation in atmosphere of the different localities.

The morphology, gram reaction and biochemical reactions of the bacterial isolates are shown in table 2.

Table 2: Morphology, Gram reaction and Biochemical reaction of Bacterial Isolates

Cell morphology	Gram reaction	Catalase	Citrate	Indole	Oxidase	Name of isolate
Rod	+	+	+	-	-	*Bacillus* species
Rod	-	+	+	-	-	*Klebsiella pneumonia*
Rod	-	+	-	+	-	*Escherichia coli*
Rod	-	+	-	-	-	*Salmonella* species
Rod	-	+	+	-	-	*Shigella* species
Cocci	+	+	+	-	-	*Staphylococcus aureus*

Table 2 shows that all the isolates were rods with the exemption of one. All of the isolates were catalase positive and oxidase negative (Table 2). The bacteria belonged to six genera identified as: *Klebsiella, Salmonella, Escherichia coli, Shigella, Staphylococcus* and *Bacillus* (Table 2).

The majority of these bacteria have also been reported by preceding researchers (Rhodes and Kator, 1988). The microbiological quality of the river, estuaries and seashores from which shellfish are harvested influence the microflora of shellfish samples (Adams and Moss, 2005). The primary microbial load on ready-to eat foods is important; but factors such as processing, storage and display for sale may influence the microbiological load of ready-to-eat foods at the point of sale (Beuchat and Ryu, 2004). Although drying reduces water activity and destroys bacteria in the course of heating, post processing contamination can occur especially during handling and transportation of processed periwinkle to point of sale(Obire, Nwosu and Wemedo, 2017).

Staphylococcus species was isolated from roasted samples of *Tympanotonus fuscatus*. Studies have suggested that the presence of *Staphylococcus* species on ready-to-eat food may be as a result of improper handling, cross contamination and poor temperature control (Christiansen and King, 1991). Market shellfish vendors use their bare hands during measuring and constantly dip their fingers into basins containing fresh and dry seafood, even different types of shellfish during saling which lead to cross contamination of the roasted periwinkle. Food handlers with hand infection or with cold or with sore throat may transfer enterotoxigenic strains of *Staphylococcus* to food. When given optimum conditions, it grows, generate toxins and cause staphylococcal intoxication. Growth to levels above 10^6cfu/g is required for toxin formation and since *Staphylococcus aureus* is a mesophilic organism, some degree of temperature abuse precedes intoxication (FAO/WHO, 2003).

Isolation of *Bacillus* species and *Shigella* species indicated that the seafood was contaminated from wherever they were harvested from. The display of the roasted dried periwinkle meat without any form of packaging could also be attributed to contamination. Being frequently displayed and uncovered, the shellfish meat will become prone to contamination from bacterial origin. Some strains of *Bacillus* (e.g. *Bacillus cereus*) and *Staphylococcus aureus* are known enterotoxin producers (Bryant, 2007). The inherent danger in the association of *Bacillus cereus* and *Staphylococcus aureus* with or without their metabolic products in various foods, without further heat treatment is the possible outbreak of serious food-borne illness. Keeping processed seafood for retail free of contamination with *Staphylococcus* species is best ensured by observing proper food

handling practices involving minimal contact with human skin. The infectious dose of *Shigella* is low, approximately 10 to 100 cells (FAO/WHO, 2003), therefore its presence in food must be avoided. Isolation of *Salmonella* species from the shellfish samples can be attributed to possible chronic carriers, from feaces to other persons by the oral-feacal route, which may be water-borne, food borne or by contact with hands and other formites.

The findings of the study show that microbial counts were generally lower for samples from Opolo market compared to Kpansia and Swali markets.

The morphological characteristics of fungal isolates are shown in table 3.

Table 3: Morphololological Characteristics of fungi isolated from samples

Growth Medium	Colony Morphology	Microscopic Appearance	Identity
PDA	Yellowish–green mycelium	Brush-like conidia, Septate branching, Conidiophore was, smooth.	*Penicillum* species
PDA	Effuse black colony	Simple septate and branched conidia in chain.	*Aspergillus niger*
PDA	Greenish mycelium	Conidiophore with vesicles, unbranched Conidiophores in chain.	*Aspergillus flavus*
PDA	Whitish grey mycelium	Sporangiophore Branched with spored sporangium. Rhizoids not present.	*Mucor* species

Four (4) fungal isolates were obtained from the dried periwinkle samples, *Aspergillus flavus, Aspergillus niger, Penicillium* species and *Mucor* species (Table 3). They belonged to three genera *Penicillium, Aspergillus* and *Mucor*. Isolation of *Aspergillus* species indicated that the dried periwinkle was contaminated by air, water or soil because the spores are commonly found in air, water or soil. Isolation of *Penicillium* and *Mucor* species indicated that the dried periwinkle was contaminated from air to which the dried periwinkle was exposed to.

Table 4: Mean and standard deviation of total microbial counts

Sample location	Total viable count (10^{-5}cfu/g)	Total colifom count (10^{-5}cfu/g)	Total *Salmonella Shigella* count (10^{-5}cfu/g)	Total fungi count (10^{-5}cfu/g)
Opolo Market	23.6667 ± 12.58306	17.0000 ± 7.21110	5.6667 ± 5.03322	3.6667± 2.51661
Swali Market	37.0000 ±10.58301	20.3333 ± 9.07377	8.0000 ± 5.56776	5.6667 ±3.05505
Kpansia Market	25.3333 ± 11.01514	18.3333 ± 9.07377	7.6667 ± 4.50925	4.3333 ±2.51661

Table 4 shows that there were no significant difference in total viable count between one market and another at 5% level of significance ($p>0.05$). Also, there was no significant difference in total viable count between one seller and other sellers.

Percentage (%) and frequency of occurrence of microbial isolates: The percentage (%) frequencies of the different bacterial and fungal isolates are shown in table 5 - 6.

Table 5: Frequency and Percentage (%) occurrence of bacterial isolates from dried periwinkle

Isolate	Frequency of Occurrence	Percentage (%) of Occurrence
Bacillus species	8	28.5
Staphylococcus aureus	7	25
Shigella	6	21.4
Echerishia coli	5	17.9
Klebsiella pneumonia	1	3.6
Salmonella species	1	3.6
Total	**28**	**100**

The percentage (%) occurence of the bacterial isolates were: *Bacillus* species (28.5%) > *Staphylococcus aureus* (25%), *Shigella* species (21.4%), *Escherichia coli* (17.9%), *Salmonella* species (3.6%) and *Klebsiella pnuemoniae* (3.6%) (table 5).

Table 6: Frequency and Percentage (%) of occurrence of fungi in roasted dried periwinkle

Isolate	Frequency of Occurrence	Percentage (%) of Occurrence
Mucor species	5	41.6
Aspergillus niger	2	16.7
Aspergillus flavus	3	25
Penicillum species	2	16.7
Total	12	100

The percentage (%) occurence of the fungal isolates were: *Mucor* (41.6%), *Aspergillus flavus* (25%), *Aspergillus niger* (16.7%), *Penicillium* (16.7%) (Table 6).

The occurrence of *Bacillus, Shigella, Staphylococcus, Klebsiella, Escherichia coli, Salmonella, Aspergillus,* and *Mucor* species are pinpointing the high pathogenicity and health hazard in consuming the dried periwinkle. Due to the soaring demand of this sea food (periwinkle) and the health hazard associated with microorganisms isolated from them as revealed in this study, additional concentration should be paid to safety through proper storage and handling processes and it is important that periwinkle should be properly cooked before consumption.

Conclusions

The result identified fungi and bacteria as the microorganisms associated with dried periwinkle. Some of these microorganisms are pathogenic and are able to cause chronic illnesses in human if ingested. Contamination is common due to processing, storage and handling which is the major source of cross contamination. This study indicates that this dried periwinkle is not safe for consumption.

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