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MICROBIOLOGICAL ASSESSMENTOF NIGER DELTA SHELL SEA FOODS; PERIWINKLE (*Tympanotonusfuscatus*), OYSTER (*Crassostreavirginica*) AND VEINED RAPA WHELK (*Rapanavenosa*) FROM CRUDE OIL POLLUTED SITE.

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Abstract:
An assessment of the various parts of shell sea food periwinkle (Tympanotonusfuscatus), oyster (Crassostreavirginica) and veined rapa whelk (Rapanavenosa) crude oil polluted site in Niger Delta communities, Nigeria. The result showed that these shell sea foods contain unacceptable levels of bacteria, it also shows that the shell of the oyster has the highest contamination followed by the periwinkle and then the veined rapa whelk and it ranged as follows: Oyster (1.02 x10^{8}cfu/g) > Perewinkle (7.25x10^{8}cfu/g) > veined rapa whelk (7.15x10^{8}cfu/g), and for the cap/inner shell fluid, the veined rapa whelk has the highest contamination followed by periwinkle and then the oyster and it ranged as follows: veined rapa whelk(cap) (1.06x10^{8}cfu/g) > periwinkle (cap) (6.55x10^{8}cfu/g) > oyster (inner shell fluid) (5.3x10^{7}cfu/ml), for the intestine the whelk has the highest contamination followed by the periwinkle and then the oyster and it ranged as follows: vein rapa whelk (1.36x10^{8}cfu/g) >periwinkle (1.09x10^{8}cfu/g) > oyster (8.75x10^{8}cfu/g). Fungal evaluation revealed the shell (external body) of periwinkle have the highest (1.50x10^{7}cfu/g) while oyster and veined rapa whelk have the same level of contamination (1.15x10^{7}cfu/g); for the cap/inner shell fluid periwinkle has the highest contamination followed by oyster and then vein rapa whelk ang it ranged as follows: periwinkle (cap)(8.0x10^{6}sfu/g) > oyster (inner shell fluid)(7.5x10^{6}sfu/ml) > vein rapa whelk (cap)(6.0x10^{6}sfu/g), for the intestine the veined rapa whelk have the highest contamination followed by oyster then periwinkle and it ranged as follows: whelk (1.0x10^{7}sfu/g) > oyster (9.0x10^{6}sfu/g) > periwinkle (6.0x10^{6}sfu/g). The bacteria isolated from various part of these shell sea foods include: Escherichia coli, Shigella sp, Salmonella sp, Citrobacter sp Proteus sp, Micrococcus sp, Bacilli sp, and Staphylococcus aureus.The cumulative percentage (%) frequency of the bacterial isolates were: Staphylococcus aureus (17.3%) >Bacillus sp. (15.1%) >Klebsiella sp. (14.1%) >Vibrio sp. (10.8%) >Shigella sp. (10.3%) >Proteus sp. (8.1%) >Escherichia coli = Serratia sp. = Micrococcus sp. (7.6%) >Salmonella sp. (6.5%) >Citrobacter sp. (3.8%). The fungi isolated include: Penicillium sp, Aspergilus sp., Physariumcinereum, Mucorsp., Cryptococcus neoformas, Neurophora sp. and Candidasp. having cumulative percentage (%) frequency as follows: Aspergillus sp. (58.8%) >Physarium cinereum (23.0%) >Mucor sp. (7.3%) >Candida sp. = Neurospora sp. (3.6%) >Penicillium sp. (3.0%) >Cryptococcus neoformans (0.61%). The occurrence ofBacillus, Staphylococcus,Vibrio, Escherichia coliand Candidasp. are indicative of high pathogenicity and health hazard in consuming these sea foods. The need to critically re-examine our public health standard in the crudeoil polluted communities is advocated.

Key word: *shell sea foods, periwinkle, whelk, oyster, Staphylococcusaureus, Vibrio*

INTRODUCTION

Sea foods are vital source of food in Niger Delta. Main sea foods consumed in the region include periwinkle, shrimps, finfish etc, which are important sources of protein. Fish constitute over 40% of the animal protein consumed by an average Nigerian compared to meat and it is relatively less expensive. This account for the mass preference for fish products. It has been reported that fish is the major occupation in Niger Delta[1].Sea foods have high nutritive base therefore this provides a good medium for the growth of microorganisms leading to food poisoning, cholera and Salmonellosis [2] when consumed. The most prevalent being bacteria and fungi, As a result of pollution of water bodies, pathogenic organisms may be

introduced to these aquatic ecosystem from which these sea foods are harvested. Sources of pollution vary and could include faecal contamination too. 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.Microorganisms associated with these sea foods (periwinkle, oyster and veined rapa whelk) include *Escherichia coli, Citrobacter sp,Salmonella paratyphi, Staphlococcus aureus, Shigella sp, Bacillus cereus, Vibrio* sp.However, studies on the microbiological quality of shell fishes have shown that they harbour many pathogenic microorganisms. Most times, the accumulation and concentration of pathogenic microorganisms and toxic materials are usually from untreated human waste and industrial effluents that find their way into the water bodies that are inhabited by these shell fish.
These sea foods has been implicated in outbreak of food-borne diseases in many part of the world especially in Niger Delta where it is highly consumed. These illnesses include hepatitis, typhoid fever and other digestive disorder. Shell fishes concentrate in water bodies and industrial waste, hence the likeliness of high pathogen level and toxic contaminants which can present health hazard to consumers[3].This study was designed to assess the microbiological quality of periwinkle, oyster and veined rapa whelk.

PERIWINKLE (*Tympanotonusfuscatus*).The word periwinkle comes from the earlier Elizabethan words 'PENNY WINKLE' meaning winkles or small whelks that cost only a penny per handful, periwinkle is therefore defined as a creeping evergreen plant with light blue flower or an edible sea-snail with shell [4].
Classification of Periwinkle Scientific classifications of periwinkle are as follows;
Kingdom: Animalia
Phylum: Mollusca
Class: Gastropoda
Genus: *Tympanotonus*
Specie: *fuscatus*

Anatomy: Periwinkle has a calcareous shell ranging in size of between 10-30mm. It is coiled in a series of complete turns or whorls of increasing diameter around the central ascur known as columella, the body whorl terminates at a large oval opening. The thin disc like proteinaceous operculum serves as a door to close the aperture when the soft parts are withdrawn into the shell. The head is an anterior protuberance dorsal to the foot. It bears two sensory cephalic tentacles with a lateral eye on the base of each. The snout is no retractile and is thus not a proboscis.The mantle cavity is enclosed by the antle whose anterior edge is the mantle skirt, the skirt form a collar. The osphradium is located inside the mantle cavity, is a simple pigmented ridge of sensory epithelium. It is not an elaborate gill-like structure; the gill is a large long pile brown oval organ lying medial to the osphraduim. The intestine exists in the stomach and winds through the visceral mass where it is hidden from view [4].

Habitat of Periwinkle: Periwinkles are found in the shore, muddy habitat and estuaries. *Tympanotonus fuscatus* is found in the intertidal area of the mangrove edges and therefore could be handpicked. They inhabit quite waters, where the substratum is muddy and rich in detritus. It is found in most brakish water creeks and mangrove swamps [5].

Movement in Periwinkle: Periwinkle move on a muscular, fleshing foot and lubricated by a film of mucus. When not achieved it often nestles in a crack or gully and during low tide when it is exposed to the air, it can seal the gap between its shell and the rock with mucus to prevent desiccation [5].

Mode of Feeding in Periwinkle: Periwinkle is amphibious and can survive long period without water, making use of reserved food. Periwinkles are fed by grazing along the surface on which they live. They use their radius to scrap algae from rocks, or pick up algae from the film that covers the surface of mud of estuaries or bay.

Reproduction in Periwinkle: The sexes are separate (individual are either male or female) and fertilization occurs internally after copulation. The female lays about 1000-10,000 eggs in gelatinous capsules that usually contains around their eggs (although up to nine eggs per capsule has been known), free–swimming veligar larvae hatch after a few days, the young periwinkle attain sexual maturity at two or three years of age and may live up to ten years [6].

Processing and uses of periwinkle: Periwinkle are harvested from the mangroves of estuaries, washed and boiled. The soft body is winkled out from the shell with a pin after boiling. The fresh periwinkle are soaked in water to retain freshness and consumed within a day or two. Periwinkle are eaten as food and serve as a source of cheap crude protein in diet, dehydrated claim flavour powder has been produced and is used in formulated foods and snacks. The protein value exceeds the amount

in many food legumes and compares favourably with the level or the best of these pulses [6]. The shells are used in construction work and are known to complete favourably with stone used in construction [7].Periwinkles contains omega-3, which is believed to be responsible for low prevalent of heart related disease among people in the riverine areas. Periwinkle can be used as absorbents for the removal of pb^{2+} from aqueous solution [8]. Periwinkle is known to be supplement dietary protein for poultry toiler better than fish meal.

Periwinkle preservation: The practice of preserving food can be traced from prehistoric times, when vegetables and fruits were dried, cereal grains were parched and fish and game were salted and dried. The post harvest technologies on fish preservation have been remarkable and led to reduce a stage and improved eating quality of fish. The best way to preserve periwinkle still remain sun drying, oven drying or storage at constant temperature or about 10^0c in a refrigerator.

Microorganisms Associated with periwinkles: Periwinkles dues to their soft skin 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. Microorganisms such 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 [9].

OYSTER (*Crassostreavirginica*). An oyster is a bivalve (meaning two shells) and belongs to a group of animals with shells called molluscs. The two valves are thick and irregular in shape and appear a purplish/grey on the outside and white inside. The shorter of the valves is called the right valve (top shell below), and the longer, more rounded valve is called the left valve.

Classification of oyster

Kingdom: Animalia
Phylum: Mollusca
Family: Filibranchia
Genus: *Crassostrea*
Specie: *virginica*

Anatomy of Oyster: The shell of the oyster consist of two calcareous valves joined by a resilient hinge ligament. Valves are asymmetrical, the left being larger and more deeply cupped than , because the oyster invariabl settles on its left valve, the right valve is always uppermost.Internal organs are covered with a fleshy fold of tissue called the Mantle or pallium

Habitat of Oyster: Oyster reefs and beds maybe intertidal or subtidal biogenic Structures formed by oyster living at high densities and building a Habitat with significant surface complexity. Reefs and bed are accreting through the continuing deposition of shell material which is in turn degraded at varying rates. In some places it is likely that vertical accretion maybe restricted by tidal exposure, leaving a non-accreting reef.

Mode of Feeding in Oyster: Suspension feeding is a common mode of food collection in severalgroups of aquatic organisms, including bivalves. Feeding inbivalves involves pumping of water through a set of ctenidia,removal of particle from suspension, and transport of collectedmaterial to the mouth. The traditional theory of removal by thectenidia is based on a mucociliary concept [10].

Reproduction in Oyster: The sexes are separate, but the change sex every year. After spawning, the oyster enters a period of sexual rest, at that time the reproductive organ shrinks, and it is impossible to determine the animal's sex.In June, oyster prepare for spawning, they are then said to be ''milky'' (the meat takes on a whitish appearance). Temperature triggers spawning, once the water temperature reaches 20^0C, the female oyster clap their valves together and release millions of eggs into the marine environment. The male do their part as well, releasing an even greater number of sperm.Fertilization takes place in the water, and 24 hours later, a larva capable of free movement travels with the tidal currents.

Uses of Oyster: Oyster are primary source of food and the shell is a great alternative to gravel, crushed oyster shell can be used for cover material for paths, patios, courtyard and driveway. Oyster shell help neutralize soil acidity for tomato and vegetable garden.

Preservation of Oyster: Due to limited facilities and extreme climate conditions, smoking is carried out as an inexpensive option for preservation in less developed countries to reduce and avoid post-harvest loss. The preservation process, which combines smoking with salting, drying and heating, gives the product a characteristics and desirable flavour.

Microorganisms associated with Oyster: Oyster can contain harmful bacteria, because they are filter feeders, they concentrate anything present in the surrounding water. They contain high bacterial load of human pathogens in the warm months, most notably *Vibrio vulnificus* and *Vibrio parahaemolyticus*. In these cases the main danger is for immunocompromised individuals, who are unable to fight off infection and can succumb to septicaemia, leading to death. *Vibrio vulnificus* is the most deadly seafood-borne pathogen.

VEINED RAPA WHELK(*Rapavenosa*): is a large predatory marine snail that is native to marine and estuarine waters of the western pacific, from the sea of Japan, yellow sea, East China sea and Bohai sea, (Richerson 2006).It is now also establish in many parts of the world.

Classification of Veined Rapa Whelk
Kingdom: Animalia
Phylum: Mollusca
Class: Gastropoda
Subfamily: Rapaninae
Genus: *Rapana*
Species: *venosa*

Anatomy of Veined Rapa Whelk: The shell of the *Rapanavenosa* is globose (rounded) and heavy, possessing a very short spire, a large body whorl, a strong columella and a deep umbilicus. The aperture is a large and roughly ovate, ornamentation is present externally as axial ribs, smooth spiral ribs ending in blunt knobs at both the shoulder and body whorl, and internally as small elongated teeth disposed along the outer lip margin. The height of the shell can reach up to 180mm.

Habitat of Veined Rapa Whelk: Veined rapa whelk favours compact sandy bottom, in which they can burrow almost completely. The native habitat of this species is a region of wide annual temperature ranges, comparable to other localities, fleeing cooled water in the winter, this species may migrate to warmer, deeper waters thereby invading cool surface waters. This fertile sea snail is extremely versatile, tolerating low salinities[11].

Mode of Feeding in Veined Rapa Whelk: Veined rapa whelk are carnivorous selectively predatory gastropods which main diet consist of a variety of other mollusc species. Prey are chosen by the whelk according to the species and size [12].Most snails feed by drilling a hole into their bivalve prey, but rapa whelks usually smother their prey by wrapping around the hinged region of the shell and feed by introducing their proboscis between the opened valves. The whelk can also secrete a thick mucus that may or may not contain biotoxins to weaken the prey.

Reproductio in Veined Rapa Whelk It is dioecious gastropod with separate sexes. Mating occurs duringwinter and spring. Masses of egg cases are laid in April to late July.The egg cases are attached to hard substrates and may contain1,000 developing embryos. One female adult can lay multiple eggcases throughout summer, upon hatching the larvae areplanktotrophic. The variable duration of the planktonic periodallows for a variety of disposal strategies by the species thereby facilitating its invasions and spread.

MATERIALS AND METHODS
Sampling Area: The samples were collected from crude oil polluted environment in Niger Delta, Nigeria.
Sample A: Nembe, Bayelsa state
Sample B: Buguma, Kalabari community
Sample C: UnyeadaAndoni community

COLLECTION OF SAMPLE
The samples used for this analysis were periwinkle, oyster and veined rapa whelk. They are marine sea food mostly found and eaten in the Niger Delta region.The samples were bought from sellers from different market in Port Harcourt and was put in sterile polythene bags and taken to the laboratory for analysis within 2 hours after purchase

Media Preparation

Nutrient Agar: The medium was prepared by weighing 11.2g of nutrient agar into 500ml Erlenmeyer flask. 400ml of distilled water was gradually added to it. This was brought to boiling to dissolve completely by heating it over Bunsen burner flame for 30 minutes. The medium was sterilized at 121^0C for 15 minutes using the autoclave at 15psi. The medium was allowed to cool down to 45^0C and 15ml of the medium was poured into sterile petri dishes. The plates were allowed to set and dried in an oven before used.

Sabouraud Dextrose Agar: The medium was prepared by weighing 62g of sabouraud dextrose agar and dissolved in 1000ml of distilled water. And then sterilized by autoclaving at 121^0C for 15 minutes at 15psi.

Isolation and identification of test organisms: Aliquot (0.1ml) of the sample was transferred into sterile agar plates in duplicates. Uniformly spread with sterile glass spreader (spread plate method) and incubated in inverted position at 37^0C for 24 hours.

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 48 hours of growth at 30^0C. The morphology was determined by examination of plates directly under the microscope at low (10x).

Microscope Examination: A portion of each discrete colony was thinly smeared in a drop of water on a clean grease-free glass slide with the aid of a sterile wire loop and allowed to air dry. It was heat fixed by passing over the flame. The fixed smear was flooded with crystal violet stain for 1 minute and washed off with tap water. Lugol's iodine was used in flooding it and allowed to stay for 1 minute and rinsed with tap water. The smear was decolourized with alcohol and rinsed immediately with tap water. The smear was then counterstained with safranin-O for 1 minute, then it was washed off with tap 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-O.

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.

Microscopy: Several coloured colonies were selected from the incubated plate for identification using the following procedure: a wet mount slide was prepared by transferring a small amount of the culture with a dissecting needle or inoculating loop to make a slide. This was covered with a cover slip and examined under low power (x10) or high power (x100) objective.

STOCK SOLUTION

Ten percentage glycerol solution was prepared in McCartney bottles and autoclaved at 121^0C for 15 minutes, allowed to cool, then the pure cultures were inoculated into each McCartney bottle, until the clear colourless solution turns turbid and were stored in the refrigerator. This served as pure cultures for subsequent characterization.

RESULTS

Estimation of bacterial and fungal count on different parts of shell sea food

Estimation of total heterotrophic bacteria and total fungi using standard plate count of the various samples from Port Harcourt were shown in Table 1- 2.

Table 1: Total heterotrophic bacteria count of periwinkle, oyster and veined rapa whelk samples

Sample ID		Periwinkle	Oyster	Veined rapa whelk
External body (cfu/g)	Plate 1	1.25×10^8	9.8×10^7	5.6×10^7
	Plate 2	2.0×10^7	1.05×10^8	8.7×10^7
*Cap (cfu/g)/inner shell fluid (cfu/ml)	Plate 1	7.8×10^7	4.5×10^7	6.2×10^7
	Plate 2	5.3×10^7	6.1×10^7	1.50×10^8
Intestine (cfu/ml)	Plate 1	1.53×10^8	1.25×10^8	1.22×10^8
	Plate 2	6.6×10^7	5.0×10^7	1.50×10^8

*cap applies to periwinkle and vein rapa whelk while inner shell fluid is for oyster (cap absent in oyster)

Table 2: Total fungal count of periwinkle, oyster and veined rapa whelk

Sample ID		periwinkle	oyster	Veined rapa whelk
External body (sfu/g)	Plate 1	1.4×10^7	1.4×10^7	1.5×10^7
	Plate 2	1.6×10^7	9.0×10^6	8.0×10^6
*Cap(sfu/g)/inner shell fluid(sfu/ml)	Plate 1	1.1×10^7	6.0×10^6	7.0×10^6
	Plate 2	5.0×10^6	7.0×10^6	5.0×10^6
Intestine (sfu/ml)	Plate 1	7.0×10^6	7.0×10^6	8.0×10^6
	Plate 2	5.0×10^6	1.1×10^7	1.2×10^7

*cap applies to periwinkle and vein rapa whelk while inner shell fluid is for oyster (cap absent in oyster)

Percentage (%) frequency of microbial isolates

The percentage (%) frequency of the different bacterial and fungal isolates were shown in table 3-4.

Table 3: Percentage (%) occurrence of bacterial isolates from periwinkle, oyster and veined rapa whelk.

Isolate	No. of Occurrence	Percentage (%) of Occurrence
Staphylococcus aureus	32	17.3%
Escherichia coli	14	7.6%
Salmonella sp	12	6.5%
Vibrio sp	20	10.8%
Citrobacter sp	7	3.8%
Serratia sp	14	7.6%
Micrococcus sp	14	7.6%
Bacillus sp	26	15.1%
Proteus sp	15	8.1%
Shigella sp	19	10.3%
Klebsiella sp	26	14.1%
	185	100%

Table 4: Percentage occurrence of fungi inperiwinkle, oyster and veined rapa whelk.

ORGANISM	OCCURRENCE	PERCENTAGE (%) OF OCCURRENCE
Aspergillus sp	97	58.8%
Penicillium sp	5	3.0%
Physarum sp	38	23.0%
Crytococcus neoformas	1	0.61%
Mucor sp	12	7.3%
Candida sp	6	3.6%
Neurospora sp	6	3.6%
Total	165	100

Table 5: Frequency of total heterotrophic bacteria from periwinkle, oyster and veined rapa whelk.

		Staphylococous aureus	*Escherichia coli*	*Vibrio sp*	*Citrobacter sp*	*Serratia sp*	*Micrococcus sp*	*Bacillus sp*	*Proteus sp*	*Shigella sp*	*Klebsiella sp*	*Salmonella sp*
	Sample ID											
y s t e r	E X B	$6.0x10^6$	$3.0x10^6$	$1.0x10^6$	-	-	$4.0x10^6$	$5.0x10^6$	-	$2.0x10^6$	$3.0x10^6$	-
	I S F	-	$4.0x10^6$	-	$1.0x10^6$	-	$5.0x10^6$	$7.0x10^6$	$4.0x10^6$	-	$8.0x10^6$	$4.0x1$
	I N T	$5.0x10^6$	$2.0x10^6$	$4.0x10^6$	-	-	-	$2.0x10^6$	-	-	-	$1.0x1$
iwinkle	E X B	$7.0x10^6$	$1.0x10^6$	$5.0x10^6$	$3.0x10^6$	$5.0x10^6$	$2.0x10^6$	$3.0x10^6$	$5.0x10^6$	$7.0x10^6$	$4.0x10^6$	-
	C A P	$4.0x10^6$	-	$1.0x10^6$	$2.0x10^6$	-	$6.0x10^6$	$1.0x10^6$	$1.0x10^6$	-	-	$2.0x1$
	I N T	$2.0x10^6$	-	$4.0x10^6$	-	$5.0x10^6$	-	$5.0x10^6$	-	-	-	$1.0x1$
h e l k	E X B	$3.0x10^6$	$2.0x10^6$	-	$1.0x10^6$	$1.0x10^6$	-	-	$2.0x10^6$	$6.0x10^6$	$5.0x10^6$	$2.0x1$
	C A P	-	-	$5.0x10^6$	-	$2.0x10^6$	$2.0x10^6$	$1.0x10^6$	-	-	-	-
	I N T	$5.0x10^6$	$2.0x10^6$	-	-	$1.0x10^6$	-	$2.0x10^6$	$3.0x10^6$	$4.0x10^6$	$6.0x10^6$	$2.0x1$

Key: EXB = External body, ISH = Inner Shell Fluid, CAP = Periwinkle or Whelk Cap .
INT = Intestine

Table 6: Frequency of total fungi from periwinkle, oyster and veined rapa whelk.

Samples	Sample ID	*Aspergillus terreus*	*Aspergillus fumigates*	*Aspergillus niger*	*Aspergillus flavus*	*Physarum cenerum*	*Crytococcus neofomas*	*Mucor sp*	*Penicillium sp*	*Candida sp*	*Neurospora sp*
O y s t e r	E XB	1.0x107	5.0x106	-	-	$3.0x106$	-	-	-	-	-
	I S F	-	$8.0X10^6$	$7.0X10^6$	-	$3.0X10^6$	-	-	$5.0X10^6$	-	-
	I N T	-	$2.0X10^6$	-	-	$1.0X1O^7$	-	-	-	-	-
Periwinkle	E X B	-	$6.0X10^6$	$3.0X10^6$	-	-	-	-	-	$3.0X10^6$	
	C A P	-	-	$1.0X10^7$	$6.0X10^6$	$5.0X10^6$	-	$7.0X10^6$	-	$2.0X10^6$	-
	I N T	-	-	-	$5.0X10^6$	-	-	$4.0X10^6$	-	-	$6.0X10^6$
W h e l k	E X B	-	-	$1.0X10^7$	-	$1.0X10^7$	-	-	-	-	-
	C A P	-	$1.0X10^6$	$1.0X10^7$	$8.0X10^6$	$5.0X10^6$	-	-	-	-	-
	I N T	-	-	$1.0X10^6$	$2.0X10^6$	$1.0X10^6$	$2.0X10^6$	$1.0X10^6$	$1.0X10^6$	-	-

Key: EXB = External body, ISH = Inner Shell Fluid CAP = Periwinkle or Whelk Cap
INT = Intestine

DISCUSSION

In this study of the microbiological investigation of shell sea food, the various part of the sample were assessed for microbial contamination. It was discovered that the oyster shell has the highest contamination followed by the periwinkle and the veined rapa whelk, for the cap/inner shell fluid, the veined rapa whelk has the highest contamination, followed by

the periwinkle and then the oyster and for the intestine the it was discovered that the veined rapa whelk has the highest contamination, followed by the periwinkle and the then oyster Table 1-2.

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 [13]. It was discovered that this fresh sea foods accumulate high level of bacteria resident in soil and water in addition to organisms from faecal pollution and other waste products.

Houses in the Riverine areas of Nigeria lack proper toilet facilities and out-houses are used as toilets with faeces deposited directly into rivers, streams and swamps. The indicator organisms imply that these sea foods are hazardous to consume and the various gram negative bacterial isolated from these sea foods including *Escherichia coli* suggest that consumers could easily succumb to gastroenteritis, food intoxication and hepatitis A infection [14].

The bacteria isolated from various part of these shell sea foods include: *Escherichia coli, Shigella sp, Salmonella sp, Citrobacter sp Proteus sp, Micrococcus sp, Bacilli* sp, and *Staphylococcus aureus.*The cumulative percentage (%) frequency of the bacterial isolates were: *Staphylococcus aureus* (17.3%) >*Bacillus* sp. (15.1%) >*Klebsiella* sp. (14.1%) >*Vibrio* sp. (10.8%) >*Shigella* sp. (10.3%) >*Proteus* sp. (8.1%) >*Escherichia coli = Serratia* sp. = *Micrococcus* sp. (7.6%) >*Salmonella* sp. (6.5%) >*Citrobacter* sp. (3.8%). The fungi isolated include: *Penicillium sp, Aspergilus* sp., *Physariumcinereum, Mucor*sp., *Cryptococcus neoformas, Neurophora* sp. and *Candida*sp. having cumulative percentage (%) frequency as follows: *Aspergillus* sp. (58.8%) >*Physarium cinereum* (23.0%) >*Mucor* sp. (7.3%) >*Candida* sp. = *Neurospora* sp. (3.6%) >*Penicillium* sp. (3.0%) >*Cryptococcus neoformans* (0.61%) Table 3-4. The sea foods used in this study are important commercial molluscs that are widely eaten by mankind from time immemorable especially by people of the Riverine areas, as a good source of protein [15]. Variation in the microbial load between the shellfishes can be attributed to the variation in micro environmental condition encountered by these organisms .

Most of the microbial isolates in this study have been isolated from periwinkle in the previous study. Some of the organisms isolated are known indigenous bacteria flora in water body and they include *Vibrio sp,* hence are common and widely distributed where these sea foods inhabit, the non-indigenous bacteria such as *Stapylococcus aureus, Escherichia coli* and *Salmonella sp* are known to be pathogenic. All the organisms isolated have health implication for human except *Micrococcus virians,*(Table 5) which have not been associated with human infections [1].

Escherichia coli is a pathogenic strain producing diseases of the gut which may vary in severity from extremely mild to severe, and possibly life-threatening, depending on a number of factors such as type of pathogenic strains, susceptibility of victim and degree of exposure. The isolation of the opportunistic pathogen *Candida* sp, (Table 6) is of great health concern.

The principal symptoms of salmonellosis (non-typhoid infection) are non-bloody diarrhea, abdominal pain, fever, nausea, vomiting which generally appear 12-36 hours after ingestion. There is evidence for a minimum infection dose (M.I.D) of as little as 20 cells (Varnam and Evans 1991), while other studies have consistently indicated $>10^6$ cells. The disease caused by *S. aureus* is intoxication and common symptoms may appear within 2-4 hours of consumption of contaminated of foods, include nausea, but in severe cases, dehydration can lead to shock and collapse. Shigellosis (earlier name was bacillary dysentery) which is an infection of the gut.

The diseases associated with *Vibrio sp* are characterized by gastroenteritis symptoms varying from mild diarrhea to the classical cholera, with profuse watery diarrhea. These organisms are likely to have been introduced into the environment by bathers and surfers who use these creeks for recreational purposes or from industrial and domestic waste deposited into the creeks.

The microbial load of the fresh samples, from the results obtained, when compared with the food and drug administration (FDA, 1986) standards for standard plate count <500,000/g following harvest of shellfishes for consumers' safety indicated that these fresh sea foods are not safe for consumption.

Good sanitary practices, maintenance of bacteriological standard for the quality of the growing waters, further processing (preservation) before consumption is necessary to improve the quality of shellfishes.

CONCLUSION

The result revealed fungi and bacteria as microorganisms that are associated with periwinkle, oyster and veined rapa whelk. Some of these microorganisms are pathogenic and are capable of causing chronic illnesses in human if consumed. Post harvest contamination is common due to processing, storage and handling which is the major source of cross contamination. Safety indicates that these fresh sea foods are not safe for consumption rather it should be well cooked before consumption.

RECOMMENDATION

Owing to the high demand of these sea foods and health hazard associated with microorganisms isolated from them as revealed in this study, it is important that sea foods products should be properly cooked.

1. Introduction and enforcement of microbial guideline as a way of protecting consumers appears to be highly desirable.
2. More attention should be paid to safety through proper storage and handling procedure.
3. Oilcompaniesoperating in area should avoid untreated waste disposal into the river.

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