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BACTERIAL CONTAMINATION OF SOME EDIBLE SHELLFISH HARVESTED FROM KALARUGBANI CREEK, RIVERS STATE, NIGERIA

N. N. Odu[1], H. O. Njoku[1], N. J. P. Igwiloh[1] and C. O. Akujobi[2]
[1]Department of Microbiology, University of Port Harcourt, PMB 5323 Port Harcourt, Rivers State, Nigeria
[2]Department of Microbiology, Federal University of Technology, PMB 1526, Owerri, Imo State, Nigeria
Corresponding author: Dr. C. O. Akujobi

ABSTRACT

The bacteriological quality of raw shucked oyster (RSO), raw extracted periwinkle (REP), overlying water (OLW) and mud flat samples (MFS) obtained from Kalarugbani Creek in Rivers State was investigated. The parameters investigated were the total viable count (TVC), salmonella-Shigella counts (SSC), total faecal coliforms (TFC) and total coliform counts (TCC). There were variations in the values of these parameters analyzed, which were sample dependent. Of all the samples analyzed, RSO had the highest microbial counts except for TFC and TCC in which periwinkle has higher counts. Statistical analysis of the mean total viable counts showed high significant differences among the four samples across various months at ($p \leq 0.05$). The microbial counts were lower in the wet season and higher in the dry season. Correlation analysis of the seasonal variations of the total viable count for the various samples showed a very weak correlation between the two seasons for MFS and OLW ($r = 0.499$ and $r = 0.515$, respectively), while those of REP and RSO were strongly correlated between the two seasons ($r = 0.69$ and $r = 0.607$, respectively). The microbial counts obtained from this study were found to be higher than the specified standard limits ($1x10^5$ cfu/g) for bacteria by International Commission on Microbiological Specification for Foods (ICMSF) and United States Food and Drug Administration (USFDA). The results of this study show the presence of organisms of public health concern and highlight the need for maintenance of quality standards in the processing of these shellfish. The data obtained will be useful for the development of food safety schemes and policies.

Keywords: Oyster, periwinkle, shellfish, molluscs, creek, Nigeria

INTRODUCTION

Shellfish is a popular term for aquatic and marine invertebrate animals possessing hard outer covering or shell. The term shellfish applies especially to edible species that constitute very important part of the diet in many parts of the world. Shellfish are plentiful and easily collected food source that can be found in coastal zones as well as rivers and lakes around the world. Shellfish comprises of crustaceans, which include, shrimps, crabs, lobster, crayfish and molluscs (bivalves squids, snail) and posses single (univalve) or double (bivalves) shell for a covering (Adebayo-Tayo and Ogunobi, 2008).

These shellfishes are obtained from the sea and eaten as food; thus, they are termed seafood. Seafood forms a major source of protein to both riverine dwellers and the public at large. Seafood is readily available in the Niger Delta region of Nigeria and many other parts of the World (Odu 1989; Ovuru and Alfred-Ockjya, 2001). Fresh seafood is highly perishable and their shelf life is substantially limited by microbiological spoilage (Mu *et al.*, 1997). Microbial growth in fresh seafood posses potential hazards to the general public, especially when pathogenic microrganisms are involved (McCarthy and Shugan, 1990). The aquatic environment and the methods employed in harvesting, handling, processing and distribution of seafood may adversely affect their wholesomeness and microbial safety.

It is established that the aquatic environment from which they are harvested is continually polluted by both natural factors and industrial activities, which result in the occurrence of many polluted shellfish. Some of the pollutants generated from such industrial activities which have direct impact on the aquatic environment and human health are toxic heavy metal (Mandany *et al.*, 1996).

In several developing countries in African, there is a strong economic incentive derived from a sustained demand for shellfish as an animal protein source and this is particularly so in Ghana, Nigeria and Cameroon. However, in these countries harvesting bivalves has little or no regulatory

mechanisms in place and this is further exacerbated by poor sanitary facilities which require extra attention to curtail the incidence of shellfish borne diseases.

Due to the health hazard inherent with the consumption of bivalves many developed countries have enacted regulations based on the microbiological analysis of water and or bivalves flesh. Most of these regulations use coliform counts as indicator of faecal contamination (West and Coleman, 1986; Vilalobos and Elguezabal, 2001). Bivalves are regarded as potential hazardous food because of their inherent tendency to bioaccumulation of pathogenic bacteria and toxic metals, through filter feeding (Hatha *et al.,* 2005). The ingestion of bivalves has been frequently associated with food related infectious disease (Vieira *et al.,* 2003).

The word oyster is used as a common name for a number of distinct groups of bivalve molluscs which live in marine or brackish habitats. Oyster is locally called "'Mgbe" in Rivers state, Nigeria. Oyster is a shellfish that belongs to the family Ostreidae and the phylum Mollusca. Oysters are classified into three genera Ostrea, Crassostera, and Pycnodonta. In Nigeria, these bivalves are found in large number in the riverine area around the Niger Delta coastline (Ukwade, 1990; Odu, 1989). Oysters are cultivated in large numbers in the Philippines, Japan and the United State of America, thus, it plays an important role in the food supply in various parts of the world (Clem, 1988). Oysters are filter feeders drawing water in over their gills through the beating of cilia. Suspended plankton and particles are trapped in the mucus of a gill and from there are transported to the mouth where they are eaten, digested and expelled as faeces or pseudo-faeces.

Periwinkles are gastropods belonging to the family Cerithiidae. They are considered to be the most common and dominant molluscs in the brackish waters of West Africa (Nickles, 1950; Jamabo and Chinda, 2010). Periwinkles are small animals with thick spiral shell. There are about 30 species of periwinkle widely distributed in cold and temperate region and is locally called Isam. They are invertebrates and belong to the phylum Mollusca and a class Gastropod (Jamabo and Chinda, 2010). Some commonly known species are *Pachymelania bryronensis, Pachymelania fusca and Tympanotonous fuscatu*s. Around the Niger Delta region of Nigeria, periwinkle has been found where salinity is as low 1.5%, the hydrogen ion concentration between 6.5 and 7.1 and temperature range between 27 and 34^{o}C.

The species is a delicacy in most riverine communities as it provides a relatively cheap source of animal protein and its shell can be used as a source of calcium in animal feed and for construction purposes (Odu, 1989). The concentration of micro organism in bivalves can be tens to hundreds times as high as that in their growing water (FDA, 1989) consumer and public health regulatory agencies are concerned about the pathogenic organism found in bivalves that are generally used as indicators for bivalves quality and for domestic pollution in shellfish-growing water (Clem, 1988; Feng *et al.,* 2002).

This study was conducted to isolate and characterize bacteria associated with edible molluscs (Periwinkle and Oyster), overlying water, in Kalarugbani Creek, Okirika Local Government Area of Rivers State, Nigeria.

MATERIALS ANS METHODS

Samples collection: The samples for this study were obtained from Kalarugbani creek in Okirika LGA of Rivers State, Nigeria. The samples were raw extracted periwinkle (REP), raw shucked oyster (RSO), mud flat samples (MFS) and overlying water (OLW). Samples were taken once every month for seven months (July – January, 2010). Up to 300 pieces each of oyster and periwinkle were taken in sterile containers and analyzed within 3 hours of collection. Overlying water was obtained using sterile bottles, while mud flat samples, taken at the site where the shellfish were harvested, were obtained using sterile Ziploc plastic bag.

Extraction of molluscs: Raw oyster was extracted from the shell using sterile stainless knife as described by APHA (1970). The raw periwinkles were extracted using the method of APHA (1970) as modified by Odu (2010). Briefly, the periwinkle shells were cracked using a small sterile hammer on an improvised sterile anvil. The periwinkle flesh was individually extracted from the broken shell using sterile forceps and transferred into sterile containers.

Bacteriological analysis of the samples: Duplicates (50 g each) of the seafood samples (RSO and REP) were homogenized in 450 ml sterile 0.1% peptone water in Seward Medical Stomacher Homogenizer at 120 rev/min for 2 minutes. Tenfold serial dilution of the homogenates were made in sterile peptone water and 0.1 ml inoculated in duplicates onto nutrient agar, Salmonella-Shigella agar ant thiosulphate citrate bile salt sucrose agar plates respectively for viable, Salmonella-Shigella and Vibrio counts.

For the overlying water (1 ml of the water sample) was dispensed into 9 ml of sterile 0.1% peptone water in duplicates. Tenfold serial dilution was made and plates inoculated as described above for viable, Salmonella-Shigella and vibrio counts (Obire *et al.,* 2002).
Mudflat samples were weighed and 25 g was placed in 25 ml of 0.1% peptone water and vortexed (Undofong *et al.,* 2008). Viable, Salmonella-Shigella and Vibrio counts were obtained as previously described. All plates were incubated at 37^{o}C for 24 hours. At the end of incubation period, representative isolates were picked and purified. They were identified using their morphological and biochemical characteristics (Adjei-Boateng *et al.,* 2009).
Total coliform/faecal coliform counts were also performed on the samples by the Most Probable Number (MPN) technique as described by West (1980). Ten grams each of REP and RSO samples was transferred to the sterile homogenizing bag and homogenized with 90 ml of sterile peptone water. Samples were serially diluted and used for the determination of total/faecal coliform counts.
Physicochemical parameters of overlying water: The overlying water samples were subjected to physicochemical analysis. The parameters analyzed were salinity, temperature and pH. Salinity of the water samples was determined using a refract meter (Antergo 28). The temperature was determined on site using mercury-in-glass thermometer while the pH was measured using a multiple meter model U-10 micro (Horiba Limited, Japan) as described by Deekea *et al.* (2010).

RESULTS

The result of this study shows that there were variations in bacterial contamination which was sample dependent. It was observed that raw shucked oyster had the highest level of bacterial count while the overlying water had the least count (Table 1). The total bacteria population of the samples varied from 5.3×10^6–1.6×10^7cfu/g for raw extracted periwinkle, 7.0×10^6 - 2.1×10^7cfu/g for raw shucked oyster, 4.8×10^6 -1.2×10^7 cfu/ml for over lying water, 3.1×10^6 - 7.4×10^6 cfu/g mud flat sample. The Salmonella – Shigella counts varied from 4.4×10^3- 1.3×10^4 cfu/g for raw extracted periwinkle, 5.1 x10- 1.6×10^4 cfu/g raw shucked oyster, 5.1 x10- 1.6×10^4 cfu/ml for over lying water, 2.9×10^3 - 6.7×10^3 cfu/g for mudflat sample.

Table1: Total counts of microbial groups in the various samples

Samples	Months	TVC(cfu/g/ml)	SSC(cfu/g/ml)	TC(MPN/g/ml)	FC(MPN/g/ml)
REP	JULY	8.1×10^6	4.8×10^3	7	3
	AUG	7.1×10^6	8.0×10^3	15	4
	SEPT	6.6×10^6	6.0×10^3	36	11
	OCT	5.3×10^6	4.4×10^3	210	<3
	NOV	8.4×10^6	7.3×10^3	290	21
	DEC	1.2×10^7	9.0×10^3	240	28
	JAN	1.6×10^7	1.3×10^4	460	21
RSO	JULY	8.7×10^6	5.9×10^3	210	3
	AUG	8.0×10^6	7.4×10^3	28	7
	SEPT	7.3×10^6	5.1×10^3	150	3
	OCT	7.0×10^6	5.6×10^3	240	6
	NOV	9.1×10^6	8.2×10^3	460	28
	DEC	1.8×10^7	1.0×10^3	460	21
	JAN	2.1×10^7	1.6×10^4	1100	98
OLW	JULY	6.0×10^6	4.4×10^3	460	<3
	AUG	6.9×10^6	3.2×10^3	93	7
	SEPT	4.8×10^6	4.9×10^3	35	<3
	OCT	5.0×10^6	5.5×10^3	15	<3
	NOV	5.6×10^6	5.6×10^3	210	15
	DEC	9.0×10^6	7.0×10^3	240	75
	JAN	1.2×10^7	9.1×10^3	210	98
MFS	JULY	4.9×10^6	3.1×10^3	210	43
	AUG	5.6×10^6	3.9×10^3	460	20
	SEPT	3.1×10^6	2.9×10^3	210	9
	OCT	3.6×10^6	3.4×10^3	27	3
	NOV	6.1×10^6	6.1×10^3	150	15
	DEC	6.6×10^6	6.2×10^3	210	20
	JAN	7.4×10^6	6.7×10^3	210	38

The total coliform count ranges from 28 – 1100 MPN/g for raw shucked oyster, 7 – 460 MPN/g for raw extracted periwinkle, 15 - 460 MPN/g for overlying water and 27 - 460 MPN/ml for mudflat sample. The total faecal counts ranged from 3 - 98 MPN/g for raw shucked oyster, 3 – 28 MPN/g for raw extracted periwinkle, 3 - 98MPN/ml for overlying water to 2 - 43 MPN/g for mud flat sample.

The bacteriological quality (TVC, SSC, TCC and TFC) of the various samples in the wet season (July – October 2010) and dry season (November- January 2010) showed a seasonal variation in which counts were higher in the dry season than the rainy season. Generally, the counts obtained in January were higher than those obtained in the other months except in total count of overlying water and faecal count of raw extracted periwinkle in which counts obtained in December were higher than those obtained in the other months.

Statistical analysis of the mean total viable count showed a significant difference at ($p<0.05$) between the samples in the various months. Correlation analysis of the seasonal variation of the total viable for the various samples, OLW ($r=0.515$), MFS ($r=0.499$), REP ($r=0.69$), RSO ($r=0.607$) showed that there was a weak correlation between the two seasons for MFS and OLW, while the REP and RSO are strongly correlated in both seasons.

There were variations in the bacterial species isolated in the various samples (Table 2). The organism isolated from raw shucked oyster and raw extracted periwinkle were *Escherichia coli Bacillus sp*, *Staphylococcus aureus, Proteus vulgaris*, *Pseudomonas sp, Salmonella sp, Enterobacter aerogenes, Shigella sp, Klebsiella sp, Micrococcus sp, Enterobacter sp, Citrobacter sp, Proteus vulgaris.* The organism isolated from mudflat sample and overlying water sample were *Escherichia coli, Bacillus sp, Proteus sp, Pseudomonas aeruginosa, Micrococcus sp, Enterobacter sp, Klebsiella sp, Acinetobacter sp, Salmonella sp, Acinetobacter sp, Enterobacter sp, Micrococcus sp, Citrobacter sp, Flavobacterium*.

The physiochemical parameter of the overlying water shows that the pH values of the overlying water ranges from 6.51 - 6.78 in the wet season and 6.83-7.02 in the dry season (Figure I). There was a sudden decrease in pH between July and August, which started increasing steadily from August and culminated in the highest pH of 7.02 obtained in January. There was also seasonal variation in the ambient temperature in Kalarugbani creek as depicted in this study. Dry season temperatures were slightly higher than those of the wet season (Figure II). The temperature values range from 24.75 - 26.05ºC for the wet season and 26.85 - 27.61ºC in the dry season.

Figure III showed that the salinity values range from 1.094- 2.71 % in the wet season and 2.89-3.79 % in the dry season.

Table 2 Microbial isolates from RSO, REP, OLW and MFS

Samples	Microbial Isolates
RSO	*Escherichia coli, Bacillus sp*, *Staphylococcus aureus*, *Proteus vulgaris Pseudomonas sp, Salmonella sp, Enterobacter aerogenes, Shigella sp, Klebsiella sp.*
REP ,	*Staphylococcus aureus, Bacillus sp, Escherichia coli, Micrococcus sp, Enterobacter sp ,Citrobacter sp Klebsiella sp ,Salmonella sp Shigella sp, Proteus vulgaris.*
OLW	*Escherichia coli, Bacillus sp, Proteus sp, Pseudomonas aeruginosa, Micrococcus sp, Enterobacter sp,Klebsiella sp, Acinetobacter sp, Salmonella sp.*
MFS	*Acinetobacter sp, Enterobacter sp, Micrococcus sp, Klebsiella sp, Salmonella sp, Proteus sp, Citrobacter sp, Escherichia coli, Flavobacterium*

Key: RSO – Raw shucked oyster; REP- Raw extracted perwinkle; OLW- overlying water; MFS- Mud flat sample.

DISCUSSION

Periwinkle and Oyster are important commercial molluscs that are widely eaten by mankind especially by people of the riverine areas in Nigeria which serve as good sources of protein. Mollusca shellfish, especially periwinkle and oyster are considered as potentially hazardous food because of their inherent tendency to bioaccumulate pathogenic bacteria and toxic metals through filter feeding. It was understood that the inappropriate disposal of raw and partially treated sewage was a principal reason for increasing incidence of shellfish-borne illness (Odu, 1989; Whitman and MacNair, 2004). Sewage contamination of filter–feeding bivalve caused a well-documented human health risks.

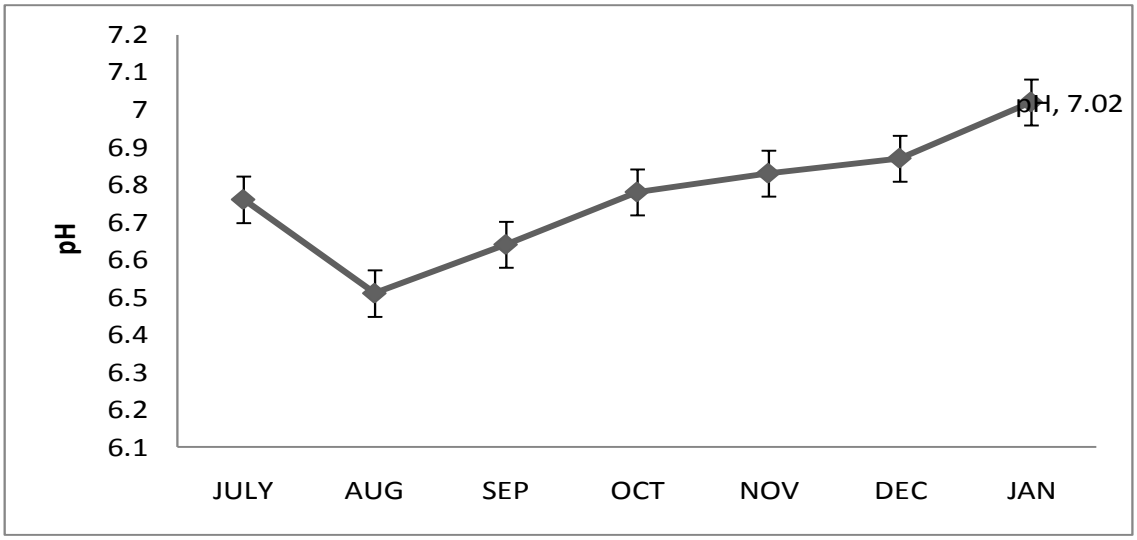

Fig. I: Monthly variation of pH of the overlying water. (Values represent the mean of two determinations. Error bars indicate standard errors of mean).

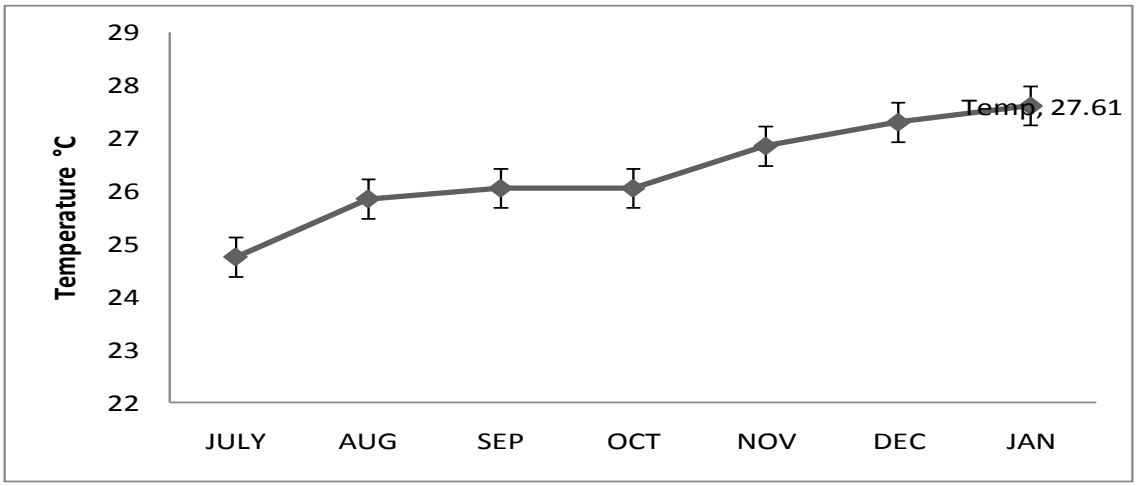

Fig. II: Monthly Temperature variation of the overlying water. (Values represent the mean temperature determination. Error bars indicate standard errors of mean).

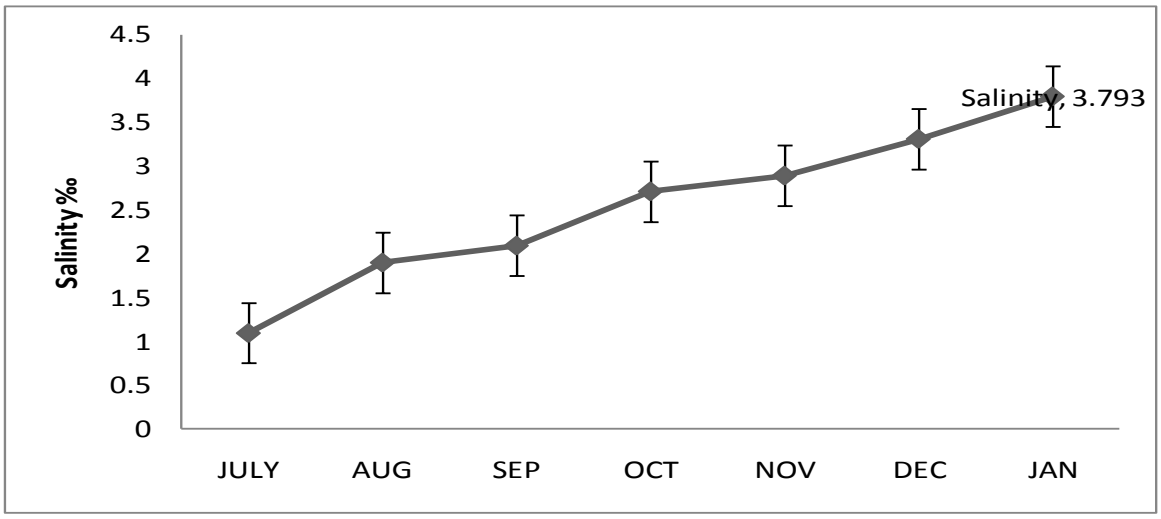

Fig. III: Monthly variation of salinity of the overlying water. **(**Values represent the mean of two determinations. Error bars indicate standard errors of mean)

This is due to microrganisms transmitted by the faecal-oral route, particularly when the shellfish are consumed raw or lightly cooked. Several factors influence the microbial quality of seafood.

Seafood is however contaminated by several microrganisms from their aquatic environment (McCarthy *et al.,* 1990). This was evident in the high bacterial load obtained in the various samples in this study. The bacteriological quality (TVC, SSC, TCC and TFC) of the various samples in the wet season (July –October 2010) and dry season (November 2009 - January 2010) showed a seasonal variation in which counts were higher in the dry season than the rainy season. The total viable counts for the various samples showed that raw shucked oyster had higher counts. this may be due to the fact that oyster which is a deposits feeder are known to ingest sediments and use organic matter and microrganisms in the sediment as food (Jeffery, 1995).

Oysters are known to live in fresh water, which support the proliferation of broader spectrum of microrganisms (Nester *et al.,* 1993). It may also be as a result of the fact that oysters are filter feeder and are able to accumulate the bacteria in their tissue to level of 4 to 7 times higher than that of surrounding (Hatha *et al.,* 2005). The result of the present study is in agreement with the work of other researchers (Odu, 1989; Kriengsag, 1992; Adebayo-Tayo and Ogunjobi, 2008; Odu *et al.,* 2010). The mudflat sample had the lowest total viable counts. Microbial communities in mudflat may have been influenced by many abiotic factors including various types of organic and inorganic nutrients, sediments physical properties and environment condition and may be negatively impacted by human activity (Mosher *et al.,* 2006).

In general the microbial count of these samples was higher in the dry season than the wet season and this may be due to the increase in dilution of the estuarine water by rain fall. Depaola *et al.* (2005) and Adjei–Boateng *et al.* (2009) reported that the level of contamination of shellfish depends on the extent of pollution in the growing water since this water may be contaminated with untreated wastes and as filter feeder, there is a great tendency that these shellfishes will accumulate a number of food poisoning and pathogenic organism from water impacted by sewage pollution and bio-toxins produced by some of the phytoplanktons that they feed on, which accumulate in their tissues. When ingested as food, cause a disease called paralytic shellfish poisoning in humans.

Most of the microrganisms isolated from this study have been isolated from shellfish in other studies (Kriengsag, 1992; Adebayo-Tayo and Ogunjobi, 2008; Odu *et al.,* 2010).The incidence of microorganisms in shellfishes depends on the quality of the water from which the animal is obtained. Various bacterial isolates were obtained in RSO, REP, OLW and MFS. The occurrence of enteric organisms in these shellfishes is an indication of the pollution of their overlying water with untreated faecal waste and sewage. These organisms are common in seafood and have health implication in man.

The presence of *E. coli* in these shellfish is an indication of faecal contamination as *E. coli* are known to be associated with gastrointestinal tract of warm blooded animals. *E coli* are the causative agent of diarrhoea, dysentery, haemolytic uremic syndrome, bladder and kidney infection, septicaemia, pneumonia and Meningitis (Kumar *et al.,* 2004). Although *E. coli* contamination of tropical seafood is quite common, the distribution of different pathogenic types in seafood is poorly studied. *E. coli* is frequently used as an indicator organism for faecal contamination in food and water. These species is in most cases quite harmless but some strains are highly pathogenic to humans especially in infants in the developing countries. The enteropathogenic *E. coli* (ETEC) are commonly implicated in diarrhoea (Crane *et al.,* 2006).

Contrary to *E. coli*, *Samonella* spp are general pathogenic to humans and represents one of the main causes of food borne illness worldwide (Galanis *et al.,* 2006). Salmonella is one of the most important food-borne pathogen being responsible for about half of the reported cases and outbreak of food borne diseases (Butt *et al.,* 2004). It is generally believed that like *E. coli*, *Salmonella* is associated with a number of non-human hosts e.g. reptiles (Winfield and Groisman, 2003). *Salmonella* has been detected in shellfish (Adebayo-Tayo *et al.,* 2006) in the gut of Tilapia and crab (Lyer and Shrivastava, 1983; Ogbondeminu, 1993) and causes new-born meningitis and infantile diarrhoea. *Salmonella* sp. are the causative agents of Salmonellosis in humans who are only reservoirs of this organism (Nester *et al.,* 1995). Seafood in particular has been recognized as potential vectors (Wilson and Morre, 1996; Heinitz *et al.,* 2000).

CONCLUSION

The results of this study show the presence of organisms of public health concern and highlight the need for maintenance of quality standards in the processing of these shellfish. The data obtained will be useful for the development of food safety schemes and policies. There is cause for concern for shellfish culturist, processors and exporters as failure to meet the standards of importing countries may lead to serious economic losses. Possible measures to help improve microbial quality

and safety of shellfish could be the treatment of wastes/effluents from domestic and industrial sources before disposal. This would constitute additional feasible strategy for quality control in the shellfish industry.

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