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The Significance of Pathogenic Bacteria in the Gut of Swimming Crab, *Callinectes* sp Obtained from Lagos Lagoon and Market Samples Stored at Freezer Temperature (0°C)

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Abstract: An investigation was conducted to isolate and characterize pathogenic bacteria in the gut of swimming crab (*Callinectes*) obtained from Lagos lagoon (28±2°C) and market frozen samples (0°C). The log₁₀ of cfu/ml total value of 7.2 and 4.83 cfu/ml was obtained from fresh lagoon and market samples respectively. Pathogens isolated were *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella* sp, *Streptococcus agalactiae*, *Vibrio* sp, *Staphylococcus aureus* and *Micrococcus* sp. *Escherichia coli* and *Bacillus subtilis* were more preponderant than other pathogens with log₁₀ cfu/ml values of 5.90 and 3.0 (for Lagoon and market samples) and 5.81 and 4.47 (for lagoon and market samples) respectively. However, the log₁₀ of cfu/ml for market samples for *Bacillus subtilis* was higher than *Escherichia coli*. On blood agar supplemented with sheep red blood cells, most isolates except few were β- and α-haemolytic, demonstrating that they could be pathogenic strains. The degree of haemolysis were *Bacillus cereus* 34 (76%), *Escherichia coli* 46 (78%), *Salmonella* sp 33 (76.7%), *Vibrio parahaemolyticus* 40 (93%), *Staphylococcus aureus* and *Bacillus subtilis* were more haemolytic than *Micrococcus* sp, *Streptococcus agalactiae* and *Proteus vulgaris*. The occurrence of these pathogens in the swimming crab is of epidemiological and health significance.

Key words: *Salmonella*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, epidemiological

INTRODUCTION

Crabs are crustaceans found in virtually all parts of the world. In tropical waters, *Callinectes* species are common and most of the landed crabs belong to this genus. Crabs are popular dietary components of many Nigerian dishes. Because of its high calcium content it is recommended for pregnant women. However, they have been implicated in incidences of food intoxication and infection. Crabs and shrimps have been implicated in *Vibrio parahaemolyticus* food poisoning (Silker, 1986) cholera (Baine *et al.*, 1974) salmonellosis (Frazier and Westhoff, 1988) shigellosis (Piexotto *et al.*, 1979) and Yersinia food infection (Silker, 1986). Other pathogenic microbes has been associated with food poisoning and infection in sea foods; *Bacillus cereus* enterotoxin (Granum *et al.*, 1993; Johnson, 1984).

Deaths from staphylococcal food poisoning have been reported (Bergdoll, 1990). This investigator's report also asserted that the offending organism, *Staphylococcus aureus* grow rapidly and produces enterotoxins between 66°F and 99°F (20°C and 37°C) and that the

staphylococcal enterotoxins are highly resistant to heat. Bergdoll (1979, 1990) reported that the normal temperature used in cooking will not destroy the toxins and foods containing staphylococcal enterotoxins usually look and taste normal.

The present study was designed to determine the occurrence of pathogenic and health-threatening bacterial species in the gut of the swimming crab (*Callinectes* sp) and to stress the health implication of their presence in this edible seafood.

MATERIALS AND METHODS

Samples of crab of the species *Callinectes* variety were purchased from supermarkets (frozen) and lagoon front of the University of Lagos, in Lagos metropolis. The fresh samples were collected in new sterile polythene bags in the morning and transported to the laboratory immediately for analysis.

Different enrichment and selective cultural methods were used to determine the abundance of bacteria in the gut of the swimming crab. The medium employed were

among others, Thiosulphate Citrate Bile Salts (TCBS; Oxoid), MacConkey agar, Nutrient agar, *Salmonella-Shigella* agar, Blair-Parker agar, Eosin Methylene Blue agar (EMB), Tryptose Soy Agar (TSA), Tryptone, Bile salt, X glucuronide medium (TBX, Oxoid) for the cultivation of the pathogens etc.

Isolation of the gut from the crab samples: Using a sterile scalpel and other dissecting instruments under aseptic conditions, the crabs were dissected and the guts obtained and kept in sterile microbiological tubes prior to inoculation into appropriate media.

Isolation of pathogens from the gut samples: About 10.0 g of the dissected guts samples were blended with 90 ml of Phosphate Buffered Saline (PBS). Serial dilution was subsequently made from this stock for the enumeration of bacterial pathogens. For the isolation, identification and enumeration of various bacterial species the methods of Maugeri *et al.* (2004), Olutiola *et al.* (1991) and Oyewole (1990) were employed. Morphology of the isolated organisms was noted and selected colonies from NA, MCA, were transferred to agar slants as pure cultures and stored at 4°C for further studies. Isolates were identified according to the procedure of Oyewole (1990); spore staining, catalase test, IMVC test, sugar fermentation test, nitrate reduction, oxidase test, indole test (Olutiola *et al.*, 1991).

***Escherichia coli*:** For the isolation of *Escherichia coli*, Concentrated samples were inoculated onto plates of Tryptone, Bile salts, X glucuronide medium (TBX; Oxoid) and incubated at 44°C for 18-24 h. Blue colonies were counted and the isolates were identified by using the API 20E system (bioMérieux). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 11228 were used as positive and negative control strains respectively.

***Vibrio*:** The total number of *Vibrio* spp. was obtained by directly inoculating the concentrated samples onto plates of Thiosulphate Citrate Bile Salts (TCBS; Oxoid) and incubating at 37°C for 24 h. Yellow and green colonies were isolated and identified at genus and species level (Alsina and Blanch, 1994a,b; Maugeri *et al.*, 2004). The isolates were confirmed using the API 20E system (bioMérieux). The nonpathogenic *Vibrio furnissii* strain NCTC 11218, isolated by Taylor and Barry (1981) and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Identification of *B. cereus*: All colonies that were rough in texture, turquoise to peacock blue in color, surrounded by greyish zones of egg yolk precipitate and mannitol negative were picked from plates with the highest dilutions for identification and confirmation.

These isolates belonging to the '*Bacillus cereus* group' were identified using staining procedures described by Holbrook and Anderson (1980) for detecting lipid globules and spore location. Differential biochemical tests were carried out as described by Harmon (1982). Motility, hemolytic activity on trypticase soy sheep blood agar, rhizoid growth on nutrient agar and the presence of toxin crystals were determined.

Blood haemolysis of isolates: The method of Samie *et al.* (2007) was used. Briefly, sheep red blood was incorporated into blood agar base (Oxoid, Basingstone, England) after sterilization and cooling to 50°C. Pure cultures of the isolates were streaked on the prepared blood agar plates and incubated at 37°C for 24 h. Plates were thereafter observed for β , α and no haemolysis. Also the percentages β - and α -haemolysis were determined for each bacterial type.

RESULTS AND DISCUSSION

Table 1 Number in Log₁₀ and types of colony forming units of bacteria in the gut of crab samples from Lagos lagoon (28°C) and those stored at freezer temperature (0°C).

In Fig. 1 the results are average values of all analysis. The abundance of bacteria measured in log₁₀ of cfu/ml of fresh crabs from the lagoon was 7.2, which were higher than cfu/ml for the frozen market samples of 4.83. Interestingly, all the isolates from the lagoon samples were also isolated from the frozen market samples. This means freezing does not actually eliminate the pathogens but only helped to reduce their numbers probably below the infective dose. However, it is not inconceivable that potent toxins, which might have been elaborated before and during freezing, may be detrimental to health of consumers when foods are consumed.

Fig. 1 shows the abundance of each pathogen obtained from the crabs. CRB2 (*Escherichia coli*) and CRB4 (*Bacillus subtilis*) occurred more than all other isolates with values of 5.90 and 3.0 (for lagoon and market samples) and 5.81 and 4.47 (for Lagoon and market samples) respectively. However, the cfu/ml for the market samples for CRB4 (*Bacillus subtilis*) was higher than for CRB2 (*Escherichia coli*). The occurrence of large numbers of *Escherichia coli* and *Bacillus subtilis* in these samples is due to the daily and uncontrolled discharge of untreated human wastes into the lagoon. The pathogenic bacteria isolated from the gut of crab include *Bacillus cereus* (CRB1), *Escherichia coli* (CRB2), *Proteus vulgaris* (CRB3), *Bacillus subtilis* (CRB4), *Salmonella* sp (CRB5), *Streptococcus agalactiae* (CRB6), *Vibrio parahaemolyticus* (CRB7), *Staphylococcus aureus* (CRB8) and *Micrococcus* sp (CRB9).

Table 1: Results of Blood haemolysis by isolates (28°C)

Microbial species	No. of Strains tested	No. haemolytic	Type of haemolysis			% haemolysis	
			β	α	No	β	α
<i>Bacillus cereus</i>	45	42	34	9	2	76	2.0
<i>Escherichia coli</i>	59	50	46	4	9	78	6.8
<i>Proteus vulgaris</i>	45	18	15	3	27	33	6.7
<i>Bacillus subtilis</i>	58	48	38	10	10	65.5	17.2
<i>Salmonella sp</i>	43	36	33	3	7	76.7	7.0
<i>Streptococcus agalactiae</i>	40	25	14	11	15	35	27.5
<i>Vibrio parahaemolyticus</i>	43	43	40	3	0	93	7.0
<i>Staphylococcus aureus</i>	40	38	35	3	2	87.5	7.5
<i>Micrococcus sp</i>	45	32	20	12	13	44.4	26.7

Table 2: Results of Blood haemolysis by isolates (refrigerator stored, 0°C)

Microbial species	No of Strains tested	No. haemolytic	Type of haemolysis			% haemolysis	
			β	α	No	β	α
<i>Bacillus cereus</i>	42	36	33	3	6	78.6	7.1
<i>Escherichia coli</i>	30	26	20	6	4	66.7	20
<i>Proteus vulgaris</i>	39	21	14	7	18	35.9	18
<i>Bacillus subtilis</i>	45	32	23	9	13	51.1	20
<i>Salmonella sp</i>	35	32	30	2	3	85.7	5.7
<i>Streptococcus agalactiae</i>	34	27	13	14	7	38.2	41.2
<i>Vibrio parahaemolyticus</i>	36	33	30	3	3	83.3	8.3
<i>Staphylococcus aureus</i>	30	28	25	3	2	83.3	10
<i>Micrococcus sp</i>	33	22	16	6	11	48.5	18.2

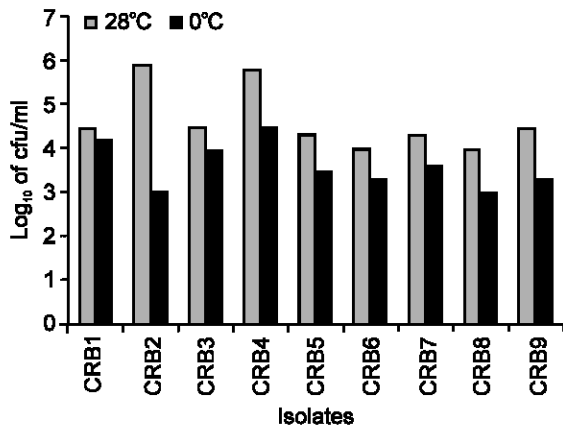


Fig. 1: Composite Bar Chart of Log₁₀ of cfu/ml of bacterial pathogens isolated from the gut of the swimming crab (*Callinectes* sp) obtained from Lagos lagoon (28°C) and frozen market samples at 0°C

In blood haemolysis test, *Bacillus cereus* 34 (76%), *Escherichia coli* 46 (78%), *Salmonella sp* 33 (76.7%), *Vibrio parahaemolyticus* 40 (93%), *Staphylococcus aureus* and *Bacillus subtilis* were more haemolytic than *Micrococcus sp*, *Streptococcus agalactiae* and *Proteus vulgaris* were least haemolytic with values of 20 (44.4%), 14 (35%) and 15 (33%) respectively for samples obtained from the open lagoon at 28°C (Table 1). Similar degree of haemolysis was recorded for isolates obtained from market samples stored at 0°C (Table 2)

with percentage haemolytic values ranging from *Vibrio parahaemolyticus* 30 (83.3%), *Staphylococcus aureus* 25 (83.3%), *Salmonella sp* 30 (85.7%), *Bacillus cereus* 33 (78.6%) and *Escherichia coli* 20 (66.7%). *Bacillus subtilis*, *Micrococcus sp*, *Streptococcus agalactiae* and *Proteus vulgaris* had lowest percentage haemolytic values of 23 (51.1%), 16 (48.5%), 14 (38.2%) and 14 (35.9%) respectively. Few numbers of each bacterial isolates were β-haemolytic except for *Streptococcus agalactiae* (market samples) where the percentage β-haemolytic strains were more than α-haemolytic strains. It must be stressed that the exponential value of isolates from open lagoon was higher than those from the market samples at 0°C. So the percentage values of crabs stored at 0°C should not be compared to those of lagoon samples in the real sense of it as these values are actually lower than those of stored samples.

The bacterial species isolated from the gut of the swimming crabs are usually found as pathogens associated with the guts of warm-blooded animals. Frazier and Westhoff (1988); Owhe-Ureghe *et al.* (1993) in their studies have implicated *Bacillus cereus* and *Bacillus subtilis* in infections and food poisoning. The pathogenesis of *Escherichia coli* and its occurrence as faecal contaminant has been reported (Frazier and Westhoff, 1988). Other workers have reported virulence and pathogenic *E. coli* strains in different animals. In humans (Reid *et al.*, 2000; Kaper, 2004); in avian (Audouin gulls) (Carmada *et al.*, 2007); in poultry (JanBen *et al.*, 2001). Hussein (2006) reported the prevalence and pathogenicity of shiga toxin-producing *E. coli* in beef and their products. Schmid-Hempel and

Frank (2007) observed in their study that in *E. coli* as small as 10 cells are infectious enough to establish disease condition, whereas the dosage can be very high in other pathogens like *Vibrio cholerae* (10^3 - 10^8 cells). The presence of *E. coli* is an indication of the pollution status of Lagos lagoon water where the crabs were harvested. Frazier and Westhoff (1988) and Owhe-Ureghe *et al.* (1993) have repeatedly mentioned *Salmonella* spp as enteric pathogens and a source of food borne infections. *Streptococcus agalactiae* belongs to the pyrogenic (pus-producing group that causes mastitis in cow (Frazier and Westhoff, 1988). Owhe-Ureghe *et al.* (1993) asserted that *Staphylococcus aureus* is pathogenic and produce enterotoxins that enhance their pathogenic effect. The isolation of *Vibrio parahaemolyticus* from the gut of crab is in line with the finding of Frazier and Westhoff (1988) that isolated this organism from the alimentary canal of mammals and classified them as potential pathogens. Also Silker (1986) in his study implicated this pathogen as a potential pathogen to consumer of raw shrimps. In addition, Vongxay *et al.* (2006, 2008) reported the prevalence, pathogenesis and occurrence of pandemic *Vibrio parahaemolyticus* in clinical samples and seafood in China, thereby re-emphasizing the public health significance of this pathogen. Pan *et al.* (2007) in their analysis of foods in China isolated haemolysin-related genotypes of *Vibrio parahaemolyticus* from seafood and clinical samples. Beecher *et al.* (1995) in their study observed that *Bacillus cereus* produces distinct exotoxin-mediated and emetic food poisoning syndrome as well as a variety of non-gastrointestinal infections. Also Rusul and Yaacob (1994) working on selected foods isolated *Bacillus cereus* and toxins associated with its growth on foods. Though *Micrococcus* spp occur as commensals, they have also been implicated in opportunistic infections especially in immunocompromised systems such as HIV patients. They have also been implicated in recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis and cavitating pneumonia (Microwiki, 2008). Due to their metabolic versatility, they are capable of utilizing wide range of unusual substrates such as pyridine, herbicides, chlorinated biphenyls and oil. They are also involved in the detoxification or biodegradation of many environmental pollutants. Hence their occurrence in the lagoon and hence the gut of the swimming crab is understandable. (Microwiki, 2008). Farley *et al.* (1993) and Farley (2001) revealed that *Streptococcus agalactiae* can cause Group B streptococcus infection. It causes bacterial septicaemia of the newborn which can lead to death or long-term sequelae such as hearing loss. It can cause neonate meningitis, invasive group B streptococcal disease of adult in pregnant, elderly or immunosuppressed. Barbaras *et al.* (2005) and Farley (2001) stressed the danger of group B agalactiae

infection in newborns and proffer how it can be prevented. Ip *et al.* (2006) isolated *S. agalactiae* serotype III from nonpregnant adults and describe their invasiveness. *Proteus vulgaris* inhabit the intestinal as well as urinary tract of humans and animals and cause urinary and wound infections. The production of urease by this organism causes UTI obstruction as the urease precipitate organic and inorganic compounds which lead to struvite stone formation.

The colony forming units observed for crabs stored at low temperature (0°C) further strengthen the assertion that low temperature arrests microbial load of foods. It is advised that crabs when harvested from the lagoons or other sources be frozen at temperature of between 0°C and subzero since toxin production by the pathogens especially *Staphylococcus aureus* will be stalled (Bergdoll, 1979, 1990). It should be emphasized that the habit of dumping untreated human excreta into the lagoon as commonly practiced should be discouraged. The Federal Government of Nigeria should pass laws or amend existing ones, prohibiting the discharge of untreated human sewage into the lagoon and, in the event of circumvention, impose stiff penalties to defaulters. The presence of potentially pathogenic bacteria in seawater associated with seafood in this case crabs, can have serious ecological, public health and epidemiological implications. Consequently, consumption of raw and partially cooked *Callinectes* sp. (swimming crab) pose serious danger to consumers of this protein and calcium-rich sea food.

Conclusion: In conclusion, our findings show that the swimming crab, *Callinectes* harbour quite a large variety of life-threatening pathogen bacteria. We are not aware if there had been any report on this subject anywhere regarding the presence of the identified pathogens in the gut of the swimming crab. Reports available so far are those on other seafood especially shrimps and lobsters. With the consortium of pathogens isolated from this crab (which serves as source of protein and mineral ions) it is advised that appropriate campaign is organized by the Nigerian Government to educate the populace on the need to cook crabs properly before consumption. The Ministry of Health should be more functional in this regard.

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