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Prevalence of *Vibrio parahaemolyticus* in Penaeus Monodon (Fabricius, 1798) from the Douala Coastal Waters of Cameroon: Implication for Food Safety

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Abstract: *Seafood constitutes an important food component and a cheap source of protein in developing countries. Shrimps are prone to bacterial contamination in their environment and have been frequently implicated in Vibrio parahaemolyticus infection.*

Of the 121 Penaeus monodon collected from coastal beaches in Douala, 79(65.3%) were contaminated with Vibrio spp. Presumed Vibrio sp. were identified by standard biochemical tests and further confirmed by API 20E kit.

A total of 150 Vibrio parahaemolyticus were isolated from Penaeus monodon and predominated in the gills40%, 22.7% from hepatopancrea and 37.3% from intestines. V. parahaemolyticus was high in Shrimps from Essengue beach (56%). V. parahaemolyticus occurrence was high in the dry season in Youpwe beach (32.6%) while low in Essengue beach (22%). Meanwhile in the wet season Essengue beach recorded high prevalence (32.7%) of V. parahaemolyticus as opposed to 20.7% in Youpwe beach. A high prevalence of haemolytic activity (80.4%) was observed when V. parahaemolyticus was grown on 5% human blood agar. Complete haemolysis predominated with a prevalence of 47.8% while partial haemolysis (32.6%) and non- haemolysis (19.6%). This study has shown that the Vibrio parahaemolyticus isolated from Penaeus monodon of the Douala coastal waters is pathogenic and can pose a health risk when consumed without proper cooking.

Keywords: *Penaeus monodon, Vibrio parahaemolyticus, haemolytic activity, Douala, API 20 E kit*

Abbreviations: *TDH: thermostable direct haemolysin, TRH: TDH-related haemolysin, API: Analytical Profile Index, TCBS: Thiosulphate Citrate Bile salt Sucrose*

1. INTRODUCTION

Shrimps are aquatic animals from either fresh or marine environment belonging to the phylum Arthropoda, class: Malacostraca, order: Decapoda, family: Penaeidae and the genus of interest is *Penaeus* where the marine type falls**.**

Shrimps, as crustaceans constitutes an important food component for a large section of the world population, and more so in developing countries where shellfish forms a cheap source of protein [1]. Seafood are prone to bacterial contamination especially filter feeders such as shellfish which concentrate the bacteria in their filtration systems, and therefore, are regrettably suited to trap all bacteria and viruses, pathogenic or otherwise, that live in the water [2,3].

Vibrio parahaemolyticus is a gram -negative halophilic bacillus, curved and motile with a single flagellum. It is a facultative anaerobic organism that naturally inhabits estuarine and coastal waters worldwide [4, 5]. It has been isolated from sediment, suspended particles [6] and from a wide variety of marine organisms [7], such as Crustaceans [8-10] and Molluscs [11].

Shellfish has been frequently implicated in *Vibrio parahaemolyticus* infection. *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* are the leading cause of seafood-borne bacterial gastroenteritis in the United States [12]. The food borne infection is usually associated with the consumption of raw or undercooked seafood. Most Vibrios secrete enterotoxins in foods, water, or in the gastrointestinal tract [13].

Food-borne illness is defined as a disease which is caused through the consumption of contaminated food [14]. Other than *Vibrio spp*, pathogens such as *Campylobacter*, *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* 0157:H7 have been reported to be responsible for major food borne outbreaks worldwide [14]. Pathogenic *Vibrio* especially *Vibrio parahaemolyticus* have been a public health concern for seafood consumers and have been the cause of import bans, detentions and rejections in international fish trade [3].

Vibrio parahaemolyticus has been implicated in gastroenteritis following the consumption of seafood in the U.S [12]. Typically, within 24 hours after eating contaminated seafood, *Vibrio parahaemolyticus* causes acute, self-limiting gastroenteritis characterized by diarrhea, abdominal cramps, nausea, vomiting, fever and chills which lasts for 1-3 days.

Vibrio parahaemolyticus has been identified in a variety of seafood including shrimps, crabs, oysters and clams due to its halophilic characteristic and seawater habitat throughout the world including in Italy [4,15,]; in Germany [16], in USA [12,5], in India [17-22], in Iran[23-25], in Malaysia [26-29, 11], in Egypt [30, 10], in Nigeria [8, 31-32], in Cameroon, from fresh water prawn [33]. *Vibrio parahaemolyticus* has been reported from cultured shrimps, hatcheries and aquaculture ponds [34-37].

In Nigeria, *Vibrio parahaemolyticus* is the top causative agent among all reports of food poisoning out breaks in recent years [8, 31].

Cameroon, whose name originated from the Portuguese word for prawn has very little information on the hygiene of Shrimps. There has been very little information on the exportation of shrimps from Cameroon waters in the international market. One of the reasons could be related to paucity of data on the quality of the seafood. Given that cholera outbreaks were reported in this region [38], therefore effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food-borne illness [39, 40]. The microbiology safety of food and water is achieved by ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication [40].

In Cameroon, *Penaeus monodon* is one of the most delicious seafood sold in top hotels and restaurants, monitoring *Vibrio parahaemolyticus* from *Penaeus monodon* and the waters is therefore crucial. Due to limited information available on Vibriosis associated with the consumption of *Penaeus monodon* in coastal waters around the commercial and touristic city of Douala, Cameroon, the aim of this study was to investigate the prevalence of *Vibrio parahaemolyticus* and test its haemolytic properties in order to generate information on risks that may be associated with the consumption of poorly processed *Penaeus monodon*.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in two beaches from the rural area south of the Douala lagoon complex. This lagoon- creek is south from Youpwe into the marine environment. This covers the only division that is rural in Douala area called the Youpwe district. The population of the two villages: Essengue and Youpwe are characterized mostly by fishermen and women and small business men. Douala area has been achieved largely through an uncoordinated program of land use management based on internal filling up of aquatic undeveloped terrain and the absorption of rural settlement. It is indeed common to find such areas having large concentration of people who have to cope with the inadequacies and problems of poorly managed environments in the area. These poorly reclaimed area now experience varying degree of perennial flooding and inundation in the Youpwe district [41]. The settlers of Essengue village live far from the Essengue beach as opposed to the settlers of Youpwe who live closer to the Youpwe beach market.

The climate of the region belongs to the Equatorial regime of a particular type or to the Cameroonian regime [42]. It is characterised by a long rainy season (March–October) and a short dry season (November –February). Heavy rainfall (approximately 4 000 mm per year) with high and stable temperatures (annual average temperature is 26.7 °C), and a high humidity throughout the year approaching 100%, are typical for this region. During the monsoon season, the region has a low wind gust with the exception of its phases of onset (April–May) and withdrawal (September–October), which are accompanied by relative violent storms [43].

2.2. Sample Collection

A total of one hundred and twenty- one (121) shrimps of the *Penaeus monodon* were collected in the Douala coastal water from May 2014 –April 2015: 50 from Youpwe beach and 71 from Essengue beach. A volume of 1 ml of water sample was pipetted into 9 ml of alkaline peptone water and the tubes were wrapped with aluminum foil paper. The shrimps were packed in sterile polythene bags. The samples were placed in an ice box and transported the same day to Yaoundé and processed at the Food Safety Laboratory, Biotechnology Center, Nkolbission, University of Yaoundé I for analysis.

2.3. Sample Processing

The entire shrimp was weighed. The gills were aseptically removed from the shrimp using a pair of sterile scissors and forceps. The gills were weighed, homogenized in 5 ml of sterile distilled water in a mortar and pestle. A volume of 1 ml of the homogenate was pipetted into 9 ml of alkaline peptone water tubes and further incubated at 30^0 C for 18 hours in an incubator (G-cell, Italy). A volume of 1 ml of the culture was pipetted and a tenfold serial dilution up to 10^{-3} was performed. The 1ml of the inoculum of the 10^{-3} dilution was used to inoculate Thiosulphate Citrate Bile salt Sucrose (TCBS) agar (Liofilchem, Italy) plates by the spread plate technique.

The procedure was repeated for intestines and hepatopancreatic organs with the exception that the skin surface was disinfected by wiping with 75% alcohol [44]. Water samples in alkaline peptone water were incubated for 18 hours and a tenfold serial dilution was performed up to 10^{-3}. A volume of 1 ml from this dilution was used to inoculate TCBS agar by the spread plate technique and incubated for 24 hours at 30^0 C.

2.4. Bacteriological Isolation and Identification

Discrete medium to large green colonies were aseptically isolated and subcultured onto fresh nutrient agar plates by the streak technique in order to obtain pure cultures of the isolates. The isolates were stored in 20% glycerol in Trypic soy broth medium for further analysis.

Discrete identifiable colonies were carefully examined macroscopically for morphologic and colony characteristics presumptively identified by motility, gram reaction, oxidase, catalase and salt tolerant and confirmed by commercially available miniaturized systems Analytical Profile Index (API) 20 E kits (BioMerieux SA, Marcy L'Etiole France). The bacterial suspensions protocol was slightly modified because the recommended 0.85% NaCl medium did not yield good identification. The bacterial suspensions were prepared in 5 ml of 2% NaCl solution instead of recommended 0.85% NaCl medium. The incubation time and temperature were maintained within the limits prescribed by the supplier (37 ± 2^0 C). The results were interpreted against a reference using the API 20E catalogue version 4.0 data base.

2.5. Test for Haemolysis

Colony of the identified isolates were subcultured onto freshly prepared 5% blood agar (Columbia agar containing human blood) plates and streaked to obtain discrete colonies. The plates were incubated at $30\ ^0$C for 24 hours after which the colonies were examined for zone of inhibition around the colony or greenish appearance colony (haemolytic activity).

3. RESULTS

3.1. Prevalence and Distribution of *Vibrio Parahaemolyticus* in *Penaeus Monodon*

The total rate of contamination was 65.3%. Out of the 36 water samples analysed, 5(13.9%) were contaminated, and with the isolation of *Vibrio parahaemolyticus*.

A total of 150 *Vibrio parahaemolyticus* were obtained from the contaminated *Penaeus monodon*. Of these, 60(40%) was isolated from the gills, 34(22.7%) from the hepatopancrea, and 56(37.3%) from the intestines (Fig. 1). These results demonstrate that the gills were the most contaminated with *Vibrio parahaemolyticus* followed by the intestines and then the hepatopancrea.

When studying the prevalence of *Vibrio parahaemolyticus* from shrimps of the sampling sites, it was noted that Essengue beach recorded higher frequency of occurrence of *Vibrio parahaemolyticus* 84(56%) than Youpwe beach 66(44%) (Fig. 2).

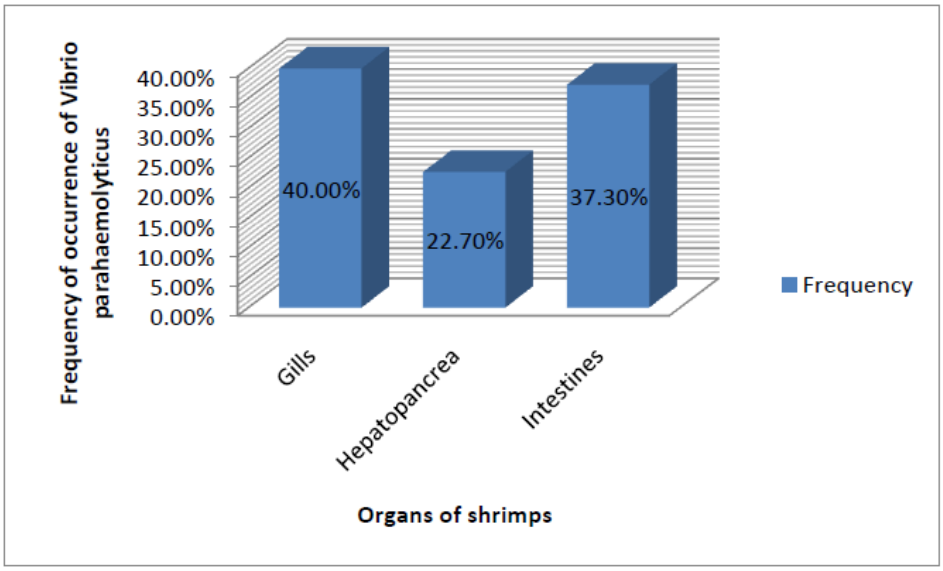

Fig1. *Frequency of occurrence of Vibrio parahaemolyticus from the organs of shrimps from the two sampling sites (Essengue and Youpwe beaches)*

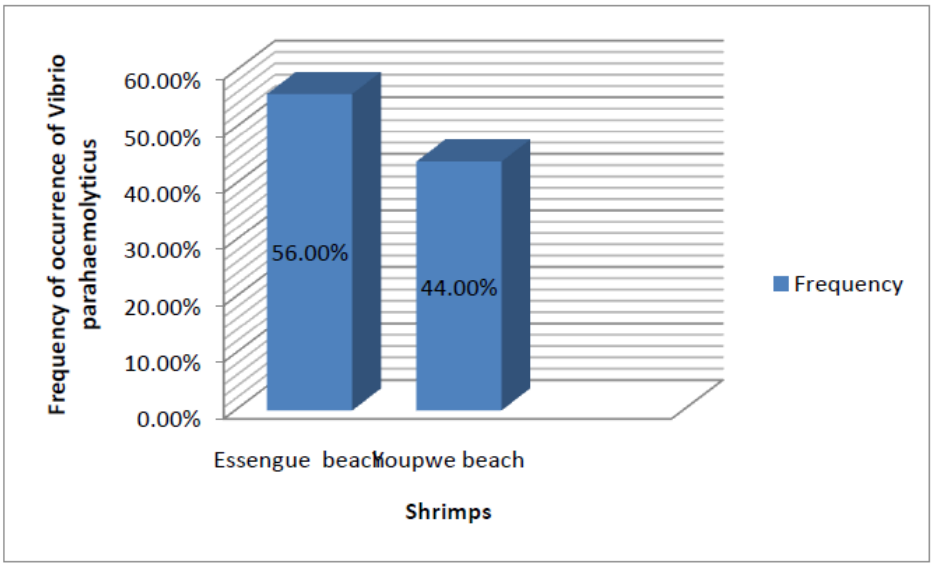

Fig2. *Frequency of occurrence of Vibrio parahaemolyticus from shrimps of the two sampling sites (Essengue and Youpwe beaches)*

3.2. Seasonal Variation of the Prevalence Vibrio Parahaemolyticus in Penaeus Monodon

When analysed by season of collection, the results demonstrate that there was a seasonal variation of the frequency of occurrence of *Vibrio parahaemolyticus* in the sampling sites. We observed that the Youpwe beach had a high frequency of occurrence of *Vibrio parahaemolyticus* during the dry season and low during the rainy season while Essengue beach recorded high frequency of occurrence during the rainy season and low during the dry season (Fig. 3)

The general trend of season variation of the frequency of occurrence of *Vibrio parahaemolyticus* has shown that shrimps in the wet season were more contaminated with *Vibrio parahaemolyticus* than those of the dry season (Fig. 3).

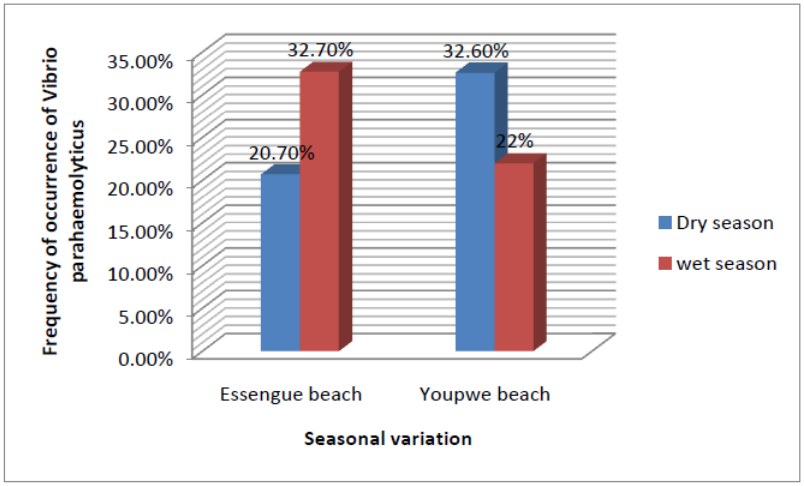

Fig3. *Seasonal variation of Vibrio parahaemolyticus from the two sampling sites (Essengue and Youpwe beaches)*

3.3. Haemolysis Test

Out of 46 *Vibrio parahaemolyticus* species that were tested for haemolysis on 5% human blood agar, 37 (80.4%) species showed either Complete (β) haemolysis (Fig. 4) or partial (α) haemolysis (Fig.5). Nine species (19.6%) showed no haemolysis (γ). Out of the 37 *Vibrio parahaemolyticus* that exhibited haemolysis, 22(47.8%) of the species manifested Complete (β) haemolysis and 15(32.6%) exhibited partial (α) haemolysis (Table 1).

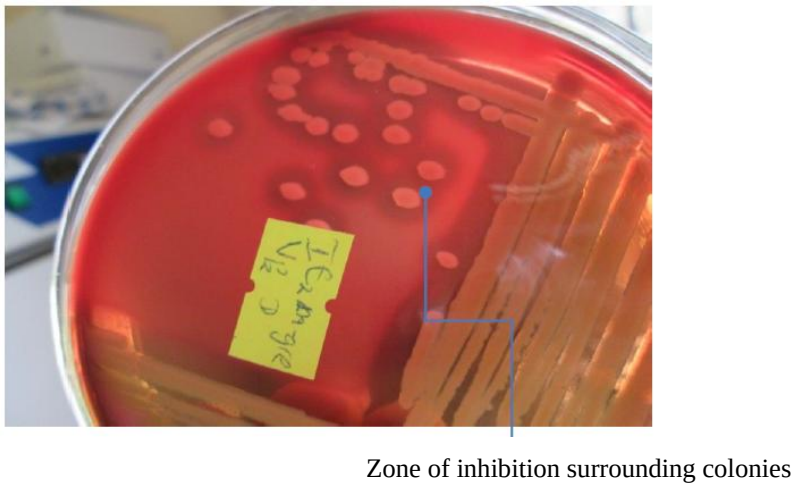

Fig4. *Diagram of haemolytic activity: complete haemolysis (β) of Vibrio parahaemolyticus on 5% blood agar*

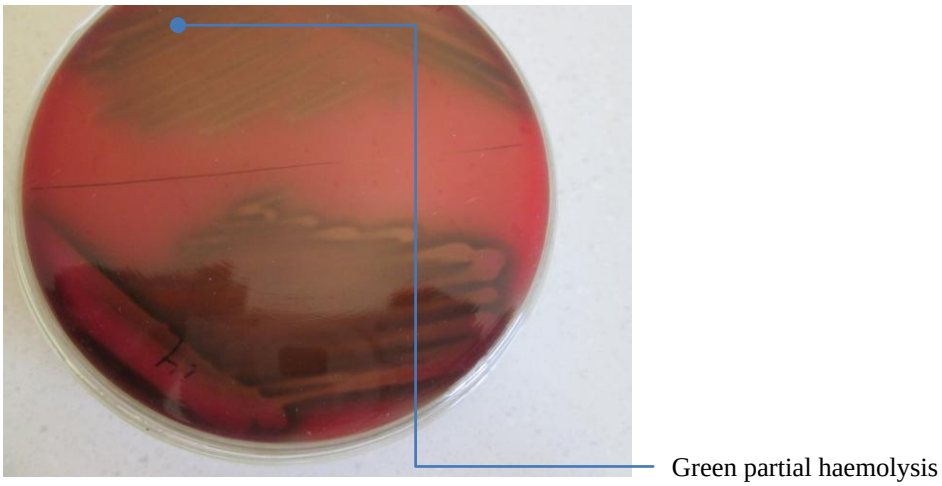

Fig5. *Diagram of haemolytic activity: partial haemolysis (α) of Vibrio parahaemolyticus on 5% blood agar*

Table1. *Haemolysis and Non haemolysis of 5% human blood in blood agar base by Vibrio parahaemolyticus*

Types of Haemolysis	Frequency of occurrence (percentage %)
Partial haemolysis (α)	15(32.6)
Complete haemolysis (β)	22(47.8)
Non Haemolysis (γ)	9(19.6)

Finally, the result has demonstrated that there were more *Vibrio parahaemolyticus* strains that exhibited complete haemolysis of red blood cells in blood agar than partial haemolysis (Table 1). One stain of *Vibrio parahaemolyticus* that produced urease exhibited partial haemolysis of red blood cells in blood agar.

4. DISCUSSION

In this study, *Vibrio parahaemolyticus* was isolated from *Peneaus monodon* collected from Douala coastal region. This study showed that 150 *Vibrio parahaemolyticus* were obtained from 79(65.3%) *Penaeus monodon* giving a high contamination rate of the seafood in this area. The findings of this study corroborate with that of Gopal *et al*. [18], Srinivasan and Ramasamy, [19], Adebayo-Tayo *et al*. [31], Sudha *et al*. [22] respectively who isolated *Vibrio parahaemolyticus* with increasing prevalence (6.9%, 11%, 18.9%, 68.1%) in seafood. Other authors have reported the presence of *Vibrio parahaemolyticus* in shrimps, crabs, sediments and pond water [17, 27, 4, 20, 31, 45, 21, 5, 46]

The current study also demonstrated that the gills were the most contaminated with *Vibrio parahaemolyticus* followed by intestines and then the hepatopancrea. These findings agree with the reports of Hua and Apun, [47] who isolated *Vibrio parahaemolyticus* from sediments, Shrimps of aquaculture farm in Malaysia. The presence of *Vibrio parahaemolyticus* in seafood could be attributed to the fact that shrimps are filtered feeders, they concentrate the bacteria as water containing food particle passed through the gills which served as a sieve during feeding. As the water passes through the digestive tract other bacteria that escape the filtration at the gills are been eliminated by the digestive tract enzymes and those that could not be eliminated will find themselves at the intestine. The findings of this study did not agree with the findings of Gomez-Gil *et al*. [48] who reported high prevalence of bacteria species in the hepatopancrea followed by intestine in healthy juveniles of *Penaeus vannamei*.

Other authors have claimed that bacteria are not commonly found in the hepatopancrea of healthy shrimps because they are prevented from entering by the gastric sieve which exclude particles larger than 0.1 mm [49]. It has been suggested that the sieve may combine with the digestive enzymes to prevent bacteria gaining access to or colonizing the hepatopancrea and therefore the presence of bacteria may represent a failure of these mechanisms [50]. However, it may be possible for bacteria to enter the hepatopancrea by other routes. In our study the appearance of most of the hepatopancreas were mostly yellowish, as compared to the characteristic reddish brown appearance of a healthy shrimp. This could explain why we isolated *Vibrio parahaemolyticus* species in the hepatopancrea. Given that the shrimps were caught in the wild and the fishermen might not have gotten good transporting conditions, there is also a possibility of autolytic enzymatic activities occurring, encouraging the outgrowth of *Vibrio parahaemolyticus* that gained entrance into the hepatopancrea.

When analyzing the distribution of Vibrio parahaemolyticus from sampling sites, Essengue beach recorded higher prevalence (56%) of *Vibrio parahaemolyticus* than Youpwe beach (44%). This could be due to the fact that the sample size of Essengue beach was higher than that of Youpwe. Essengue and Youpwe beaches are landing sites from the same coastal water so a change in sample size and transporting conditions could account for the difference in prevalence of the *Vibrio parahaemolyticus*.

There was a different in the prevalence of the seasonal variation of *Vibrio parahaemolyticus*. Youpwe beach recorded a high prevalence in the dry season while low during the wet season and vice versa for Essengue beach. The general trend of season variation of the frequency of occurrence of *Vibrio parahaemolyticus* showed that shrimps in the wet season were more contaminated with *Vibrio parahaemolyticus* than those of the dry season. This finding contrast the findings of Xu *et al*. [51] who reported high isolation rate of *Vibrio parahaemolyticus* in aquatic products in summer (50%) and 22.7% in winter.

Looking at the fishermen in the current study from both beaches and the hygienic conditions on which these shrimps were transported, it was observed that Youpwe environ practiced poor sanitation

(personal communication), this was demonstrated by the result obtained when the number of shrimps used for sampling was small but a high prevalence of *Vibrio parahaemolyticus* was recorded in the dry season in Youpwe beach than in Essengue beach. This could suggest that shrimps from Youpwe beach may be very prone to seafood poisoning.

The reason for the high prevalence of *Vibrio parahaemolyticus* at the Essengue beach in the rainy season may be due to the fact that the beach is open to the Wouri River that could bring run off from homes and wastes from crack septic tanks into the environment, thus increasing bacteria load in water.

Some parts of the coastal area of Douala coastal region are not just a public health crisis environment but also prone to humanitarian disaster area as well. From 2010 to 2012, 23000 people contacted cholera of which 843 died [38]. Cholera and Vibriosis risk factors such as slum settlement, lack of proper social amenities and services for example, drainage; waste collection; poor hygiene and sanitation facilities, are generally spread throughout the Atlantic coast. These factors could account for the high prevalence of *Vibrio parahaemolyticus* in the study area.

In this study, 80.4% of *Vibrio parahaemolyticus* were able to haemolyse human red blood cells in blood agar. This finding is similar to that of Robert *et al*. [52] and Arunagiri *et al*. [9] who reported haemolysis of human red blood in blood agar when *Vibrio parahaemolyticus* was grown. The urease positive strains of *Vibrio parahaemolyticus* in this study also haemolysed red blood cells. Urease production in *Vibrio parahaemolyticus* has been suggested to be a marker of TDH-related haemolysin (TRH) gene but not thermostable direct haemolysin (TDH) gene [18].

Haemolysis of human red blood cell by *Vibrio parahaemolyticus* is significantly associated with enteropathogenicity of *Vibrio parahaemolyticus* [53]. *Vibrio parahaemolyticus* that are able to haemolysis red blood cell have the disease causing properties (virulence factor) within its DNA. It has been reported that *Vibrio parahaemolyticus* haemolysis of red blood cell is as a result of the presence of the TDH gene [54]. The specificity of the thermostable direct haemolysin (TDH) or/and a TDH-related haemolysin (TRH) genes of *Vibrio parahaemolyticus* has been used as a molecular marker for *Vibrio parahaemolyticus* or for evaluation of the potential pathogenicity of *Vibrio parahaemolyticus* strains [54]. These genes code for the virulence factor in the *Vibrio parahaemolyticus*, suggesting that the *Vibrio parahaemolyticus* that were isolated from shrimps in our study could be pathogenic.

Gopal *et al*. [18] reported pathogenic strains of *Vibrio parahaemolyticus* in aquaculture systems and concluded that the low but detectable frequency of TDH/TRH positive strains, that is, potentially human pathogenic *Vibrio parahaemolyticus* in shrimp environs in India suggests a probable risk for health of people consuming raw seafood.

In this study the contamination rate of *Penaeus monodon* with *Vibrio parahaemolyticus* was high (65.3%). *Vibrio parahaemolyticus* are regularly linked to human food borne infections caused by consumption of undercooked or recontaminated shellfish [30]. *Vibrio parahaemolyticus* is an organism of concern in shrimp culture because some strains are associated with diseases in shrimp [55-56] and also because strains of this species are human pathogens, causing gastroenteritis [53, 57-58] worldwide and sporadic cases of and outbreaks occur regularly in Asia as well as in other countries [59]. It has also been reported in wound infection and in septicemia [60-62].

5. CONCLUSION

This study revealed that *Penaeus monodon* was grossly contaminated with *Vibrio parahaemolyticus* and as such may constitute a potential health hazard to human. Due to the potential hazard of pathogenic *Vibrio parahaemolyticus*, it is therefore necessary to emphasise seafood hygiene. Surveillance of potential contaminant bacteria for example *Vibrio cholerae* is crucial for sustenance of the public' health since outbreaks of cholera in this area have been reported in the past three years (2010-2012).

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