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Seasonal Occurrence of Vibrios in Water and Shellfish Obtained from the Great Kwa River Estuary, Calabar, Nigeria

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Abstract The prevalence of *Vibrio* species in shellfish and their seasonal variability in the Great Kwa River estuary (GKWE) were examined. Results revealed a tri-modal peak in *Vibrio* counts, coinciding with meteorological changes and the hot periods of the year. The estuary was constantly faecally polluted, coupled with high rates of infection of shellfish by *V. parahaemolyticus* 42 (13.6%), *V. cholerae* non-01 29 (9.4%) and *V. alginolyticus* 22 (7.1%), thus posing a health risk. The observed seasonal variability and prevalence of *Vibrio* species infection are of epidemiological significance, and provide a guide for effective control of associated cholera epidemics.

Keywords *Vibrio* species · Seasonal variability

The number of cases of illness caused by *Vibrio* is on the increase in several parts of the world, especially in developing countries where sanitation is grossly inadequate. Besides increased foreign travels which are associated with greater exposure to *Vibrio*-contaminating environmental sources, the consumption of raw and improperly cooked shellfish is known to be a reason for several *Vibrio*-related illnesses (Rippey 1994). Usually, outbreaks of cholera are frequent in coastal areas where the physico-chemical parameters of the coastal estuarine waters such as temperature, salinity and organic substrate concentration affect not only the physiological state of *Vibrio cholerae*, but also its potential pathogenicity (Kaspar and Tamplin 1993; Huq et al. 2005). Several reports have indicated that *Vibrio* are indigenous to coastal waters and estuarine habitats, and have caused several epidemics in different parts of the world (WHO 1971; Kaspar and Tamplin 1993; Nasu et al. 2000). It is endemic in some developing countries with poor hygienic status (Eja 1999; Myers et al. 2003).

In some parts of Asia and Africa, e.g., Bangladesh and Nigeria, aquatic reservoirs of *V. cholerae* have been implicated in the maintenance of cholera endemicity (Utsalo et al. 1988). Shellfish (shrimps, crabs, clams, periwinkles) harvested from the Cross River estuary are known to be reservoirs of *Vibrio* species in the Cross River cholera endemic area of Nigeria (Udo 1993; Eko et al. 1994), but there is little or no information on the prevalence and seasonal occurrence of *Vibrio* species in the Cross River estuarine system. Therefore, this study was carried out to examine the prevalence of the *Vibrio* species in shellfish, and the seasonal variability of *Vibrio* in the GKRE of Calabar, Nigeria. The GKRE which constitutes an integral part of the Cross River estuarine system is located between latitudes 4°30′ and 5°00′ N, and longitudes 5°15′ and 8°45′ E and is the major estuary that receives the main drainage channel carrying runoff input from Calabar municipality (Eja et al. 2003).

Materials and Methods

Water samples were taken once a month in triplicates from five stations chosen randomly along the length of the

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GKWE, starting from its mouth at the sea to the inland from January to September, 2006. Water samples from low and high tides were collected in accordance with the recommended procedures and precaution (APHA 1985). The water samples were collected in sterilized 250 mL screw-capped bottles and conveyed in icebox at 4°C to the laboratory for analysis in 1–4 h. Shellfish specimens were randomly collected monthly from shellfish harvesters along the GKWE from January to September, 2006. The shellfish sampled were 120 shrimps (*Macrobrachium vollenhovenii)*, 98 perwinkles (*Tympanotonus fuscatus*) and 90 clams (*Egeria radiata*) representing the crustacian, univalve mollusc and the bivalve mollusc, respectively. The shellfish samples were put in polyethylene bags and immediately carried to the laboratory for processing and analysis within 1–6 h.

The diluted (10^{-4}) turbid water (1 mL) was inoculated into 9 mL MacConkey broth and incubated at 37°C for 24 h. Where there was evidence of growth in the diluted sample, 0.1 mL broth was plated onto MacConkey agar. After incubation at 44°C for 48 h, the colonies that developed were presumed to be *Escherichia coli* and counts were multiplied by the reciprocal of the dilution factor and recorded as colony-forming units (CFU/mL) of water sample (APHA 1985). The diluted (10^{-4}) water sample (1 mL) was inoculated into 9 mL alkaline peptone water (APW) for enrichment, and incubated for 6 h at 37°C. Where there was evidence of growth in the diluted sample, 0.1 mL of the APW broth culture was plated onto thiosulphate citrate bile salt sucrose (TCBS) agar and incubated for 18 h at 37°C. All yellow and green colonies were presumed to be vibrios (Cheesborough 1991) and counts were multiplied by the reciprocal of the dilution factor and recorded as CFU/mL of water sample. The shellfish samples were carefully washed with distilled water to remove sediment particles retained on their shells or mantle cavity. Each shrimp was scrubbed and the soft tissue (1.0 g) was homogenized using a well-cleaned stainless steel blender (Eja et al. 2003). The external surfaces of the clams and periwinkles were disinfected with 70% ethanol (Udo 1993). The samples were then individually scrubbed and the soft tissues (1.0 g) were homogenized. Each homogenate was diluted (10^{-3}) in phosphate buffered saline (PBS) pH 7.0. Triplicates of the diluted homogenate (1.0 mL) were spread on TCBS and nutrient agar in order to determine counts of viable vibrios and heterotrophic bacteria respectively. The plates were incubated at 37°C for 18 h. The yellow and green colonies on TCBS agar were presumed to be vibrios and were both used as total *Vibrio* counts (Cheesborough 1991). On the other hand, all the colonies on nutrient agar were counted and used for the calculation of total heterotrophic bacterial count per gram of sample tissue. The yellow and green colonies were examined for biochemical and morphological characteristics using established procedures (Cheesborough 1991). All the biochemically identified *Vibrio cholerae* were serotyped using polyvalent 01, non-specific Ogawa and Inaba antisera, respectively. Biotyping was done on the basis of production of soluble haemolysins, haemaglutination with chicken red blood cell (RBC) and resistance to 0/129 *Vibrio* static compound (Cowan 1975; Cheesborough 1991).

Table 1 Bacterial counts

Shellfish	Total heterotrophic count ($\times 10^3$ CFU/g)	Total *Vibrio* count ($\times 10^3$ CFU/g)	Ratio of *Vibrio* count/ hererotrophic count
Shrimps	6.1 ± 0.6	1.5 ± 1.0	0.20
Periwinkle	7.6 ± 0.5	1.2 ± 0.9	0.20
Clam	9.2 ± 0.6	1.1 ± 1.5	0.12

CFU = colony-forming unit

Results and Discussion

The population profiles of total heterotrophic bacteria and vibrios per gram of tissue of sampled shellfish observed in this study are presented in Table 1 which shows the highest mean total heterotrophic bacterial count ($9.2 \pm 0.6 \times 10^3$ CFU/g) in clam, and highest total mean *Vibrio* count ($1.5 \pm 1.0 \times 10^3$ CFU/g) in shrimps. The profiles demonstrate that vibrios make up between 12% and 20% of the heterotrophic bacteria in shellfish tissue, indicating very high health risk associated with the consumption of raw or improperly cooked shellfish in Calabar coastal city.

The monthly counts of *E. coli* and *Vibrio* in the water samples from the GKWE are presented in Table 2. *E. coli* counts ranged from $64.00 \pm 0.7 \times 10^4$ to $67.00 \pm 0.4 \times 10^4$ CFU/mL between January and April (i.e., the dry season period), and $6.5 \pm 1.0 \times 10^4$ to $64.75 \pm 0.3 \times 10^4$ CFU/mL in the rainy season (May to September). *Vibrio* counts ranged from $11.5 \pm 1.2 \times 10^4$ to $14.75 \pm 2.3 \times 10^4$ CFU/mL in the dry season, and 4.0 ± 3.32 to $12.25 \pm 1.3 \times 10^4$ CFU/mL in the rainy season. These high counts indicated that the water was faecally polluted and never met bacteriological standards (WHO 1971) throughout the sampling period.

The seasonal occurrence of *E. coli* and *Vibrio* in the Great Kwa River estuarine water is presented in Fig. 1. The monthly percent totals used to plot Fig. 1 were calculated from Table 2. *E. coli* counts exhibited two peaks, one in January, the second in April and gradually declined till June, after which there was a sharp decline till September when mean count was about 1.5% ($6.50 \pm 0.1 \times 10^4$ CFU/mL). On the other hand, *Vibrio* counts exhibited a trimodal peak,

Table 2 Monthly counts of E.coli and *Vibrio* in the water samples from the Great Kwa River estuary

Months of sampling	*E. coli* counts ($\times 10^4$ CFU/mL) ± SD	*Vibrio* counts ($\times 10^4$ CFU/mL) ± SD
January	65.75 ± 0.6	11.5 ± 0.9
February	64.00 ± 0.7	9.5 ± 1.1
March	66.75 ± 0.5	11.5 ± 1.2
April	67.00 ± 0.4	14.75 ± 2.3
May	64.75 ± 0.3	12.25 ± 1.3
June	63.5 ± 0.3	6.75 ± 0.9
July	26.00 ± 0.4	8.50 ± 2.1
August	9.00 ± 1.3	8.25 ± 1.9
September	6.50 ± 0.1	4.0 ± 3.2
Total	433.25 ± 5.6	87 ± 14.9

SD = standard deviation of mean; CFU = colony-forming unit

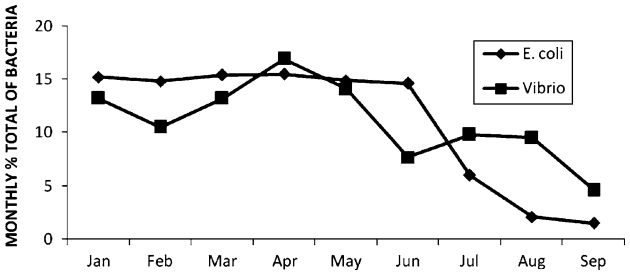

Fig. 1 Seasonal occurrence of *E. coli* and *Vibrio* in the Great Kwa River estuary

one in January, the second in April and the third in August. The peak exhibited by *Vibrio* and *E. coli* counts have been attributed to meteorological changes accompanied by corresponding changes in run-off input in the estuary (Eja 1999). Characteristically, there is occasional rainfall in Calabar between December and February whose run-off carries some fairly high load of faecal matter into the estuary, resulting in the first peaks exhibited by *Vibrio* and *E. coli* counts in January. There is occasional heavy downfall with a corresponding increase in run-off input between March and May, and this accounts for the highest peaks observed for *Vibrio* and *E. coli* counts in April. Thereafter, there is a constant heavy downfall in June through August, accounting

Table 3 Carriage rate of *Vibrio* among the shellfish harvested from the Great Kwa River estuary

Shellfish	N examined	N (%) carrying *Vibrio*	N (%) negative
Shrimps	120	59 (49.2)	61 (50.8)
Periwinkle	98	19 (19.4)	79 (80.6)
Clam	90	16 (17.8)	74 (82.2)
Total	308	94 (30.5)	214 (69.5)

N = number

for the decline in the levels of *Vibrio* and *E.coli*. The decline has earlier been reported to result from dilution, following constant heavy rainfall (Eja 1999). The third peak exhibited by *Vibrio* counts in August coincided with "August break" (a period of 2 weeks in August every year, when there is absence of rainfall, similar to dry season, with temperature rising to about 30 ± 5°C). In other reports, although the peak in *Vibrio* diarrhoea during the dry season has been attributed to water scarcity, high temperature, increased fishing and economic activity on Calabar River estuary (Utsalo et al. 1988), it has been observed in Southeast Asia that the incidence of enteropathogens increased during the wet season, and marginally higher when ponds under study were close to urban areas (Reilly and Twiddy 1992).

The observed carriage rate of *Vibrio* among the shellfish harvested from the GKRE in this study revealed that 94(30.5%) out of a total of 308 shellfish examined, were infected by *Vibrio* (Table 3). Shrimps were most frequently infected with a carriage rate of 59 (49.2%), agreeing with an earlier reported 67.9% carriage rate for shrimps harvested from an adjacent Calabar River estuary (Udo 1993). Consumption of shrimps therefore poses the highest health risk in this part of the world.

The prevalence rates of infection by *Vibrio* as presented in Table 4, were 1 (0.3%) for *V. cholerae 01 el tor*, 0 (0.0%) for *V. cholerae* 01 classical, 29 (9.4%) for *V. cholerae* non-01, 22 (7.1%) for *V. alginolyticus*, and 42 (13.6%) for *V. parahaemolytious*. This finding agrees with an earlier report which showed a high prevalence of infection due to *V. alginolyticus* and *V. Parahaemolyticus*, which was observed to be of significant public health importance, as the two *Vibrio* have been described in

Table 4 Prevalence of *Vibrio* in the shellfish harvested from the Great Kwa River estuary

Shellfish	*V. cholerae 01 el tor* No (%)	*V. cholerae* 01 classical No (%)	*V. cholerae* non-01 No (%)	*V. alginolyticus* No (%)	*V. parahaemolyticus* No (%)	Total No (%)
Shrimps N = 120	1 (0.8)	0 (0.0)	20 (16.7)	12 (10.0)	26 (21.7)	59 (49.2)
Periwinkle N = 98	0 (0.0)	0 (0.0)	4 (4.1)	6 (6.1)	9 (9.2)	19 (19.4)
Clam N = 90	0 (0.0)	0 (0.0)	5 (5.6)	4 (4.5)	7 (7.7)	16 (17.8)
Total 308	1 (0.3)	0 (0.0)	29 (9.4)	22 (7.1)	42 (13.6)	94 (30.5)

N = number of shellfish examined, No = number of samples infected

human infections (Utsalo et al. 1988). Also, the prevalence rate of infection of shellfish was observed to be highest with *V. parahaemolyticus*. This is the scenario in many coastal areas where unique strains of *V. parahaemolyticus* have been associated with many of the recent *V. parahaemolyticus* outbreaks (50–80% in Calcutta, India), including epidemics in Russia, Southeast Asia, Japan and North America (Myers et al. 2003). One such new pathogenic strain known as *V. parahaemolyticus* 03:K6, a pandemic genotype, has been discovered to possess a filamentous phage designated f237 which confers pandemic potency on the strain (Bhuiyan et al. 2002).

The next high prevalence rate of 29 (9.4%) was exhibited by *V. cholerae* non-01. Unlike *V. cholerae* 01 and 0139 serogroups which spread mainly through contaminated drinking or recreational water, and cause non-invasive epidemics of cholera in developing countries, *V. cholerae* non-01 has been reported to be invasive and transmitted to humans by ingestion of raw seafoods, causing episodes of septicaemia in some patients (Namdari et al. 2000). Besides the prevalence rates of *V. parahaemolyticus* and *V. alginolyticus*, the high prevalence rate of the non-01 *V. cholerae* poses a health risk to the coastal inhabitants of Calabar. This is more serious because shellfish harvesting is a gainful occupation in the Cross River State of Nigeria, as shellfish is one of the major delicate dishes of the inhabitants. Thus, shellfish harvesting makes Cross River State, especially Calabar coastal city, a cholera endemic area; and the endemicity is known to be maintained by shellfish which have been reported to be reservoirs of *Vibrio* (Utsalo et al. 1988; Udo 1993). Epidemics of cholera in Cross River State have coincided with the peaks of *Vibrio* in water (Utsalo et al. 1988) which occur in mid-dry season (January), between March and May, and June through August which are the periods and when shellfish commercial activities boom in the state.

It is concluded that the seasonal variability and prevalence of *Vibrio* infection are of epidemiological significance, thus effective control of cholera epidemics in Cross River State, Nigeria, could be achieved by official health workers, if they intensify effort on preventive measures at mid-dry season, periods between March and May, and between June and August.

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