CARRIAGE OF *VIBRIO* SPECIES BY SHRIMPS HARVESTED FROM THE COASTAL WATERS OF SOUTH WEST CAMEROON

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ABSTRACT

Objectives: **To determine the prevalence of *Vibrio* spp in unprocessed shrimps and their susceptibility to antibiotics.**

Design: **A prospective study of *Vibrio* spp associated with shrimps harvested from the coastal waters of South West Cameroon.**

Setting: **A laboratory based study at the Department of Life Sciences, University of Buea. Two hundred and thirty six shrimps harvested from the coastal towns of Limbe and Tiko, Cameroon, were examined for the prevalence of *Vibrio* spp using standard microbiologic procedures. The antibiotic sensitivity of isolates was determined using the Kirby-Bauer disc diffusion technique.**

Results: **Of the 236 shrimps examined, 73 (30.9%) were contaminated with *Vibrio* spp. Further, a total of 125 *Vibrio* strains were isolated from the contaminated shrimps. Of this number, 33 (26.4%) were *V. cholerae*, 55 (44%) *V. parahaemolyticus*, 34 (27.2%) *V. alginolyticus* and three (2.4%) *V. vulnificus*. Antibiotic susceptibility generally ranged from 68.8% for polymyxin B to 99.2% for gentamycin. Multiple resistant strains were noted, especially with *V. parahaemolyticus* and *V. alginolyticus***

Conclusion: **Shrimps maintain a reservoir of potential *Vibrio* spp in the coastal area of South West Cameroon. This finding is of epidemiologic and clinical significance.**

INTRODUCTION

Many members of the genus *Vibrio* including *V. cholerae, V. alginolyticus, V. anguillarum, V. parahaemolyticus, V. vulnificus, V. fluvialis* and *V. hollisae* have been implicated in both human diseases and marine animal diseases[1,2]. Infection in humans have been associated with the handling and consumption of contaminated food and drink, raw or undercooked shrimps, fish, turtle eggs, oysters, vegetables and exposure of abraded skins to contaminated environments[1,3-6]. Clinical conditions such as cholera, cholera-like gastroenteritis, acute diarrhoea, wound and ear infections have been described[7-9]. People especially vulnerable to infection include those with chronic diseases involving elevated serum iron levels, immune function abnormalities and other chronic disorders[1,10].

The association of shrimps with *Vibrio* spp has stimulated research in this area, following the observation that surface proteins are involved in *in vitro* interaction of *V. cholerae* 01 classical forms with chitin particles of crustaceans. The chitin binding proteins of *V. alginolyticus* are also known to play a pivotal role in their attachment to crustaceans[11,12] causing *Vibriosis* in shrimps[2,13,14]. In the coastal areas of Fako Division, South West Cameroon, it is common to find shrimps marketed under unhygienic conditions. This seafood considered a delicacy here, is highly consumed, thus presenting a possible exposure to *V. cholerae* and related vibrios. Recently, other investigators[5,15] in neighbouring Nigeria reported the role of shrimps in the maintenance of *Vibrio* spp in the environment of Central and Southern Nigeria. Although other studies[7,8] have strongly suggested a relation between diarrhoeal episodes and consumption of undercooked shrimps, there appears to be no report on the association of *Vibrio* spp and shrimps in the coastal area of South West Cameroon. It is against this background that the present study was carried out. We therefore report on the isolation and characterisation of *Vibrio* spp associated with unprocessed shrimps and their susceptibility to antibiotics.

MATERIALS AND METHODS

Sample collection: A total of 236 freshly caught shrimps (*Macrobranchium* spp) were bought from local fishermen in two coastal towns of South West Cameroon, namely Limbe and Tiko. These towns are approximately 21 kilometres apart. The shrimps (9-13 grams) were transferred singly into polythene bags and transported to the Microbiology Unit Laboratory of the Department of Life Sciences, University of Buea, Cameroon, within one hour and processed. When this was not possible, they were maintained at 4°C and processed as soon as possible.

Bacteriological analysis: Each shrimp was washed thoroughly (3 times) with fresh sterile water to remove sand and detritus. The head parts of each was removed with a sterile forcep and homogenised in a sterile blender containing 5ml sterile water(5). Two Bijou bottles each containing 9ml of sterile alkaline peptone water (APW), 1% and 2% sodium chloride (Nacl) respectively were inoculated with 1ml of shrimp homogenate and incubated at 37°C for 8 hours. A loopful of broth from each bottle was sub-cultured on thiosulfate-citrate-bile-sucrose (TCBS, Oxoid, Basingstoke, England) agar and incubated at 37°C for 18-24 hours(5,15,16). Colonies were presumptively identified by colony shape and pigmentation on TCBS, gram staining, cytochrome-oxidase and catalase activities, motility and susceptibility to 0/129 vibriostatic agent(16). Only oxidase-positive, gram-negative, vibriostatic susceptible colonies were subjected to the API 20E (bioMerieux, France) identification kit for speciation. The number of viable isolates was estimated as colony forming units per ml (CFU/ml) of homogenate as previously described(17).

Antibiotic susceptibility testing: The Kirby-Bauer disk diffusion test, which conforms to the recommended standard of the National Committee for Clinical Laboratory Standards (NCCLS)(18), was used as previously described(19). Briefly, a small innoculum of each pure bacterial isolate was emulsified in 3ml sterile normal saline in Bijou bottles and the density compared to a barium chloride standard (0.5 McFarland). A sterile cotton swab was dipped into the standardised suspension of bacterial cultures and used to evenly inoculate Mueller-Hinton plates (Biotec, England) and allowed to dry. Thereafter, antibiotic disks with the following drug contents: ampicillin (10 µg/ml), tetracycline (30 µg/ml), streptomycin (10 µg/ml), chloramphenicol (30 µg/ml), polymixin B (300 IU/ml) and gentamicin (10 µg/ml) (Oxoid, Basingstoke, England) were placed on the plates spacing them well to prevent the overlapping of inhibition zones. The plates were incubated at 37°C for 24 hours and the diameters were then compared with recorded standards to determine susceptibility or resistance(20,21).

Statistical analysis: The χ2 test was employed. Differences were considered significant at $p \leq 0.05$.

RESULTS

Carrier rate of shrimps: Of the 236 shrimps examined, 73 (30.9%) were found to be harbouring one or more *Vibrio* spp. The carrier rate per locality was 32.5% for Limbe and 29.3% for Tiko. This difference was not statistically significant (p>0.05) (Table 1). The microbial load varied in the contaminated shrimps. Fifty eight (79.4%) of the 73 samples had 10-20 CFU/ml of homogenate and, one (1.4%) had 100-200 CFU/ml (Figure 1).

Figure 1

Plate counts of vibrios isolated from 73 contaminated shrimps
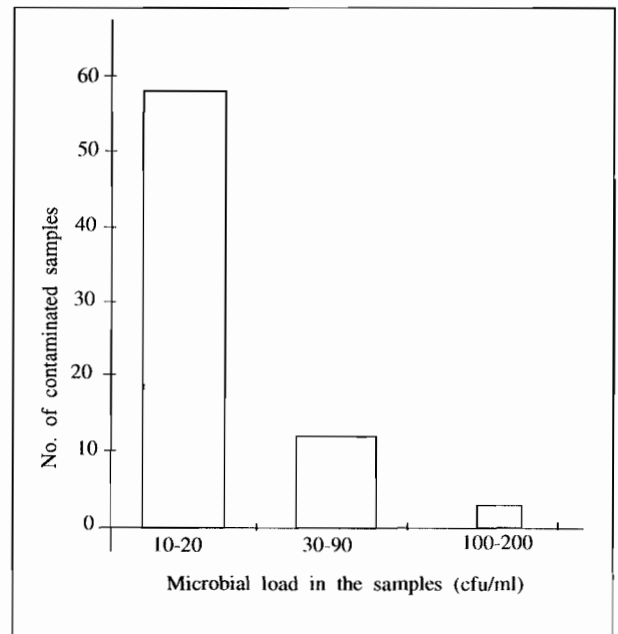
Prevalence of Vibrio spp: A total of 125 *Vibrio* strains were isolated from the 73 contaminated shrimps. Isolation rates of 61.6% and 38.4% were obtained for Limbe and Tiko, respectively.

Of the 125 isolates, 33 (26.4%) were *V. cholerae*, 55 (44%) *V. parahaemolyticus*, 34 (27.2%) *V. alginolyticus* and three (2.4%) *V. vulnificus*. These differences were, however, not statistically significant (p>0.05) (Table 2).

Table 1

Carrier rate of shrimps harvested from the Limbe and Tiko coastal waters

Locality	No. of shrimps examined	No. (%) of shrimps positive for a single *Vibrio* spp	No. (%) of shrimps positive for two or more *Vibrio* spp	No. (%) of shrimps negative for all *Vibrio* spp	Total no. (%) of infected shrimps
Limbe	120	31 (25.8)	8 (6.7)	81 (67.5)	39 (32.5)
Tiko	116	27 (23.3)	7 (6.0)	82 (70.7)	34 (29.3)
Total	236	58 (24.6)	15 (6.6)	163 (69.1)	73 (30.9)

Table 2

Prevalence of Vibrio species in contaminated shrimps

| Locality	No. of shrimps examined	No. (%) of isolates obtained				Total
		V. cholerae	*V. parahaemolyticus*	*V. alginolyticus*	*V. vulnificus*	
Limbe	39	14 (18.2)	38 (49.4)	23 (29.9)	2 (2.6)	77 (61.6)
Tiko	34	19 (39.6)	17 (35.4)	11 (22.9)	1 (2.1)	48 (38.4)
Total	73	33 (26.4)	55 (44.0)	34 (27.2)	3 (2.4)	125 (100)

Table 3

Antibiotic susceptibility patterns of vibrio species

Vibrio spp and number tested	No. (%) of isolates sensitive (S) and resistant (R) to											
	AMP[a]		TE[b]		GEN[c]		POL[d]		CHR[e]		STR[f]	
	R	S	R	S	R	S	R	S	R	S	R	S
V. parahaemolyticus	11	44	1	54	0	55	22	33	2	53	2	53
n=55	(20.0)	(80.0)	(1.8)	(98.2)	(0.0)	(100)	(40.0)	(60.0)	(3.6)	(96.4)	(3.6)	(96.4)
V. alginolyticus	10	24	0	34	0	34	10	24	6	28	4	30
n=34	(29.4)	(70.6)	(0.0)	(100.0)	(0.0)	(100.0)	(29.4)	(70.6)	(17.7)	(82.4)	(11.8)	(88.2)
V. cholerae	4	29	4	29	1	32	7	26	2	31	0	33
n=33	(12.1)	(87.9)	(12.1)	(87.9)	(3.0)	(97.0)	(21.2)	(78.8)	(6.1)	(93.9)	(0.0)	(100)
V. vulnificus	0	3	0	3	0	3	0	3	0	3	0	3
n=3	(0.0)	(100)	(0.0)	(100)	(0.0)	(100)	(0.0)	(100)	(0.0)	(100)	(0.0)	(100)
Total	25	100	5	120	1	124	39	86	10	115	6	119
	(20.0)	(80.0)	(4.0)	(96.0)	(0.8)	(99.2)	(31.2)	(68.8)	(8.0)	(92.0)	(4.8)	(95.2)

AMP[a]=ampicillin (10 μg/ml), TE[b] = tetracycline (30 μg/ml), GEN[c]=gentamycin (10 μg/ml), POL[d]=polymyxin (300I.U/ml),
CHR[e]=chrolamphenicol (30 μg/ml), STR[f]=streptomycin (10 μg/ml)

Figure 2

Antibiotic susceptibility results of the Vibrio species

AMP=Ampicillin (10 μg/ml), TE=Tetracycline (30 μg/ml), GEN=Gentamycin (10 μg/ml). POL=Polymyxin (200 I.U./ml). CHR=Chloramphenicol (30 μg/ml), STR=Streptomycin (10 μg/ml)
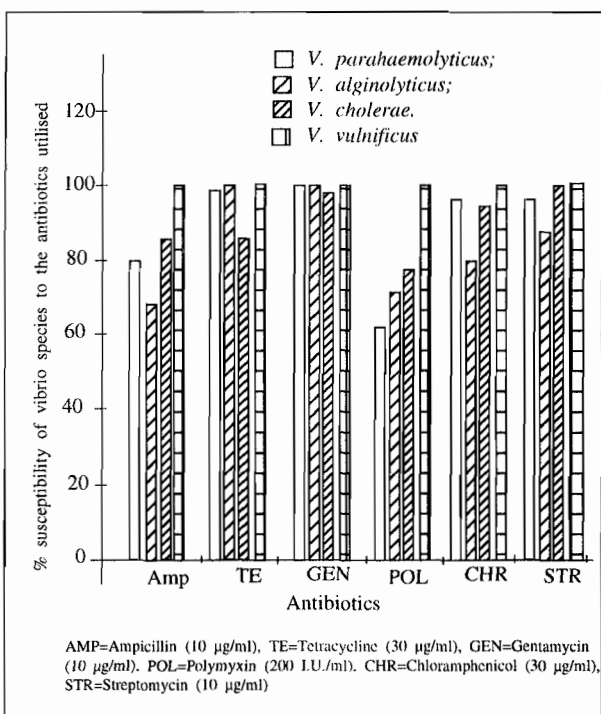
Antibiotic susceptibility results: The susceptibility results are shown on Figure 2. Percentage susceptibility ranged from 60% to 100% depending on the antibiotic and species of *Vibrio*. The lowest susceptibility (60%) was observed for *V. parahaemolyticus* to polymyxin B. Gentamycin generally exhibited good activity against the isolates. On the other hand, 86 (68.8%) out of 125 isolates were found to be resistant to at least one antibiotic while, 21 (16.8%) isolates displayed resistance to two or more antibiotics. The overall resistance rates were generally low: 20%, 4% and 0.8% for ampicillin, tetracycline and gentamycin, respectively. However, the highest resistance (31.2%) was noted for polymyxin B (Table 3).

DISCUSSION

It is universally acknowledged that *Vibrio* spp abound in the coastal waters of many regions of the world(5,16,21) where they attach to various environmental flora and fauna(11). Consequently, the coastal waters of South West Cameroon is not expected to be an exception.

In the present study, we noted that the carrier rate of shrimps to different *Vibrio* spp was 30.9% (76 out of 239). However, there was no significant difference (p>0.05) between the localities of Limbe (32.5%) and Tiko (29.3%) where the shrimps were harvested. This could be related to the fact that Limbe and Tiko are two coastal towns separated by only approximately 21 kilometres with similar sewage disposal systems, which target the ocean. Consequently, the coastal waters are over-nourished and favour the growth of *Vibrio* spp that in turn colonise

shrimps and other crustaceans. This is supported by an earlier observation(16) that a high bacterial density is correlated with coastal eutrophication.

It has been documented that *Vibrio* spp are among the major causative agents of acute diarrhoeal diseases(7,8,22). Depaolo *et al*(23) reported that the presence of high densities of *V. vulnificus* in seafood may have both ecological and public health implications. Occasioned by this, it was therefore necessary to determine the prevalence of *Vibrio* spp in the contaminated shrimps as well as their microbial loads. From the 73 contaminated shrimps, isolation rates of 26.4%, 44%, 27.2% and 2.4% were respectively obtained for *V. cholerae, V. parahaemolyticus, V. alginolyticus* and *V. vulnificus*. This is consistent with the findings of other investigators(2,5,12,15,20). However, the isolation of *V. vulnificus* in our study contrasts with that of Udo(5), but agrees with that of Amaro *et al*(20).

In this study, a plate count of 2×10^2 CFU/mL of *V. parahaemolyticus* was obtained from an infected shrimp harvested in Limbe. Although this is lower than the infective dose of *Vibrio* spp for humans, it is an indication that shrimps, which are considered a delicacy and highly consumed here, may play a role in the endemicity of *Vibrio* infections in our environment. Further, it might suggest vibriosis, a disease related to mortality and decrease in shrimp yields(2). This finding could therefore be useful for shrimp farmers in our environment.

The *Vibrio* spp encountered in our study were found to be uniformly susceptible to all the antibiotics used, ampicillin (80%), tetracycline (96%), polymyxin B (68.8%), chloramphenicol (92%), gentamycin (99.2%) and streptomycin (95.2%). This is in agreement with the findings of Udo(5). In his study, susceptibility rates representing ampicillin (60%), tetracycline (97.1%), gentamycin (100%), chloramphenicol (81.8%) and streptomycin (83.3%) were obtained. However, Shapiro *et al*(24) found most *V. cholerae* 01 isolates from western Kenya to be fully or partially resistant to streptomycin and chloramphenicol. Also, another study(20) reported resistances of isolates of *Vibrio* spp to ampicillin and polymyxin B. Though low, we however noted multiple resistance with some of our isolates, mostly to polymyxin B. This is consistent with the results of Filetici *et al*(21). Most of their isolates were resistant to colimycin (which we did not use) and ampicillin. These disparities in susceptibility patterns to standard antibiotics by *Vibrio* spp cannot be immediately ascertained but they may point to the fact that antibiotic susceptibility patterns vary with time and geographical regions as earlier reported(25,26). Antibiotic resistance is also known to be related to the transfer of resistance plasmids(27). We could not investigate this with our strains because of financial limitations. This may, however, be a subject of our next study.

On the basis of our data, we conclude that shrimps maintain a reservoir of *Vibrio* spp in our environment, thus posing a health risk to consumers of undercooked shrimps. This finding is of epidemiologic and clinical significance.

ACKNOWLEDGEMENTS

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