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Original Article

Enterotoxicigenicity profile of *Escherichia coli, Vibrio,* and *Salmonella* species isolated from well and river water sources in Oproama town in the Niger Delta, Nigeria

Asiton-a A. Dick*, Gideon O. Abu, Stella N. Ibe

Department of Microbiology, University Of Port Harcourt, Choba, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria

**Corresponding author: Asiton-a A. Dick, E-mail: asitona@yahoo.com, Telephone: +2348064131848*

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ABSTRACT: Well water is the only source of drinking water in Oproama Town in Rivers state, Nigeria. Water from these sources is consumed without treatment, and potentially poses a health risk to the local population. The Enterotoxigenicity profile of *Escherichia coli*, *Vibrio* and *Salmonella* species isolated from well and river water sources in Oproama were investigated using fluid accumulation (FA) ratio. The study revealed that Salmonella isolate (S9) from the river showed doubtful toxicity out of the ten *Escherichia coli*, ten *Vibrio* and ten Salmonella isolates. This study revealed a near-absence of enterotoxigenic *Escherichia coli*, *Vibrio* and Salmonella organisms from the waters in Oproama. More studies are required to further test the overall safety of these water sources due to the potential risk of consuming untreated water.

KEYWORDS: *Escherichia coli, Vibrio, Salmonella, Enterotoxigenicity*

INTRODUCTION

The quality and quantity of available water have implication on the health status of a community. Over 50,000 people die daily due to water borne diseases (Herschy, 1999) and mortality in children under five years from water related diseases annually is estimated to be about 4 million in developing countries (USAID, 1990; Warner, 1998). Worst still, 2.3 billion people worldwide have mortality and morbidity associated with water related ailment (WHO, 1997). These statistics though alarming definitely have impact on developmental efforts (Olshanky *et al*., 1997).

In the last two decades, there has been an increase in the number of reported case of water-borne diseases such as typhoid fever, cholera and dysentery which are caused by the enterotoxin produced by *Salmonella*, *Vibrio* and *Escherichia coli*. Little is known about the predisposing factors that might be responsible for such disease upsurge in Nigeria. Well water is the only source of drinking water in Oproama Town, in Asari-Toru Local Government Area, Rivers State, Nigeria. In these regions, medical attention is lacking and there is no previous information on the bacteria content and their enterotoxigenicity. Hence, this investigation was carried out as a baseline study to investigate the presence of enterotoxigenic *Escherichia coli*, *Vibrio* and *Salmonella* in the water.

MATERIALS AND METHODS

Sampling

Water samples were collected from seven (7) hand-dug wells that used extensively as sources of drinking water and other domestic purposes and at three (3) source points along the Oproama River. *Escherichia coli*, *Vibrio* and *Salmonella* sp. in well water and river water were isolated using membrane filtration. The membrane filters were placed on Eosin methylene blue agar for *Escherichia coli*, Thiosulphate Citrate Bile Salts Sucrose (TCBS) for *Vibrio* species and *Salmonella* and *Shigella* agar for *Salmonella* species. All the plates in duplicates were incubated at 44°C (*Escherichia coli*), 35°C (*Vibrio* species) and 37°C (*Salmonella* species) for 24-28 hours. They were further identified based on biochemical test (Cheesbrough, 1984).

Preparation of Inoculum

Enterotoxic material was prepared by inoculating tryptic soy broth with stock cultures of ten (10) *Escherichia coli*, *Vibrio* and *Salmonella* isolates from the water samples (i.e. one isolate from each station). Also, clinical isolates of *Escherichia coli* (C21) and *Salmonella typhi* (BFC2) were obtained as control from the Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt. The broth was incubated at 37°C for twenty-four (24) hours in a Shaker (Stuart, Orbital Incubator S150). The cultures were then centrifuged (800 Centrifuge, B. Bran Scientific and Instrument Company, England)

at 4000 x g for 35 minutes and the supernatant was used for inoculation of the rats.

Suckling rats test for enterotoxigenicity

Enterotoxigenicity tests were carried out according to Dean et al., (1972) and Obi and Nzeako (1980). Suckling rats (Wistar) were obtained from the Animal Farm, Department of Biochemistry, University of Port Harcourt, Choba. The suckling rats (1-3 days old) were separated from their mothers shortly before the test and placed randomly in groups of three (3). For each of the thirty (30) cultures (*Escherichia coli*, *Vibrio*, and *Salmonella* sp.), three rats were inoculated orally with 0.1 ml of the culture using syringe without the needle. All the inoculated animals were kept at room temperature (25°C) for four hours after which they were killed with chloroform. The gut (intestine) was surgically removed from the body and each weighed separately. The fluid accumulation (FA) ratio of (weight of entire intestine/total body weight-weight of intestine) of each animal was calculated. The gut-to-weight ratio was >0.065.

RESULTS AND DISCUSSION

The enterotoxigenicity responses after 4 hours in infant Wistar rats orally infected with isolates from water sources are presented in Tables 1-3. Table 1 shows that all the animals infected with *Escherichia coli* isolates (E1-E10) did not show any toxicity based on the fluid accumulation (FA) ratio after 4 hours post-inoculation. The FA ratio ranged from 0.032 to 0.053. Table 2 shows that all the animals infected with *Vibrio cholerae* isolates (V1–V10) did not show any toxicity based on the fluid accumulation (FA) ratio after 4 hours post-inoculation; with the FA ratio ranging from 0.03-0.055. The results also show that of all the animals infected with *Salmonella typhi* isolates (S1-S10), only S9 isolate showed some doubtful toxicity (0.079) based on the fluid accumulation (FA) ratio after 4 hours post-inoculation. The FA ratio ranged from 0.044-0.079 (Table 3).

The transmission of disease through consumption of faecally contaminated waters particularly in developing countries has long been recognized and documented (WHO, 1996; 1997). The role of *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi* in cases of diarrhoea have been established and reported to be of substantial degree of morbidity and mortality among children and adults (Feachem et al., 1983). Diarrhoea on it is own is characterised by loss of fluids and electrolytes, hypokalaemia and acidosis (Tilkian et al., 1979). Organisms colonise the small intestine epithelial surfaces, where they elaborate a protein enterotoxin which cause net fluid secretion into the lumen (Finkelstein et al., 1973). Intact animals models used to study pathogenesis of *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi* generally mimic the diarrhoeal symptomology. Net fluid secretion in response to oral or intra-intestinal inoculation with viable organisms or enterotoxin has been demonstrated in infant rabbit, suckling mice, rats, hamsters (Dutta and Habbu, 1955; Dean et al., 1972; Koupal and Diebel, 1975; Takeda et al., 1977).

TABLE 1 **Enterotoxigenicity of *Escherichia coli* cell-free culture**

Isolate code	Mean gut weight	Mean body weight minus gut weight (g)	Gut weight/ Body weight	Result
E1	0.193	5.854	0.032	-
E2	0.207	5.903	0.035	-
E3	0.263	5.554	0.047	-
E4	0.270	6.160	0.043	-
E5	0.287	5.326	0.053	-
E6	0.227	5.853	0.038	-
E7	0.267	5.893	0.045	-
E8	0.243	6.040	0.040	-
E9	0.276	5.591	0.049	-
E10	0.257	5.903	0.043	-
C21	0.333	4.61	0.071	+
TSB	0.270	4.357	0.061	-
Uninoculated	0.210	4.390	0.049	-

- Non-toxicity
+ Doubtful toxicity
++ Clear toxicity
E1-E10 *Escherichia coli* from Stations 1-10
C21Control *Escherichia coli* from BMSH
TSB Tryptic Soy Broth

The results obtained in this study revealed that with the exception of one *Salmonella* isolate (S9) with weak toxicity, all the *Escherichia coli*, *Vibrio* and *Salmonella* toxins studied did not induce fluid accumulation (toxicity) in the suckling animals (wistar rats) model when the rats were sacrificed after 4 hours post-inoculation. It had been reported that *Escherichia coli* enterotoxin elicit fluid accumulation within 4 hours (Dean et al., 1972), *Vibrio cholerae* in while, *Salmonella* toxin elicit a maximum response at 2.5 hours challenge (Koupal and Diebel, 1975). The non-responsiveness of these enterotoxins may either be due to differences in toxin preparation or differences in the stability of the toxin in solution (Takeda et al., 1978). Also, it could be that mechanisms other than production of enterotoxin are responsible for their pathogenic activity, although the isolates are from environmental sources (drinking water and river). Again, the doubtful positive result obtained with S9 (*Salmonella* from station 9) may indicate the production of small amounts of enterotoxin by this isolate (Dean et al., 1972).

The presence of even non-pathogenic *Escherichia coli*, *Vibrio* and *Salmonella* organisms in water used for drinking purposes is also a matter of concern because the virulence gene regulation is influenced by environmental factors.

TABLE 2 Enterotoxigenicity of *Vibrio sp* cell-free cultures

Isolate code	Mean gut weight	Mean body weight minus gut weight (g)	Gut weight/ Body weight	Result
V1	0.220	4.920	0.044	-
V2	0.273	4.937	0.055	-
V3	0.237	4.926	0.048	-
V4	0.160	5.177	0.030	-
V5	0.183	5.280	0.034	-
V6	0.210	6.107	0.032	-
V7	0.200	5.930	0.030	-
V8	0.197	5.343	0.036	-
V9	0.147	4.950	0.029	-
V10	0.183	4.677	0.039	-
TSB	0.270	4.357	0.061	-
Uninoculated	0.210	4.390	0.049	-

- Non-toxicity
+ Doubtful toxicity
++ Clear toxicity
V1-V10 *Vibrio* sp from Stations 1-10; TSB Tryptic Soy Broth

TABLE 3 Enterotoxigenicity of *Salmonella sp* cell-free cultures

Isolate code	Mean gut weight	Mean body weight minus gut weight (g)	Gut weight/ Body weight	Result
S1	0.383	6.092	0.062	-
S2	0.279	5.530	0.050	-
S3	0.323	6.541	0.049	-
S4	0.335	6.244	0.053	-
S5	0.258	5.830	0.044	-
S6	0.337	6.069	0.055	-
S7	0.258	5.734	0.045	-
S8	0.312	6.100	0.051	-
S9	0.435	5.464	0.079	+
S10	0.262	5.467	0.047	-
BFC2	0.353	4.554	0.077	+
TSB (Broth)	0.270	4.357	0.061	-
Uninoculated	0.210	4.390	0.049	-

- Non-toxicity
+ Doubtful toxicity
++ Clear toxicity
S1-S10 *Salmonella* sp from Stations 1-10
BFC2 Control *Samonella* spp from BMSH
TSB Tryptic Soy Broth

CONCLUSION

This study revealed a near absence of enterotoxigenicity of *Escherichia coli*, *Vibrio* and *Salmonella* organisms from the waters in Oproama. However, since hand-dug wells are the only sources of drinking water, more studies are required to further test the overall safety of these water sources due to the potential risk of consuming untreated water. Apart from infection from consuming untreated water, there is also the added risk of cross contamination of foods and utensils and the danger of contamination during handling.

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