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Studies on the Pathogenic Characteristics of Non-01 *Vibrio cholerae* Isolated from Streams in Nigeria

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Abstract: The presence of non-01 *Vibrio cholerae* in water samples and their pathogenic characteristics were studied. Three hundred (300) water samples were collected from streams and examined bacteriologically according to standard methods. Out of 300 waster samples, 36 (12%) samples were positive for non-01 *Vibrio cholerae*. Experimental animals were orally infected with pathogenic non-01 *Vibrio cholerae* isolates and the electrolytes (sodium, potassium and chloride ions) and haematological (haemoglobin, white blood cells and packed cell volume0 levels were determined. Potassium ion (K^+) decreased by 3.2 mMol L^{-1} (42.9%) and sodium ion (Na^+) decreased by 63.8 mMol L^{-1} (50.8%) while chloride ion (Cl^-) decreased by 50.2Mol L^{-1} (49.6%) after 96 hours post infection. Haemoglobin (Hb), Packed Cell Volume (PCV) and White Blood Cells (WBC) values obtained in experimental animals increase! d from control value up to the seventh day and thereafter decreased. Significant differences (ANOVA, $p<0.05$) were observed in the electrolyte loss but not in HB, PCV and WBC values during the monitoring period.

Key words: Stream, non- 01 *Vibrio cholerae*, infection, pathogenic characteristics

INTRODUCTION

Water plays an important role in the transmission of pathogenic organisms. These pathogenic agents enter water bodies through agricultural, domestic wastewater or animal and human waste. Human and agricultural wastes are often discharged directly or indirectly into water sources such as rivers, streams, lakes and wells thereby polluting the water supplies and making them unsafe for consumption and domestic uses. Most pathogenic bacteria transmitted by water *include aeromonas* sp., *Campylobacter* sp., *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *Yersinia* sp.[1].

Vibrio sp. belong to the family Vibrionaceae. They are all Gram-negative motile rods with curve or comma shape. The habitat of *Vibrio* species is generally aquatic. *Vibrio* species have been isolated from fresh water, saline and waste water in various countries[2-4]. Isolation of *Vibrio* species from water main for domestic or drinking uses is of public health importance.

Vibrio cholerae is an important pathogen that causes cholera. It can cause an acute intestinal disease characterized by profuse rectal loss of water and electrolytes and if untreated may lead to death. The action is mainly due to the exotoxin produced which effects the epithelial cells resulting in watery diarrhoea[5]. Other *Vibrio* species include *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus* and *V. mimicus*. *V. parahaemolyticus* is halophilic marine organisms responsible for many outbreaks and food poisoning associated with seafood. *Vibrio minicus* has been established as a pathogenic member of the *Vibrio* found to be the agent responsible for various types of human illness[6].

Vibrio species are attracting renewed scientific interest. New information is being gained on the mechanism of pathogenesis, immunity, epidemiology and transmission. *Vibrio cholerae* which do not agglutinate polyvalent 01 antiserum but does so with the polyvalent H antiserum is classified as non-01 *Vibrio cholerae*. Since transmission of infectious *Vibrio* species is encouraged by inadequate water supply and excreta disposal and more generally by poverty, it therefore seemed of particular interests to undertake a study to evaluate the presence of non- 01 *Vibrio cholerae* in our stream water supplies and to determine their pathogenic characteristics.

MATERIALS AND METHODS

Sample collection: Three hundred (300) water samples were randomly collected from three streams located in the south-south parts of Nigeria according to the standard methods. Each water sample was taken in sterilized 2L polypropylene screw capped containers at a depth of approximately 30 cm below the surface of the water. All the samples were carried in refrigerated containers to the laboratory for analysis within 24 h.

Isolation of non- 01 *Vibrio cholerae*: Duplicate volumes of 200 mL of each water sample were filtered through membrane filters (pore size, 0.45 μm, Millipore Corp. Bedford) and the filter with retained bacteria were added to alkaline Bile Peptone Broth (BPB) (pH8.5), an enrichment medium and incubated at 37°C for 6 h. After incubation, loopful broth cultures were streaked onto Thiosulphate Citrate Bilesalt Sucrose (TCBS) agar plates. All the TCBS agar plates were incubated at 37°C and sucrose fermenting colonies were picked and sub-cultured to obtain pure culture. Thereafter the growth of each pure isolate was tested for oxidase, gram.

Stain, motility, glucose and sucrose fermentation and other biochemical tests. Only the isolates found to be oxidase positive, gram negative, motile, glucose and sucrose fermenting were considered to be potential *Vibrio* and typed using the polyvalent OI antiserum and polyvalent H antiserum. Isolates which do not agglutinate with the polyvalent OI antiserum but does so with the polyvalent H antiserum are classified as non-OI *Vibrio cholerae*.

Pathogenicity of isolate in animals: Preliminary enterotoxicity testing of isolates was first carried out using 5 day old mice[7]. Virulence of the non-OI *vibrio cholerae* isolates were measured by their ability to produce diarrhoea. Subsequently, pathogenic characteristics of isolates were investigated in healthy rabbis. Three rabbits were only administered with 0.2 nL of bacterial suspension ($6x10^7$ cfu/mL) using plastic tube that was placed above the tongue to enable the rabbit swallow the suspension slowly[8,9]. Three control rabbits were administered with 0.2 mI of sterile phosphate buffered saline. Both sets of animals were housed differently to eliminate the risk of cross-infection.

After 48, 72, 96 h, blood samples were collected from both the test and control rabbits through the heart using sterile needle and syringes. The blood samples were put in appropriate specimen containers for the estimation of White Blood Cell (WBC), Haemoglobin (Hb) level, Packed Cell Volume (PCV) and serum electrolyte (chloride, sodium, potassium ions). Blood samples were also collected every week (7, 14, 21, 28, 35, 42 days) for further estimation of WBC, Hb and PCV.

DETERMINATION OF WBC, PCV AND Hb

The methods described by Dacie and Lewis[10] were adopted in estimating Hb, PCV and WBC values. In order to estimate WBC, 20 μL of the blood sample was diluted with 0.38 mL of diluents (two or three drops of gentian violet in 2% acetic acid). With a clean Pasteur pipette the diluted sample was transferred into Neubauer counting chamber (Weber Scientific Inter. Ltd. Susex, England). The chamber was placed on a microscope stage and the WBC estimated using the X 10 objectives lenses. To determine the PCV, blood sample was transferred into heparinized capillary tube and one end of the tube was sealed with plasticine before centrifuging it at 1200 g for 5 min in a microhaematocrit centrifuge (Hawksky and Sons London). The PCV was then read with the aid of microhaematocrit scale reader. A spectrophotometer (Spectronic 20D, Milton, Roy, Ltd. USA) was used to read the haemoglobin (Hb) at wave length of 625 nm after diluting 0.05 mL of blood sample with 10 mL diluents (0.2 g! of potassium ferricyanide and 0.5 g potassium cyanide in 1000 mL of distilled water). Each sample was determined in duplicate and the mean value recorded.

Determination of serum chloride, sodium and potassium ions concentration: The flamephoitometer (Gallenkamp Co. Ltd England) was used to determine the sodium and potassium ions concentrations of blood serum according to the manufacturers instruction. Briefly 0.1 mL of serum was diluted in 9.9 mL deionized water in clean glass tube. The sample was then read in a flamephotometer using 590 mm and 770 mm light filters for the determination of sodium and potassium ions, respectively. The concentration of chloride ions were determined by titrating mercuric solution from a microburette into glass tube containing mixture of 0.2 mL serum sample, 1.7 mL distilled water and drops of diphenylcarbazone indicator[11].

RESULTS

Thirty six (12%) strains of non- OI *Vibrio cholerae* were isolated in this study. The preliminary enterotoxicity test indicated that the strains were pathogenic as they were able to produce diarrhoea and cause death in groups of 5- day old mice.

Levels of sodium,. potassium and chloride ions obtained in both control and rabbits orally infected with pathogenic isolates of non- OI *Vibrio cholerae* are shown in Table 1-3. Potassium ions (K^+) concentration decreased from a control level of 5.6 mMol L^{-1} to 3.2 mMol. L^{-1} (42.9%) after 96 h following infection (Table 1). Sodium ions (Na^+) concentration of 129.6 mMol L^{-1} for the control decreased to 80.7 mMol L^{-1} (37.7%) at 72 h and 63.8 mMol L^{-1} (50.8%) after 96 h of infection (Table 2) The concentration of chloride ion decreased from control level of 99.5 mMol L^{-1} to 82.3 Mol L^{-1} (26.5%), 61.4 mMol L^{-1} (38.3%) and 50.2 mMol L^{-1} (49.6%) at 48, 72 and 96 h, respectively following infection (Table 3). Significant differences (ANOVA, $p<0.05$) were observed in the electrolyte (sodium, potassium and chloride ions) loss by rabbits infected with non-OI *Vibrio cholerae*.

Haemoglobiun (Hb), Packed Cell Volume (PCV) and White Blood Cell (WBC) values of infected rabbits increased from control levels up to the seventh day and

Table 1: Concentrations of potassium ions obtained in blood serum of experimental animals orally infected with non-OI *Vibrio cholerae*

Animals	Time (h) following infection	Concentration of ions (mMol L^{-1})	Percentage decrease against control (%)
Control	0	5.6	-
Infected	48	5.0	10.7
Infected	72	4.3	23.2
Infected	96	3.2	42.9

Table 2: Concentrations of sodium ion obtained in blood serum of experimental animals orally infected with non- OI *Vibrio cholerae*

Animals	Time (h) following infection	Concentration of ions (mMol L^{-1})	Percentage decrease against control (%)
Control	0	129.6	-
Infected	48	108.4	16.4
Infected	72	80.7	37.7
Infected	96	63.8	50.8

Table 3: Concentrations of chloride ions obtained in blood serum of experimental animals orally infected with non-OI *Vibrio cholerae*

Animals	Time (h) following infection	Concentration of ions (mMol L^{-1})	Percentage decrease against control (%)
Control	0	99.5	-
Infected	48	82.3	26.5
Infected	72	61.4	38.3
Infected	96	50.2	49.6

then decreased. With analysis of variance (ANOVA, $p<0.05$) there is no significant difference observed in the Hb, PCV and WBC during the monitoring periods.

DISCUSSION

The occurrence of non- OI *Vibrio cholerae* and their pathogenic characteristics were investigated. Isolation of non-OI *Vibrio cholerae* from clinical samples has been reported[12,13]. In this study, 36 strains of non- OI *Vibrio cholerae* were isolated. Although *Vibrio* species have been isolated in tropical waters[4], however, over the past years not much awareness has been gained for the potential role of non- OI in the differential diagnosis of disease. Unlike the virulence of *Vibrio cholerae* OI which mechanism is essentially due to the effects of a single factor known as cholera toxin, non-OI *Vibrio cholerae* cannot be associated with a single factor[14]. The report of widespread cholera like epidemic caused by non-OI *Vibrio cholerae* 0139 in India[15] brought about interest for the organism.!

The production of diarrhoea in 5 day old mice confirmed the virulence of non-OI *Vibrio cholerae* isolates in this study and which is an indication of enterotoxic activity. The role of *Salmonella* sp., enterotoxigenic *Echerihia coli*, *Shigella* sp. and *Vibtio cholerae* in cases of diarrhoea have been established and known to be responsible for substantial degree of morbidity and mortality[16,17]. Electrolyte levels in rabbits orally infected with non-OI *Vibrio cholerae* isolates decreased as observed in sodium potassium and chloride ions concentration. Sodium ion decreased by 49.6% from control values after 96 h post-infection. The electrolytes loss induced in rabbits were significant when compared with normal controls. The pathogenic isolates possibly may have released some enterotoxic factors which induced the loss of sodium, potassium and chloride ions in the experimental animals[18].

The haemoglobin, packed cell volume and white blood cell values increased in animals infected with non- OI *Vibrio cholerae* up to the seventh day and thereafter the values of these parameters decreased. The possible explanation for the increase in white blood cells observed within the seventh day of infection is due to the response of the white blood cells to bacterial infection. The white blood cells are known as the soldiers of the body system that attack infectious microorganisms in order to prevent disease[19] and this account for their increase in the blood samples. However the increase in haemoglobin levels and packed cell volumes observed within the same period could be as a result of loss of water associated with diarrhoea that led to haemoconcentration. On the other hand the subsequent decrease observed after the seventh day in these parameters were probably due to the adverse effect of the non-OI *Vibrio choleras* on animals.

The results of this study provide information on the presence in stream water supplies of non-OI *Vibrio cholereae* associated with pathogencity in humans. Dinking of such water supplies may stimulate sodium, chloride, potassium ions loss and haematological disorder in body system. Therefore water supplies especially from streams should be boiled to avoid any waterborne diseases.

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