AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JUNE. 2002 ISSN1595-689 Vol 3 No2
AJCEM/2002022/2216

PATHOGENIC POTENTIALS OF ESCHERICHIA COLI ISOLATED FROM RURAL WATER SUPPLIES

[1]Nwachukwu, E. And [2]Otokunefor, T.V.

Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike. P.M.B. 7226, Umuahia, Abia State, Nigeria.

Department of Microbiological University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.

Electrolyte and haematological parameters in rabbits infected with pathogenic isolates of Escherichia coli from rural water supplies in Rivers State, Nigeria, where monitored. Rabbits were orally infected with suspension containing $3x10^7$ cfu /ml of Escherichia coli to induce diarrhoea, and the electrolyte (sodium, potassium and chloride ions) levels as well as the haemoglobin (hb) packed cells volume (PVC), and total white blood cell count (WBC) were determined after 48, 72, 96 hours and post infection following standard procedures. Subsequently blood samples were collected every week for 6 weeks for further estimation of WBC and HB and PVC. Results of the electrolyte (sodium, potassium and chloride ions) levels obtained revealed that significant amount of electrolytes were lost after 96 hours post-infection. Potassium ions concentration decreased by 57.2%, sodium ions by 64.6% and chloride ion concentration decreased by 59.9% as compared to the normal control rabbits not infected with E. coli.

Haematological changes were observed with an increase in haemoglobin concentration, total white blood cells count and packed cell volume from those of normal control up to day four and then decreased steadily during the monitoring period.

INTRODUCTION

Presence of *Escherichia* coli in water supply indicates faecal contamination. *Escherichia coli* has been reported in a variety of infections including gastroenteritis, urinary tract infection (Baker and Breach, 1980. Chessbrough, 1984), haemolytic colitis and haemolytic uremic syndrome (Karmali et al., 1983). The organism is also a leading cause of diarrhoea in developing countries (Alabi *et al.*, 1991) and responsible for a considerable degree of morbidity and mortality among children and adults in poor communities (WHO).

E.coli that cause diarrhoea belong to 4 major groups namely enteropathogenic E.coli (EPEC), enterotoxigenic E.coli (ETEC), enteroinvasive E.coli (EIEC) and enterohaemorrhagic E. coli (EHEC) (WHO, 1980). A number large of outbreaks of diarrhoea illness due to pathogenic *E.coli* have been related to contamination of water and death rates of 5-10 percent may be experienced among untreated infants or children (*Feachem et al*, 1983).

The pathogenicity of bacteria subsequently leading to clinical manifestation at various sites depends on certain properties of bacteria. The virulence of pathogenic organism depends on serotype and the strain specificity, infective dose and on host status (*Jawetz et al.*,1989). The incubation period is generally short and clinical symptoms occur within 5 days (usually 1-3 days) of ingesting of bacteria. Some bacteria toxins stimulate intestinal secretion, which results in the loss of electrolyte and fluid leading to dehydration. Several diarrhoea have been reported to lead to a daily loss of 5-10 litres of fluid together with large amounts of sodium, potassium and chloride ions which are responsible for a considerable degree of mortality (Weinberg, 1985). This therefore, underscores the need to embark on a study to fully elucidate the effects of pathogenic E.coli on the electrolyte and haematological parameters of experimental animals in vivo.

In this we report on levels of sodium, potassium, chloride ions, haemoglobin, packed cell volume and total white blood cell counts in rabbits orally infected with pathogenic E.coli isolated from rural drinking water sources.

MATERIALS AND METHODS

Sample collection

Using sterile 250ml glass bottles, water samples were collected from boreholes, wells, streams and rivers in the rural communities of Rivers State, Nigeria. The method for waste collection was as described by standard methods for the examination of water and wastewater (APHA, 1992).

Isolation and identification of isolates

Membrane filter technique (APHA, 1992) was employed. Water sample (200ml) was filtered under vacuum through a sterile 0.45pm pore size filter membrane (HAWG, Millipore Corp, Bed ford, U.S.A). Upon completion of filtration process, the membrane filter with retained bacteria was immediately removed with sterile forceps and placed on to eosin methylene blue (EMB) agar and incubated at 37 for 48 hours.

Identification of isolates was done following previously reported scheme (Chessbrough 1984).

Estimation of bacteria suspension.

The McFarland's standard method of estimation of bacteria suspension as described by Campbell et al. (1970) was adopted. The

* *Corresponding Author*

concentration of the bacterial suspension was determined by comparing it with McFarland's standard tube containing barium sulphate (varying amount of 1% barium chloride solution and 1% sulphuric acid) of desired concentration. McFarland's table for standardization of bacterial suspension was used to calculate the bacterial concentration.

Enterotoxicity Test

The isolates were screened in order to test the ability to induce diarrhoea. The sucking mouse enterotoxin activates assay as described in the manual for laboratory investigation of acute enteric infection (WHO, 1987) was adopted. The isolates were tested for their ability to produce diarrhoea in 5 days old mice. Each mouse was administered orally with bacterial suspensions ($3x10^7$ cfu/ml) and placed on a white filter paper in a cage. The infected mice and controls (not infected) were left at room temperature for 3 days and during which the faeces were observed for diarrhoea. E.coli controls strain ATCC 25922 was incubated in the study.

Inoculation of Experimental Animals.

Twelve healthy rabbits weighing 2.5 to3.0kg and without any history of clinical diarrhoea or illness were obtained from animal house of Michael Okpara University of Agriculture, Unudike. The inoculation of rabbit was carried out as described by Nielsen et al. (1995) and Pina et al (1993). Each of the six healthy rabbits was inoculated orally with 0.2ml ($6x10^7$ cfu/ml) suspension of pathogenic E.coli using plastic tube placed above the tongue and close to the throat to allow the rabbit swallow the suspension slowly. Six control rabbits were inoculation rabbits were housed differently in the laboratory to eliminate the risk of cross-infection.

Preparation Of Blood For Analysis.

After 48, 72 and 96 hours post inoculation, blood samples was collected from the inoculated and control rabbits through the heart by cardiac puncture (Campbell et al., 1970) using sterile needle and syringes. The blood samples were put in appropriate specimen containers for the estimation of electrolyte (sodium, potassium, chloride ions), haemoglobin (Hb), packed cell volume (PVC) and white blood cell count (WBC).

Subsequently blood samples were collected every week for 6 weeks for further estimation of WBC, Hb and PVC.

ESTIMATION OF SERUM ELECTROLYTE.

The blood serum samples were used to estimate chloride, sodium and potassium ions. The mercuric nitrate titration method of Schales and Schales as described by Raphael et al . (1976) was adopted for the estimation of the serum chloride. With a glass pipette, 0.2ml of blood serum was added to 1.8ml of distilled water in a clean test tube.

Therefore, 0.6ml of biphenyl carbazone indicator was added and titrated with mercuric nitrate solution from a microburette. An end point was obtained when the original salmon red colour turned to faint purple colour. The nitration of the standard chloride solution was also carried out as the test sample was determined in duplicates and the mean recorded.

For the estimation of sodium and potassium ions, flame photometer (GallenKamp Co. Ltd, England) was used and operated according to the manufacturers instruction. Measured volume (9.9ml) of deionised water was introduced into a universal bottle followed by 0.1ml of test blood serum. The bottle was capped and the content mixed by inversion. Sodium light filter (590nm) or potassium light filter (770nm) was inserted in the flame photometer and the test samples were read and recorded.

Estimation of haemoglobin concentration.

The spectrophotometric method as described by Dacie and Lewis (1994) was adopted. With a pipette, 10ml of haemoglobin diluting fluid (0.2g of potassium cyanide in 1000ml of distilled water) was dispensed into a test tube and 0.05ml of blood sample added. A spectrophotometer (spectronic 20D,Milton Roy Ltd. USA) was used to the diluted blood sample at wavelength of 625nm, using the diluted as a blank and value recorded.

Estimation of white blood cell count.

The method described by Dacie and Lewis (1994) was employed. Twenty microlitre of the rabbit blood sample was added to 0.38ml of white blood cell diluent (2% acetic acid with few drops of gentian violet). The content was mixed and a portion transferred with the aid of clean Pasteur pipette into Neubauer counting chamber (Weber Scientific International Ltd., Sussex, England) covered with coverslip. The chamber was placed on a microscope stage and the white blood cells estimated using the x10 objective lenses.

Animals	Time (hrs) following infection	Concentration of ion (mMol/L)	% decrease against control (%)
Control	0	5.6	---------
Infected	48	4.5	19.5
Infected	72	3.3	41.1
Infected	96	3.3	57.2

Table 1 :

CONCENTRATIONS OF POTASSIUM IONS OBTAINED IN THE BLOOD SERUM OF RABBITS ORALLY INFECTED WITH PATHOGENIC ISOLATES OF E.COLI WHEN COMPARED WITH NORMAL CONTROLS.

Animal	Time (hrs) following infection	Concentration of ions (mMol/)	Percentage decrease against control (%)
Control	0	129.6	----------
Infected	48	89.2	31.2
Infected	72	66.4	48.8
Infected	96	45.9	64.6

Table 2.

CONCENTRATIONS OF SODIUM IONS OBTAINED IN THE BLOOD SERUM OF RABBITS ORALLY INFECTED WITH PATHOGENIC ISOLATE OF E.COLI WHEN COMPARE WITH NORMAL CONTROLS.

Animals	Time (hrs) following infection	Concentration of ions (Mmol/l)	% decrease against control (%)
Control	0	99.5	------
Infected	48	65.8	33.9
Infected	72	50.5	49.3
Infected	96	39.6	59.9

Table 3.

CONCENTRATIONS OF CHLORIDE IONS OBTAINED IN THE BLOOD SERUM OF RABBITS ORALLY INFECTED WITH PATHOGENIC ISOLATES OF E.COLI WHEN COMPARED WITH NORMAL CONTROLS.

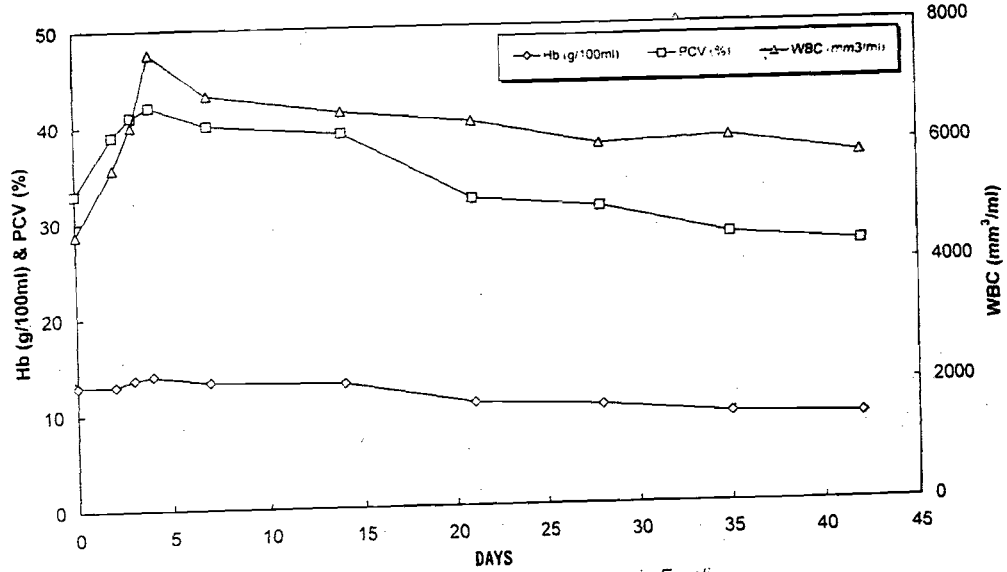

Fig. Haematological changes in rabbits orally infected with pathogenic *E. coli*

Estimation of packed cell volume

Microhaematocrit centrifugation technique as described by Dacie and Lewis (1994) was used. Capillary tube was fitted with well-mixed anticoagulated blood sample and one end of the tube sealed with plasticine. The capillary tubes containing the blood were spun at 1500rpm for minutes in microhaematocrit centrifuge (Hawksky and sons, London) and the packed cell volume read off with the haematocrit scale reader. Two independent test were carried out and the mean calculated.

Statistical analysis

Data were subjected to analysis of variance. The standard errors of difference (S.E.D) of means were multiplied by the appropriate t-statistic to test least significant difference (LSD) between means of treatment at p=0.05.

RESULTS

Electrolyte levels in the blood serum of experimental animals

Concentrations of potassium, sodium and chloride ions obtained from the analysis of blood serum collected from the infected and control rabbits are presented in tables 1-3. Potassium ions Concentration decreased from 5,6mMol/lfor the control animals to 4.5mMol/L, 3.3mMol/L and 2.4mMol/L at 96 hours respectively following inoculation (table 1). Sodium ion concentration decreased from a control value of 129.6mMol/Lto 89.3mMol/L after 48 hours following exposure to E.coli. (Table 2).

Similarly, chloride ion Concentration decreased from a control level of 99.5mMol/L to 50.5mMol/L at 72 hours and 39.6mMol/L at 96 hours of oral infection with the pathogen. Significant differences were observed in potassium, sodium and chloride ions loss at $p<0.05$.

Haematological changes in experimental animals.

The haematological responses in controls and rabbits orally infected with pathogenic E.coli shown in the figure. Haemoglobin (Hb) levels, packed cell volume (PCV) and white blood cells count showed a steady increase from control values up to the 4th day following infection. The Hb increased from 13.0g/100ml to 14.0g/100ml on the 4th day, PCV increased from 33% to 42% while WBC increased from 4600mm³/ml to 7600mm³/ml respectively on the 4th day following infection. Thereafter decrease was observed in these parameters. There is no significant difference ($p<0.05$) observed in the PCV,Hb and WBC values during the monitoring periods.

DISCUSSION

The role of E.coli in diarrhoea has been established and reported to be responsible for substantial degree of morbidity and mortality among children and adults (WHO, 1980). Diarrhoea on its own is characterised by loss of fluids and electrolytes (Tilkian et al.,1979). Results obtained in the study have revealed that experimental infection of rabbits with pathogenic isolates of Escherichia coli from drinking water source resulted in a decrease in a concentration of chloride, potassium and sodium.

Sodium ion concentration was found to decrease by 31.2,48.8 and 64.6 percentages after 48,72 and 96 hours respectively following infections with the pathogen. Potassium and chloride ions values decreased at the same time intervals when compared with the normal controls. This suggests that the infection of the rabbits with the pathogen resulted in the release of enterotoxin, which induces an outpouring of electrolyte and fluids (Robbins and Kumar, 1987). Electrolyte levels in the blood serum confirmed this.

It has been reported that loss of sodium ions from the body results in a decrease of extracellular and nervous system function (Morris, 1981), while the loss of body fluids containing large amounts of potassium ion as in diarrhoea fluids has been reported to cause hypokalaemia. Loss of chloride is characterised by acidosis.

This study has revealed tremendous loss of electrolyte that may occur in host infected with isolate of pathogenic Escherichia coli and therefore highlights the need to immediately provide portable water supplies in rural communities to prevent health hazards which could be fatal. Fluids and electrolyte depletion is one of the most grievous medical emergencies in the tropical environments in human patients (WHO, 1996). This becomes aggravated in the rural areas where there is no portable water and standard of hygiene is very poor and where medical facilities are very scarce.

As evident from the results of this study increase in haemoglobin concentration, total white blood cell count, and packed cell volume was noticed from those of control (day 0) up to the 4th day for rabbits infected with Escherichia coli and thereafter a decrease in parameters. The high values in haemoglobin concentration and packed cell volume could be due to water loss resulting from diarrhoea, which leads to haemo-concentration. The initial increase in white blood cell count values observed within the 4th day may be due to the bacteria infection. Mild to moderate elevation of the white blood cell count usually indicates disease, mainly of bacteria aetiology (Atlas, 1995). This is so because when bacteria invade the body the bone marrow is stimulated to produce and release large number of white blood cell and the number in the blood rise dramatically (Dacie and Lewis, 1994). The subsequent decrease observed in haemoglobin concentration, packed cell volume and white blood cell count after the 4th days of monitoring may be due to reduction on the severity of infection. The second possibility is that since therapy was not instituted, the rabbits generally were very ill and unable to feed normal probably due to many clinical complications (Nielsen et al., 1995). The decrease in value of these parameters observed especially in haemoglobin concentration and white blood cell count result in anaemia, immune deficiency or other haematological disorder.

The study has revealed significant loss of sodium potassium and chloride ion concentrations as well as haematological disorder in experiment animals infected with pathogenic isolates. This confirms the fact that most of the water sources in the rural communities are polluted with pathogenic bacteria. There is, therefore, the need to conduct health education continuously in the rural areas to bring about changes in individual attitudes, believes perception and values concerning water use and general environmental sanitation activities. Furthermore drilling boreholes in rural areas by government will significantly reduce the rates of water-related diseases.

REFERENCES

1 Alabi, S. A., A. Mustapha., C. L. Obi and O. A. Idegbe. 1991. Possible scheme for the bio typing of clinical strains of *Escherichia coli* in developing countries. Abstract presented at 19th conference of Nigeria Society for Microbiology, Zaria, 1-4 September 1991.

2 APHA. 1992. *Standard Methods for the Examination of Water and Wastewater.* 18th .ed. American Public Health Association Washington D.C.

3 Atlas, R. M. 1995. Principles of microbiology 1st ed. Mosby Inc. U.S.A. p 485.

4 Baker, F.J. and M.R. Breanch 1980. Medical Microbiological Techniques. 1st ed. Butterworth & co. ltd, London. 547p.

5 Campbell, D.H., J.S. Garvey, N. E . Cremer and D.H. Sussdorf. 1970 Methods in Immunology- A laboratory test for instruction and Research. 2nd ed. W. A. Benjamin, Inc. NEW York. 454p.

6 Chessbrough, M. 1984. Medical Laboratory manual for Tropical countries. 2nd ed. Buttermorths- Heinemann ltd. London .491p.

7 Dacie, J. V. and S.M. Lewis 1994. Practical Haematology. 8th ed. Churchill Livingstone. Edinburgh.

8 Feachem, R.G., D.J. Bradley. H.Garelick and D.D. Mara. 1983. Sanitation and Disease Health Aspects of Excreta and Waste water management. World Bank studies in water Supply and Sanitation, No3.
John wiley and Sons, New York.

9 Jawetz, E., L. N.Ornston, G.F. Brooks., E,A. Adelbery., J.L. Melnick and J.S. Butel. 1989. Medical Microbiology 18th ed. Lang Michael publications. Califonia. pp202-220.

10 Karmali, M.A., B.T. Steele, M Petric and C. Lim. 1983. Sporadic case of haemolytic uremic syndrome associated with Faecal cytotoxin- producing.
Escherichia coli.
Lancet, 1: 619-620.

11 Morris, D.J. 1981. The metabolism and mechanism of Action of Aldosterone.
Endocrinol. Rev 2: 234.

12 Nielsen, B. D. Baggesen, F.Bager. J. Haugegaard and P. Lind. 1995. The serological response to *Salmonella* serovars typhimurium and infactis in experimentally infected pigs. The time course followed with an indirect anti- LPS ELISA AND bacteriological examinations.
Veterinary Microbiol. 47: 205- 218.

13 Pina, M.F., R.L. Buchanam and P.H. cooke 1993. Virulence of an Escherichia O 157:H7 Sorbitol positive mutant. *Appl. Environ. Microbiol* 59: 4245- 4252.

14 Raphael, S.S., T.A. Hyde, L.D. Mellor. F. spencer, C.F.A. Culling. 1976.
Lynch's Medical Laboratory Technology 3rd ed. W.B. Saunder's Company, London.460p.

15 Robbins, S.L and V. Kumar 1987.
Basic pathology. 4th ed.
Saunders W.B Company London. Pp175.

16 Tilkian, S.M.M.C. Conover and A.G. Tilkian. 1979. Blood Chemistry Electrolytes. In: Clinical implications of Laboratory tests. 2nd ed. The C.V Mosby Company, Missouri. pp 3-26.

17 Weinberg, R.A 1985. The molecules of life.
Sci. Amer. 48:253

18 WHO. 1980. *Escherchia coli* diarrhoea. Bull World Health Org. 58(1):23-36.

19 WHO 1987. Manual for Laboratory Investigations of Acute Enteric Infections.
In: Control of Diarrhoeal diseases
CDD/83.3 Rev. I.

20 WHO 1996. Guidelines for drinking water quality. Vol.2 Recommendations.
World Health Organization.
Geneva .950p