Sodium And Potassium Ion Losses In Rabbits Infected With Strains Of Aeromonas Hydrophila: Implication For Its Roles In Diarrhoea

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ABSTRACT: The plasma levels of sodium and potassium ions were periodically (at 12 hourly intervals) estimated in rabbits orally infected with clinical and environmental Isolates of Aeromonas hydrophila. Infections with clinical isolates resulted in a 54.5% and 32.1% losses in Na^+ and K^+ ions respectively after 96 hours. Infection with the environmental isolates was however less severe resulting in a 42.7% and 16.2% depletion of plasma Na^+ and k^+ respectively 96 hours post oral challenge. It is concluded that A. hydrophila isolates from this locality are capable of causing diarrhoea as evidenced by the results. It is suggested that patients with diarrhoea require prompt treatment in order to avoid allowing decrease in plasma electrolytes which will worsening prognosis thereby increasing morbidity and mortality associated with diarrhoea diseases.
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Introduction

Aeromonas hydrophila has been implicated in both human and animal infections alike. Red leg disease of frogs, development of furunculosis or hemorrhagic septicemia in fishes, acute septicemia characterized by lethargy in snakes and lizards, pneumonia and ulcerative stomatitis which are known to cause considerable mortality in colonies have been documented in humans; and nosocomial infections have been reported in immunosuppressed patients and patients receiving antibiotic therapy (Daley et al 1981, Gracrenits and Bucher, 1983).

The enteropathogenic nature of Aeromonas strains was proposed based on the observation that patients who took antibiotics to which aeromonas strains were susceptible experienced alleviation of their gastrointestinal symptoms and that the organisms were acquired by drinking untreated water, (Holmber, et al, 1986). In some cases, the factors correlated with infections were consumption of uncooked shell fish, raw oyster; and foreign travelers gastrointestinal infections are usually self limiting in normal individuals and remains localized (singh and sanyal, 1993). Blood and mucus in the stool and other clinical implications suggested enteroinvasiveness of the organism. Besides gastrointestinal infections, eye, urinary tract infections meningitis osteomyelitis, septic arthritis, endocarditis, peritonitis among others are common human infections caused by Aeromonas species. It is the aim of this study to establish the values of sodium and potassium ion losses in rabbits infected with strains of *Aeromonas hydrophia.*

MATERIALS AND METHODS

Equipment: The equipment used in the course of this study include Autoclave, Aluminium toil, Beakers Bursen Burner, centrifuge, spectrophotometer, flame photometer, cotton wool, refrigerator, wire loop, measuring cylinder, incubator, Hot air oven, reagent bottles, Pasteur pipette, sterile petridishes, weighing Balance.

Chemicals: The following chemicals and reagents were used:

Heparinated tubes, Distilled water, nutrient agar base, phosphate Buffered saline (PBS), sodium dihydrogen monophosphate (NaH_2, $P04.2H_{2O}$) disodium hydrogen monophosphate (Na_2HP04), blood agar base.

SOURCE OF ORGANISM

The clinical and environmental isolates of Aeromonas hydrophila employed for this study were obtained from the microbiology department, Faculty of Natural sciences, Ambrose Alli University Ekpoma. The clinical isolates were obtained from the faece of an adult patient with diarrhoea visiting the health services division, Ambrose Alli University, Ekpoma. The environmental strains however were isolated from water obtained from an underground reservoir.

PREPARATION OF NUTRIENT AGAR PLATES

Six grams (6g) of nutrient agar base were measured and dissolved into 250ml of distilled water and allowed to stand. This was then heated for two minutes with occasional agitation until it was completely dissolved. The mixture was then sterilized in an autoclave at 121^0C for 30 mins.

The nutrient agar was thereafter allowed to cool to about 42^0C before pouring into plates.

PREPARATION OF BACTERIAL SUSPENSION

The slopes containing isolates were subcultured onto nutrient agar plates, using a red hot wire lop near a flame. These plates were incubated for 24 hours at 37^0c. Growth was observed and the cells were harvested using sterile phosphate buffered saline. Cells of *A. Hydrophila* was washed thrice in phosphate buffered saline and finally resuspended and adjusted to give a transmission of approximately 40% at 540nm using a spectrophotometer.

SOURCE AND INFECTION OF RABBITS

Ten albino rabbits weighing 2.5-3.2g were obtained from a local breeder in Ekpoma. They were fed with commercial diet and tap water ad initial. The rabbits were divided into two groups of 5 five each. The first experimental group received 5ml of the bacterial suspension from clinical origin, while the second group was treated similarly with bacterial suspension from environmental origin. The suspension was orally administered using a fine catheter with an external diameter of 2.5mm fitted with a syringe.

COLLECTION AND TREATMENT OF BLOOD

Blood was obtained from the marginal ear vein before *A. hydrophila* infection (0 hour) and at twelve hourly intervals up to 96 hours post oral challenge and transferred into heparinized tubes. The blood samples were centrifuged at 4000rpm for about 5 minutes. In all the cases the plasma samples were separated with the aid of a pastuer pipette, stored in plain sterile tubes and refrigerated at 4^0c. The Na^+ and K^+ concentrations were analyzed using the flame photometric method described by Thaly (1995).

METHOD USED: PREPARATION OF STANDARD CURVE FOR SODIUM ION:

A stock solution (0.5mg/ml) was prepared by dissolving 0.634g of NaCl and making up to 500ml with distilled water. From the stock solution, different working standards were prepared by pipetting different volumes into test tubes and subsequently making up to 20ml with distilled water. A blank containing 20ml distilled water was also obtained. The concentration of Na^+ in the tubes ranged from 0.1-0.5mg/ml (table 1). The flame photometer readings were obtained using a sodium filter in a digital flame photometer. The flame readings in nm were then plotted against the concentration of Na^+ (fig 1).

Table 1: Assay table for Na^+ standard curve

Tubes	Stock solution (ml)	Distilled water (ml)	Assay volume (ml)	Na^+ concentration (mg/ml)
1	20	0	20	0.5
2	16	4	20	0.4
3	12	8	20	0.3
4	8	12	20	0.2
5	4	16	20	0.1
6	Blank	20	20	0

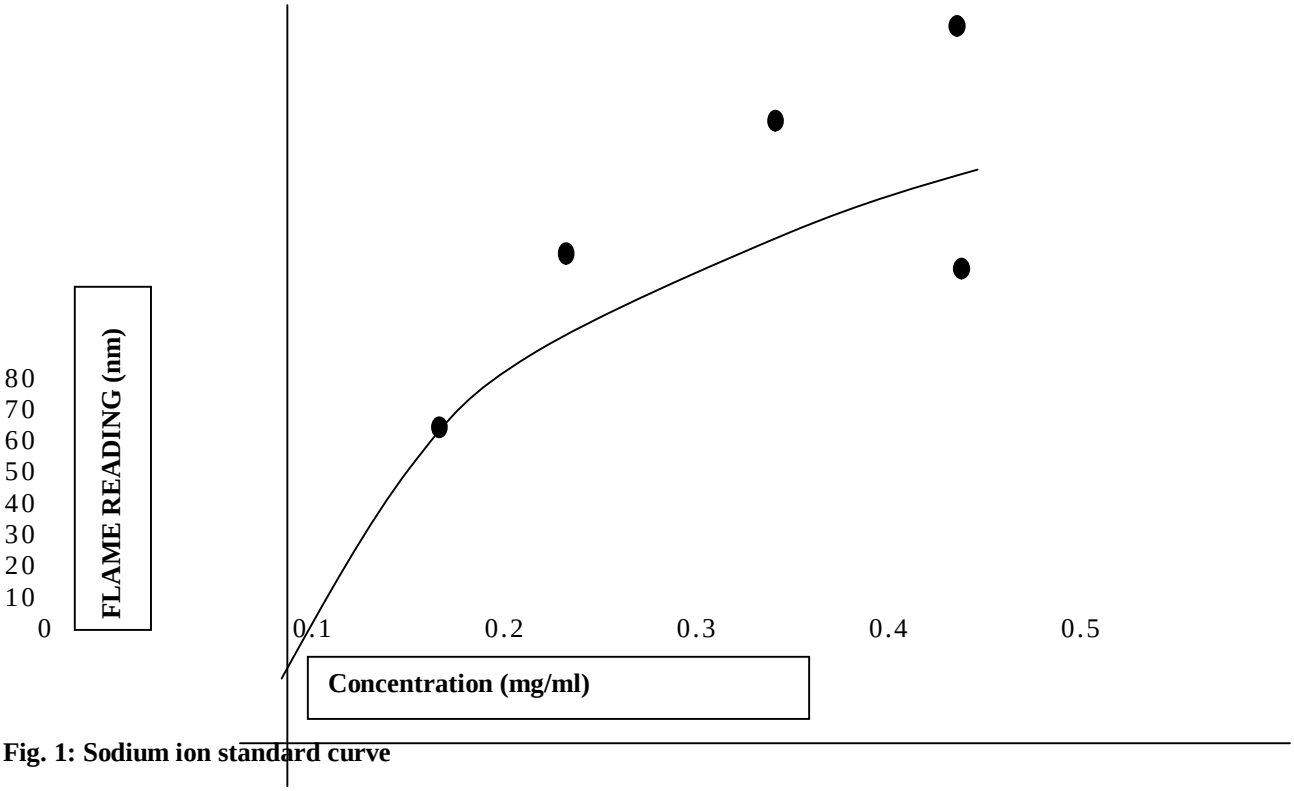

Fig. 1: Sodium ion standard curve

SODIUM (Na^+) ESTIMATED IN PLASMA SAMPLES

The plasma samples to be analyzed were diluted 100times by adding 9.9ml distilled water to 0.1ml of plasma. This diluent was analyzed for sodium using the sodium filter in the flame photometer against the distilled water blank.

PREPARATION OF STANDARD CURVE FOR POTASSIUM ION:

A stock solution (0.5mg/ml) was prepared by dissolving 0.477g of KCl and making up to 500ml with distilled water. Five (5) working standards were thereafter prepared with concentrations ranging from 0.1-0.5mg/ml K^+. Each tube was made up to 20ml with distilled water (table 2). The flame readings were then obtained from each tube after zeroing the instrument with a blank containing 20ml distilled water. The flame readings were subsequently used to plot a standard curve (figure 2).

Table 2: Assay table for Na^+ standard curve

Tubes	Stock solution (ml)	Distilled water (ml)	Assay volume (ml)	Na^+ concentration (mg/ml)
1	20	0	20	0.5
2	16	4	20	0.4
3	12	8	20	0.3
4	8	12	20	0.2
5	4	16	20	0.1
6	Blank	20	20	0

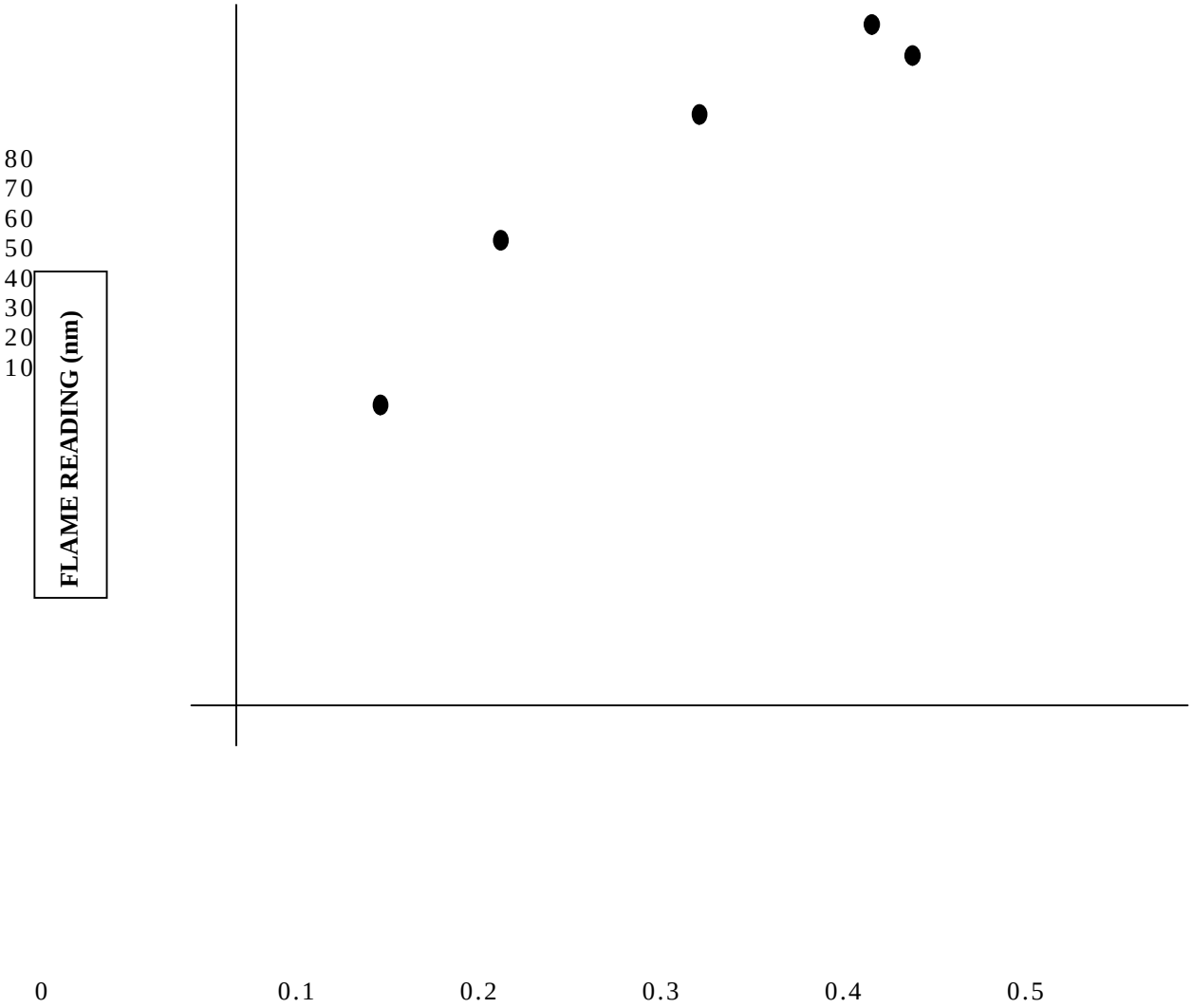

Fig. 2: Potassium ion standard curve

POTASSIUM ION (K^+) ESTIMATION IN PLASMA SAMPLES

A 1 in 500 dilution was made by the addition of 0.1ml plasma to 49.9ml of distilled water. The potassium filter was used; the flame photometer was zeroed using the distilled water blank and the values of the samples determined by extrapolation from the standard curve.

RESULTS:

Oral administration of clinical and environmental strains of *A. hydrophila* caused a general decline with time in the concentrations of plasma sodium and potassium ions in rabbits (table 3, 4). The plasma sodium ion concentration dropped from 139.6mEg/L for uninfected rabbits (0 hour) to 63.5 mmol/L 96 hours post oral challenge with the clinical strains of *A. hydrophila.* This represents a 54.54% loss of plasma sodium in the animals (table 3). The environmental strains however caused a 42.7% loss in plasma sodium ion after 96 hours.

The plasma potassium ion concentration also decreased from 7.1 to 4.8 mmol/L representing a 32.1% loss after 96 hours in rabbits infected with clinical strains of *A. hydrophila* (table3) environmental strains on the other hand caused a lesser depletion of plasma potassium ion, which feel from a control (0 hour) level of 7.1 to 5.9 mmol/L 96 hours post- oral challenge (tables 3 and 4).

TABLE 3: CONCENTRATION OF SODIUM ION IN PLASMA OF RABBITS ORALLY CHALLENGED WITH CLINICAL AND ENVIRONMENTAL ISOLATES OF *A HYDROPHILA*.

Concentration of Na^+ ions (% loss)[a] in mmol/l

Time (hrs)	Clinical isolates	Environmental isolates
12	124.7(10.7)	127.9(8.4)
24	120.6(13.6)	119.6(14.3)
36	99.5(28.7)	113.8(18.5)
48	88.9(36.4)	105.7(24.3)
60	82.1(41.2)	99.7(28.6)
72	76.2(45.2)	95.9(31.3)
84	72.2(48.3)	86.8(37.8)
96	63.5(54.5)	80.0(24.7)

[a] = % loss in Na^+ concentrate with reference to the conc. In plasma at 0 hour (control) which is 139.6mmol/L.

TABLE 4: CONCENTRATION OF POTASSIUM ION IN PLASMA OF RABBITS ORALLY CHALLENGED WITH CLINICAL AND ENVIRONMENTAL ISOLATES OF *A HYDROPHILA*.

Concentration of K^+ ions (% loss)[b] in mmol/L

Time (hrs)	Clinical isolates	Environmental isolates
12	6.7(5.0)	6.9(2.5)
24	6.3(11.5)	6.8(4.7)
36	5.6(2.8)	6.6(6.8)
48	5.4(24.4)	6.5(8.9)
60	5.3(25.6)	6.4(9.7)
72	5.0(29.6)	6.3(11.7)
84	4.9(30.4)	6.0(14.9)
96	4.8(32.1)	5.9(16.2)

[b] = loss in K^+ conc. With reference to the concentration in plasma at 0 hour (control) which is 7.1mmol/L.

DISCUSSION

The role of *A. hydrophila* in gastrointestinal diseases is controversial. This is because of the considerable difficulty in correlating enterotoxin and other virulence properties with specific symptoms of disease (Chopra et al, 1986, Holmberg et al, 1986). Epidemiological studies, (_Pitarangsi et al, 1982; Janda et al 1983, Isis et al 2000) have shown that the organism's ability to cause diarrhoea in previously healthy adults varies geographically. In Nigeria, the role of *A. hydrophila* in diarrhoea cases has been documented (Alabi and Odugbemi, 2000). In this

study, the ability of *A hydrophila* from clinical and environmental sources to cause diarrhea was determined indirectly by periodically estimating the sodium and potassium ion concentrations in plasma following oral infection of rabbits.

The rather high loss (54.5%) of Na^+, the main extracellular cation after 96 hours caused by the clinical isolates is clinically significant as diarrhoea has been indicated as one of the major causes of hyponatremia (Healy, 2000). The fact that environmental isolates caused a 42.7% loss of plasma Na^+ indicates their enterotoxigenicity and untreated water could be a considerable source of acquisition of the clinical strains (Burke et al, 1984, Millership and chattopadhyay, 1985). Potassium ions which are mainly intracellular are also lost in rabbits infected with both clinical and environmental strains of *A. hydrophila*. Over 30% is lost after 96 hours when clinical isolates were administered; while a 16.2% loss was recorded using the environmental isolates. observed in this study, is alarming and points to the epidemiological significance of environmental isolates in diarrhoea cases in our locality.

The report of Healy (2000) which showed that hypokalemia could result from diarrhoea supports these observations. The observation that the clinical strains caused significantly more electrolyte losses than their water counterparts simulates the finding of Paniagua et al (1990), who observed that isolates of *A. hydrophila* from water exhibited low virulence compared to clinical isolates. It is possible that most of the genes coding for virulence factors in this organism were particularly or fully silenced because the water environment provides very little nutrient for the organism to thrive. This view is in agreement with the report of Singh and Sanyal (1993) who showed that enterotoxin gene of environmental strains of *A. hydrophila* was induced on successive passage through rabbit ileal loop.

The fact that both clinical and environmental isolates of *A. hydrophila* from Ekpoma, a University town in Edo State, Nigeria could stimulate plasma losses of sodium and potassium ions up to the levels reactive factor by Aeromonas hydrophila J. Chin. Microbiol. 24: 631-664.

CONCLUSION AND RECOMMENDATIONS

It appears thus from this study that *A. hydrophila* isolates from this locality are associated with diarrhoea it is therefore recommended that further studies be carried out to confirm the observation here. Epidemiological study however is unlikely to give a clear-cut answer to the question of whether *A. hydrophila* and its surrogates are likely enteric pathogens or not in the absence of a detailed analysis of the virulence factors involved.

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