

Original Article

Prevalence of *Aeromonas hydrophila* in Seasonal Episodes of Gastroenteritis in Nsukka, Nigeria

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Kuwait Medical Journal 2002, 34 (1): 16-19

ABSTRACT

Background: Between April and October each year, many children of school age and some young adults in Nsukka, Nigeria suffer from gastroenteritis. The period is also the beginning (April) and end (October) of the rainy season when farms manured with faeces are flooded by running waters. With many people having diarrhoea and occasionally low grade fever at this time of the year, it became necessary to investigate the cause of the illness.

Materials and Methods: Between April and October 1996-1998, 500 loose or watery stools were collected from patients from one month to over 31 years. The stools were examined for parasites. Parasitic positive samples were excluded from the study. The samples were cultured for bacteria on 5% blood agar, 1% egg-yolk agar, all with 10µg ampicillin, MacConkey agar and alkaline peptone water. Bacterial growths on these media were identified using standard bacteriological procedures. Drinking

water and some fruits and vegetables prevalent at this time of the year were bacteriologically examined.

Results: One hundred and thirty eight samples (27.6%) grew various bacteria with *Aeromonas hydrophila*, *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* having frequencies of 65 (13%), 55 (11%), 9 (1.8%) and 9 (1.8%), respectively. Drinking water and some vegetables grew *Pseudomonas aeruginosa* and *Enterococcus faecalis*, respectively. The highest isolation occurred in the months of June and July, the period of greatest flooding of arable land. This results in food contamination.

Discussion/Conclusion: Though no enteropathogens were isolated from the fruits and vegetables examined, they contained *Enterococcus faecalis*, an organism found in faeces. They are, therefore, likely to be the sources of the infection. The association of *A. hydrophila* with gastroenteritis was highlighted.

KEYWORDS: *Aeromonas*, *Amarantus*, *Escherichia coli*, gastroenteritis, *Salmonella*, *Shigella*, *Solanum*

INTRODUCTION

The month of April each year in Nigeria heralds the onset of rainy season, which ends in October. The period is also the onset of the farming season when farmers work in their farms. People at this time of the year succumb to all forms of infection ranging from bacterial to parasitic for the state of personal and environmental hygiene is poor^[1,2]. Occasionally, there is flooding by natural waters of arable land manured with raw faeces, bursting of the domestic water mains with subsequent contamination of fruits and vegetables. At the beginning of each rainy season, some sections of the population at Nsukka, Nigeria complain of acute abdominal pain with diarrhoea and occasionally with vomiting. The town is served by an untreated water supply from deep bore holes.

Bacterial agents associated with gastroenteritis include *Salmonella*, *Shigella*, *Campylobacter*, enteropathogenic *Escherichia coli*, *Aeromonas* and *Yersinia* species^[3]. These organisms abound in fresh and marine water environments, soil and sewage

effluents from where drinking water supplies and foodstuffs get contaminated^[3,4]. The usual clinical presentation of infection due to the above agents are diarrhoea, abdominal pain, low grade fever and vomiting^[5,6]. The microbial flora of aquatic animals like fish, snakes, and leeches includes *Aeromonas spp*^[7,8].

Aeromonas hydrophila infection is found in post surgical leech application on damaged tissues with questionable arterial perfusion^[9]. It is also associated with skin and soft tissue infection following snake bite and also among patients with open wounds exposed to contaminated fresh water^[10,11,12].

Opinions differ regarding the association of *A. hydrophila* with gastroenteritis. Some investigators feel that it is a primary pathogen^[13-18] while others regard it as an opportunistic pathogen^[3,4,5,7].

With the above knowledge, we decided to investigate the causes of diarrhoea among patients reporting to the health clinics from April to October 1996 – 1998. Our aim was to determine the etiological agents of diarrhoea within the period.

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MATERIALS AND METHODS

A community health centre built for about 10,000 – 15,000 inhabitants was used for this survey. Five hundred patients passing watery or loose stools four times daily for upwards of one week were sampled. Fifty-five individuals passing well-formed solid stools were used as controls. The controls were collected over a period of three years, the duration of the study. Stools were collected every Monday and Thursday and treated as follows: One gram of each sample was briefly emulsified in 3 ml of sterile 0.85% (w/v) saline and subsequently vortexed under safety cabinet for 30 seconds. Organic debris was allowed to settle down for five minutes. Wet mounts were prepared and examined microscopically with x20 objective followed with x40. Stools with protozoan parasites or worms were eliminated from the study. They were inoculated into alkaline peptone water (pH 8.4), which were sub-cultured after incubation onto MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, UK), 5% blood agar and 1% egg-yolk agar each containing 10 µg/ml ampicillin^[3,20]. All plates were incubated at 37 °C for 24 hours.

Colonies of bacteria producing double zones of beta haemolysis on blood agar or clear halos/precipitation on egg-yolk agar or which failed to ferment lactose on MacConkey agar were identified using API 20E (Biomérieux, France). This was followed by Collee^[3] method for the identification of *Aeromonas*, *Salmonellas* and *Shigellas*. Final identification of *Salmonella* and *Shigella* was done using their respective homologous antisera.

Environmental samples

Vegetables: Local vegetables called “Ugu”, (*Telfeiria occidentalis*), “Green”, (*Amarantus viridans*) and “Anara”, (*Solanum spp*), which are occasionally eaten without cooking and which are plentiful at this time of the year, were collected and sampled. Two hundred gram of each species, collected from open market, were washed briefly in 500 ml of

0.85% (w/v) sterile saline. The saline wash was spun down in aliquots of 20 ml at 5000 rpm for 30 minutes. One hundred microlitres (100 µl) of pooled spun deposits were plated onto media as in stool samples. Any bacterial growth was identified as in stool samples.

Water: The town is served by ten deep boreholes. Two hundred millilitres of water were collected from each borehole and from the distribution system at ten different locations. Each sample was filtered through 0.45 µm Millipore membrane (Millipore, UK) and was subsequently placed on MacConkey agar (Oxoid Ltd, Hampshire, UK). Any growth was identified as in stool specimens.

RESULTS

Stool samples

Out of 500 sampled stools, 138 (27.6%) were positive for bacteria with *A. hydrophila* having the highest prevalent rate, 65 (13.0%), followed by *Salmonella*, 55 (11.0%), *Shigella*, 9 (1.8%) and enteropathogenic *E. coli*, 9 (1.8%) (Table 1). Infection due to *A. hydrophila* and *Salmonella* was high in 6 - 15 year old, 48 (9.6%) for *A. hydrophila* and 37 (5.4%) for *Salmonella* while *Shigella* was 3 (0.6%) and only in 11-15 year old group. Infection scaled down as the age sampled increased and appeared to die out after the age of 25 (Table 1). *Escherichia coli* isolated from ages below three were serovars 0126. *Salmonella spp* isolated were *Salmonella typhimurium*, 25 (5%), *Salmonella agona*, 6 (1.2%), and *Salmonella ndola*, 10 (2%), untypable spp., 24 (4.8%). *Shigellae* were *Shigella flexneri* serovar 6, 6 (1.2%) and *Shigella sonnei*, 3 (0.6%). From the control subjects (N=55), *Shigella*, 2 (3.6%), *Salmonella*, 1 (1.8%) and *A. hydrophila* 1 (1.8%), were isolated from 11-15, 16-20 and 21-25 year old respectively. No *Aeromonas*, *Salmonella* or *Shigella* were isolated from ages below five.

Fig. 1 and Table 1 represent the monthly distribution of bacteria from positive stool samples.

Table 1
Distribution of bacteria from sampled population

Ages (yr)	Samples No*	Isolated bacteria				Total isolation (%)
		<i>A. hydrophila</i> (%)	<i>Salmonella</i> (%)	<i>Shigella</i> (%)	Enteropatho-genic <i>Esch. coli</i> (%)	
< 5	101	-	-	-	9 (1.8)	9 (1.8)
6 - 10	110	30 (6.0)	17 (3.4)	-	NC*	47 (9.4)
11 - 15	75	18 (3.6)	10 (2.0)	3 (0.6)	NC	31 (6.2)
16 - 20	93	5 (1.0)	15 (3.0)	5 (1.0)	NC	25 (5.0)
21 - 25	80	8 (1.6)	12 (2.4)	1 (0.2)	NC	21 (4.2)
26 - 30	41	4 (0.8)	1 (0.2)	-	NC	5 (1.0)
Total	500	65 (13.0)	55 (11.0)	9 (1.8)	9 (1.8)	138 (27.6)

* The number sampled per age group depended on the number of patients presenting themselves to the clinic.

• Not necessary

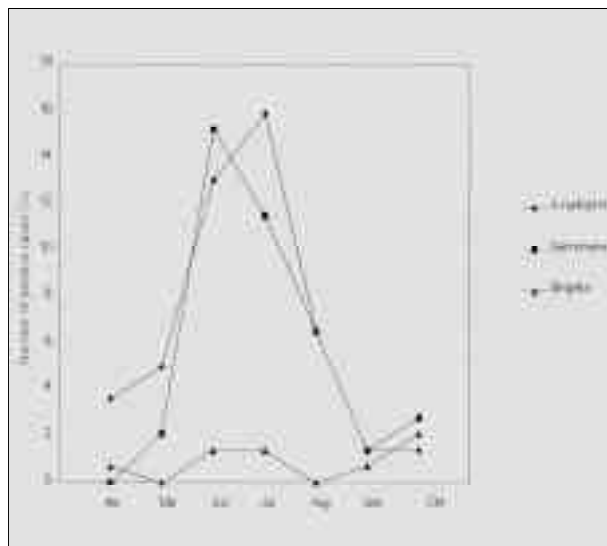


Fig. 1: Monthly distribution of bacteria from April to October 1996-1998

Table 2

Monthly distribution of bacteria from positive stool samples

Month	<i>A. hydrophila</i> (%)	<i>Salmonella</i> (%)	<i>Shigella</i> (%)	Enteropatho- genic <i>Esch. coli</i> (%)	Total isolation per month
April	5 (3.6)	-	1 (0.7)	-	6 (4.3)
May	7 (5.0)	3 (2.1)	-	-	10 (7.2)
June	18 (13.0)	21 (15.2)	2 (1.4)	5 (3.6)	46 (33.2)
July	22 (15.9)	16 (11.5)	2 (1.4)	3 (2.1)	43 (31.1)
August	9 (6.5)	9 (6.5)	-	-	18 (13.0)
September	2 (1.4)	2 (1.4)	1 (0.7)	-	5 (3.6)
October	2 (1.4)	4 (2.8)	3 (2.1)	1 (0.7)	10 (7.2)
Total	65 (47.1)	55 (39.8)	9 (6.5)	9 (6.5)	138 (99.9)

The highest level of isolation occurred in June and July. Low level of isolation occurred in April, the beginning of rainy season and in October, the end of rainy season. *Aeromonas hydrophila* was the highest isolate among the positive samples, 65 (47.1%) followed by *Salmonella*, 55 (39.8%), *Shigella*, 9 (6.5%) and enteropathogenic *Esch coli*, 9 (6.5%) in that order.

Environmental samples

Vegetables: *Telfairia occidentalis* and *Solanium spp* grew no bacteria but *Amarantus viridans* grew *Enterococcus faecalis*.

Water: Water from the ten boreholes grew no bacteria, though two samples from the distribution system grew *Pseudomonas aeruginosa*.

DISCUSSION

Salmonella organisms are found in virtually all animals including poultry, reptiles, livestock, rodents and domestic animals from where contamination of food chain occurs through faeces. Man is eventually infected by faecal oral route^[7]. Unlike the genus *Salmonella*, *Shigella* is transmitted

by the faecal oral route, primarily by people with contaminated hands and less commonly by water or food^[7]. It is a primary paediatric infection occurring in children between one and ten years^[7]. Aeromonads, though more abundant in water, and sewage than either *Salmonella* or *Shigella*, is rarely associated with human infections in Nigeria^[21]. On the other hand, infections due to *Salmonella* or *Shigella* are common since the organisms abound in some contaminated foods eaten locally^[7,8]. Diarrhoea episodes in the subjects studied manifested as ambulatory form of illness. Children of five to ten years old attended school irregularly during the period of study compared with eleven to fifteen years old and above, who were more frequently in the school. Though *Salmonella* and *Shigella* are associated with gastroenteritis^[3,7,22,23], that of aeromonads are disputed^[5,19]. Janda^[19] postulated that there is convincing evidence that *A. hydrophila* and other aeromonads cause gastroenteritis but doubted whether some of the strains isolated from faeces were involved in diarrhoea disease.

In this study, the highest prevalence for *A. hydrophila* and *Salmonella* was found in ages between six and fifteen years while *Shigella* was in ages between 16 and 20. These findings contrast with Doedhar^[16] who found highest prevalence in patients below the age of five. Children in Nigeria start primary school at the age of five and finish at the age of ten. They enter secondary school at the age of eleven and finish at the age of fifteen. Within these ages, they move freely on their way to-and-from school and may likely eat contaminated fruits and vegetables especially mangoes and "anara" (*Solanium spp*) which are plentiful at this time of the year. It is also the period of flooding of arable lands by running waters. The highest isolations occurred in the months of June and July, the peak of the rainy season in Nigeria (Table 2). The decrease in infection rate became obvious as the ages of patients sampled increased and as the end of the rainy season approached (Table 1). This effect is attributable to the improvement in the state of personal hygiene of people at this age. Asdown^[17] found *A. hydrophila* the most common enteric pathogen of man second to *Giardia* and *Salmonella* infections. His finding was supported by other workers who postulated that the organism produced powerful proteases, cytolysins and phospholipases that could harm the microvilli of intestinal epithelium^[13,14,15,18].

In our study, all patients from whom *A. hydrophila* was isolated had diarrhoea alone or abdominal pain with mild fever. Our result showed a carrier rate of one percent (N=55) among control subjects and a prevalence of 13% (Table 1). This contrasts with the work of Asiru^[21] who had a

prevalent rate of one percent. Our work supports Collee^[3] statement that the carriage rate of *A. hydrophila* varies from 0-20% depending on the country, the state of environmental health and the standard of personal hygiene of the citizens. According to Collee^[3] and Murray^[7], *A. hydrophila* causes gastroenteritis only in immunocompromised patients. This view is apparently correct for the control subjects in this experiment were children and wards of lecturers in the university, a group regarded as privileged class in the society. However, patients with cases of gastroenteritis were wards of peasant farmers who invariably were poorly fed. In an attempt to prepare this paper, we tried to avoid the controversy concerning *A. hydrophila* as a primary or opportunistic pathogen of gastroenteritis since we never performed any *in vivo* experiment with the isolates. However, all our patients from where *A. hydrophila* was isolated as the only agent were passing stools four times daily for upwards of one week. Moreover, we are aware that some microorganisms do not always obey Koch's postulate germ theory of disease, yet some of such organisms are potential pathogens. *Aeromonas hydrophila*, as a causative agent of gastroenteritis, may belong to such a group.

CONCLUSION

We have tried to highlight the seasonal episodes of gastroenteritis in children and young adults in Nsukka, Nigeria in which infection occurred between April and October 1996-1998. The causative bacteria were *A. hydrophila*, *Salmonella*, *Shigella* and enteropathogenic *Escherichia coli*. Infection was high in June and July but waned as October approached. The source of infection was likely to have come from foods, mostly fruits and vegetables contaminated by faeces in running waters. We were not able to investigate whether *Campylobacter* or Rota viruses were one of the etiological agents of diarrhoea in the patients surveyed due to lack of reagents and appropriate isolation media for *Campylobacter*.

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