Bacterial enteropathogens and factors associated with seasonal episodes of gastroenteritis in Nsukka, Nigeria

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ABSTRACT

Each year, between April and October, many children of school age and some young adults in Nsukka, Nigeria suffer from gastroenteritis. The period covers the rainy season in this part of Africa, when manured farmland occasionally is flooded. In view of the number of people suffering diarrhoea and occasionally low-grade fever, it became necessary to investigate the nature of the bacterial agents responsible. Between April and October (1996-1998), 500 loose or watery stools were collected from patients, the ages of which ranged from one month to 31 years. Stools that contained parasites were excluded from the study. Samples were cultured on 5% blood agar and 1% egg-yolk agar (both containing 10 µg/mL ampicillin), MacConkey agar, Shigella Salmonella agar and in alkaline peptone water. Bacterial growths were identified using standard bacteriological procedures. Drinking water and some fruit and vegetables prevalent during this period of the year also were cultured. Of the 500 stool samples tested, 138 (27.6%) grew a range of organisms including *Aeromonas hydrophila* (65 [13%]), *Salmonella* spp. (55 [11%]), *Shigella* spp. (9 [1.8%]) and enteropathogenic *Escherichia coli* (9 [1.8%]). Drinking water and some vegetables grew *Pseudomonas aeruginosa* and *Enterococcus faecalis*, respectively. The highest isolation rate occurred during June and July, corresponding to the period of greatest flooding of arable land. Although no enteropathogens were isolated from the fruit and vegetables examined, they contained *E. faecalis* – an organism found in faeces. Our findings failed to explain why 72% of the samples grew no bacterial enteropathogens.

KEY WORDS: Aeromonas. Escherichia coli. Gastroenteritis. Salmonella. Shigella. Solanine

Introduction

In Nigeria, the month of April heralds the onset of the rainy season, which lasts until October. This period is also the farming season. At this time of the year many people succumb to a range of bacterial and parasitic infections, as the state of personal and environmental hygiene is poor.[1,2]

Occasionally, manured farmland is flooded by natural water, and domestic water mains burst, leading to contamination of fruit and vegetables. At the beginning of each rainy season, sections of the population of Nsukka, Nigeria, complain of diarrhoea with acute abdominal pain and occasional vomiting. The town's water supply is untreated and comes from deep bore holes.

Bacterial agents associated with gastroenteritis include *Salmonella*, *Shigella* and *Campylobacter* species, enteropathogenic *Escherichia coli*, and *Aeromonas* and *Yersinia* species.[3] These organisms abound in fresh and marine water, soil and sewage effluents, and it is from here that drinking water supplies and foodstuffs become contaminated.[3,4] The usual clinical presentation of infection with these organisms is diarrhoea, abdominal pain, low-grade fever and vomiting.[5,6]

The aim of this study is to investigate the bacterial organisms associated with diarrhoea among patients reporting to the health clinic between April and October, 1996-1998.

Materials and methods

Stool samples

A community health centre caring for up to 15 000 inhabitants was used for this survey. 500 patients passing watery or loose stools three times daily for upwards of four days were sampled, and 55 individuals passing well-formed, solid stools were used as the control group. Control samples were collected over the three-year period of the study.

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Stools were collected every Monday and Thursday. A sample (1 g) of each was emulsified briefly in 3 mL 0.85% (w/v) sterile saline and then vortex-mixed for 30 sec in a safety cabinet. Organic debris was allowed to settle for 5 min and then wet mounts were prepared and examined microscopically (x20 and x40 objectives). Samples containing protozoan parasites or worms were eliminated from the study.

The remaining samples were inoculated into alkaline peptone water (pH 8.4) and selenite F medium. Following incubation, samples were subcultured onto MacConkey agar and Shigella Salmonella agar (Oxoid Ltd, Basingstoke,

Table 1. Distribution of bacteria from sampled population

Age (yr)	Samples (No♦)	*A. hydrophila* (%)	*Salmonella* spp. (%)	*Shigella* spp. (%)	Enteropathogenic *E. coli* (%)	Total isolation (%)
< 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 attending the clinic*

• *Not necessary*

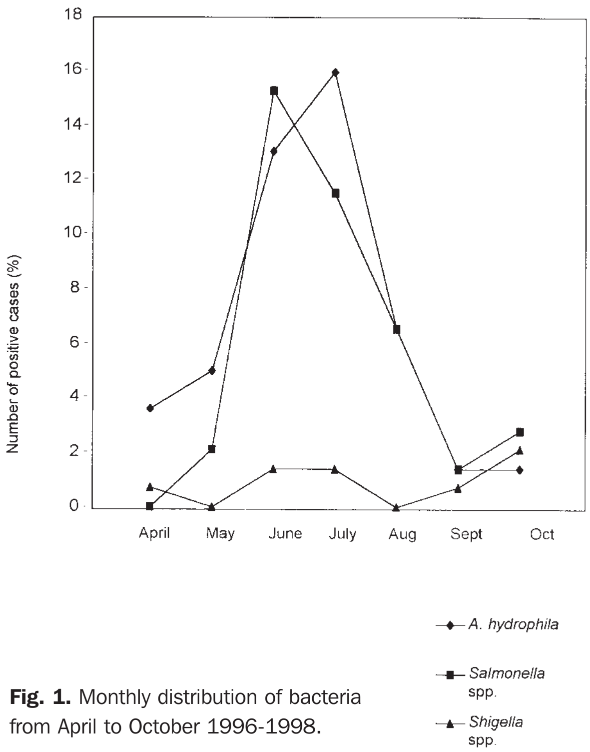

Fig. 1. Monthly distribution of bacteria from April to October 1996-1998.

Hampshire, UK), and onto 5% blood agar and 1% egg-yolk agar (both contained 10 mg/L ampicillin).[3,8] All plates were incubated at 37°C for 24 h.

Colonies that produced double zones of β-haemolysis on blood agar, clear halos/precipitation on egg-yolk agar or which failed to ferment lactose on MacConkey agar were identified using API 20E (bioMerieux, France). This was followed by Collee's method[3] for the identification of *Aeromonas*, *Salmonella* and *Shigella* spp. Final identification of *Salmonella* and *Shigella* spp. was carried out using their respective homologous antisera. Absence of reagents and appropriate media prevented examination for the presence of *Campylobacter* spp.

Environmental samples

Vegetables: Local vegetables called 'Ugu' (*Telfeiria occidentalis*), 'Greens' (*Amarantus viridans*) and 'Anara' (*Solanium* spp), which were plentiful during the study period and occasionally eaten raw, were collected and sampled. Samples (200 g) of each species, collected from the open market, were washed briefly in 500 mL 0.85% (w/v) sterile saline. Aliquots (20 mL) of the saline wash were centrifuged at 5000 rpm for 30 min and then pooled. Deposit (100 µL) was plated onto the same selection of media used for the stool samples and any bacterial growth was identified.

Water: Nsukka is served by 10 deep bore holes. Samples (200 mL) were collected from each bore hole and from the distribution system at 10 different locations. Each sample was filtered through a Millipore membrane (0.45 µm) (Millipore, UK), plated onto MacConkey agar (Oxoid), and any bacterial growth was identified.

Results

Stool samples

Of the 500 stools sampled, bacterial organisms were isolated from 138 (27.6%). *Aeromonas hydrophila* showed the highest prevalence (65 [13.0%]), followed by *Salmonella* spp. (55 [11.0%]), *Shigella* spp. (9 [1.8%]) and enteropathogenic *E. coli* (9 [1.8%]) (Table 1). Infection due to *A. hydrophila* and *Salmonella* spp. was high in the six- to 15-year-old group, being seen in 48 (9.6%) and 37 (5.4%) cases, respectively. *Shigella* spp. were isolated from just three (0.6%) cases in the 11- to 15-year-old group.

Infection diminished with increasing age and disappeared after the age of 25 (Table 1). *E. coli* isolated from children less than three years old were serovar O126. *Salmonella* spp. isolated were: *S. typhimurium*, 25(5%); *S. agona*, 6 (1.2%); and *S. ndola*, 10 (2%), with 24 (4.8%) untypable species. *Shigella* spp. isolated were: *S. flexneri* serovar 6, six (1.2%); and *S. sonnei*, three (0.6%). In the control group (n=55): *Shigella* spp., two cases (3.6%); *Salmonella* spp., one case (1.8%); and *A. hydrophila*, one case (1.8%) were isolated from the 11-15, 16-20 and 21-25 age groups, respectively. No *Aeromonas*, *Salmonella* or *Shigella* spp. were isolated from children under five.

Figure 1 and Table 2 represent the monthly distribution of bacteria from positive stool samples. The highest level of isolation occurred during June and July. Low levels occurred in April and October, corresponding to the beginning and end of the rainy season.

Table 2. Monthly distribution of bacteria from positive stool samples

	A. hydrophila (%)	*Salmonella* spp. (%)	*Shigella* spp. (%)	Enteropathogenic *E. coli* (%)	Total isolation/ 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)**

Environmental samples

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

Water: Samples taken from the 10 bore holes did not grow any bacteria but *Pseudomonas aeruginosa* was isolated from two samples from the distribution system.

Discussion

Salmonella species are found in poultry, reptiles, livestock, rodents and domestic animals, from where contamination of the food chain occurs. Eventually, humans are infected by the faecal-oral route.[3] *Shigella* species also are transmitted by the faecal-oral route, but primarily by people with contaminated hands and less commonly by water or food,[3,4] and the cause of a primary paediatric infection occurring in children.[3,6]

Aeromonads, although more abundant in water and sewage than either *Salmonella* or *Shigella* spp., are rarely associated with human infections in Nigeria.[9] On the other hand, infection due to *Salmonella* or *Shigella* spp. is common because the organisms abound in some contaminated foods eaten locally.[7,10] Episodes of diarrhoea in the subjects studied manifested as an ambulatory form of illness and became less frequent with increasing age.

Although *Salmonella* and *Shigella* spp. are associated with gastroenteritis,[3,7,11,12] the role of aeromonads in this condition is disputed.[5,13] Janda[13] postulated that there is convincing evidence that *Aeromonas hydrophila* and other aeromonads cause gastroenteritis but doubted whether some of the strains isolated from faeces were involved in diarrhoeal disease.

In the present study, the highest prevalences for *A. hydrophila* and *Salmonella* spp. were found between the ages of six and 15, while that for *Shigella* spp. was between 16 and 20. These findings contrast with Doadhar[14] who found the highest prevalence in children below the age of five.

In Nigeria, children begin primary school at the age of five and enter secondary school at age 11, finishing at age 15. They move freely on their way to and from school and may eat contaminated fruit and vegetables, especially mangoes (*Margifera indica*) and 'Anara' (*Solanium* spp.), which are plentiful at the time of the year the study was undertaken. It also corresponds to the period when flooding of farmland occurs.

The highest isolation rate occurred during June and July, the peak of the rainy season in Nigeria (Table 2). A decrease in infection rate became obvious with age and as the end of the rainy season approached (Table 1). Effect of age is attributable to an awareness of the dangers of eating uncooked, contaminated food, coupled with enhanced immunity to the infecting agents. As an enteric pathogen, Asdown[15] found *A. hydrophila* to be second only to infection with *Giardia lamblia* and *Salmonella* spp.

In the present study, all patients from whom *A. hydrophila* was isolated had diarrhoea alone or abdominal pain with mild fever. Our results showed a prevalence of 13% and a carrier rate among the control group of 1% (Table 1). This contrasts with the work of Asiru,[9] which showed *A. hydrophila* prevalence to be just 1%, but it supports Collee's view[3] that the carriage rate of *A. hydrophila* varies from zero to 20%, depending on the country, the state of its environmental health, and the standard of personal hygiene of its citizens.

According to Collee[3] and Murray,[6] *A. hydrophila* causes gastroenteritis only in immunocompromised patients. This view is apparently correct because the control group in the present study comprised children and lecturers in the university – a privileged class in Nigerian society. However, patients with gastroenteritis were mainly peasant farmers who were invariably poorly fed. During the study periods, some of the patients existed on rice cereal or oatmeal, depending on their economic or social status. Diarrhoeal episodes resolved without antibiotic treatment.

We have tried to avoid the controversy about whether *A. hydrophila* is a primary or opportunistic pathogen of gastroenteritis because we did not performed *in vivo* experiments with the isolates. However, some of the patients infected with *A. hydrophila* alone passed stools three times daily for upwards of four days. Moreover, some microorganisms do not always obey Koch's postulate germ theory of disease, yet some of these are potential pathogens. As a causative agent of gastroenteritis, *A. hydrophila* may belong to such a group.

The study's findings failed to explain why 72% (362 samples) failed to grow any bacterial pathogen. Although we did not screen for *Campylobacter* spp., due to limited resources, we feel that diets that comprise mostly fruits and vegetables may lack roughage and fibre, which help to stabilise bowel movement. Moreover, *Solanium* spp. are known to contain toxic alkaline steroids (solanine) in the foliage and in green or sprouted tubers.[16] Consumption of such vegetables may cause gastroenteritis, depending on the age and tolerance of the consumer to plant poisons. Therefore, it is likely that they, rather than bacterial agents, contributed to the diarrhoeal episodes because they can be both transient carriers of enteropathogens and causative agents of diarrhoea.

Conclusions

The study investigated the seasonal episodes of gastroenteritis in children and young adults in Nsukka, Nigeria, in which infection occurred between April and October. The causative agents proved to be *A. hydrophila*, *Salmonella* and *Shigella* spp., and enteropathogenic *E. coli*; however, the presence of *Campylobacter* spp. was not invested due to lack of resources. The rate of infection was high through June and July but waned towards October. The source proved likely to come from food – mostly fruit and vegetables – contaminated by faeces from running water. Although the study failed to explain why 72% of the samples grew no bacterial pathogens, we feel that the nature of the diet eaten during this period of the year contributed significantly to the episodes of diarrhoea. □

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