Aeromonads in acute diarrhoea and asymptomatic infections in Nigerian children

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Abstract. Stool samples of 616 asymptomatic and 296 diarrhoeic school children were compared for the recovery rate of Aeromonas spp. on ampicillin (10 µg/ml) sheep blood agar. Culture filtrates of isolates were tested for heat-stable enterotoxin by the infant mouse test and haemolysin production with 1% freshly washed rabbit erythrocytes. Stools of 9 (3.0%) diarrhoeic children yielded five strains of A. hydrophila and four of A. veronii (two each of biotypes sobria and veronii), compared to 12 (1.9%) asymptomatic children who harbored seven A. hydrophila and five A. caviae strains.

Key words: Aeromonas, Diarrhoea, Faecal carriage

Introduction

Aeromonads are waterborne, facultatively anaerobic gram-negative rods that are resistant to the vibrio-static agent 0/129 [1]. These organisms are globally distributed and autochthonous to marine, estuarine, and freshwater environments and are capable of causing disease in a wide variety of cold- and warm-blooded animals [2]. Currently, nine phenotypically distinct genospecies distributed among 12 DNA hybridization groups are recognized: A. hydrophila, A. sobria, A. caviae, A. salmonicida, A. media, A. veronii, A. schubertii, A. eucrenophila, and A. jandaei [3]. Although they are currently classified in the family Vibrionaceae, it has been proposed that aeromonads constitute a new family, Aeromonadaceae, based on 5S rRNA cataloging, 5S rRNA sequencing, and RNA-DNA hybridization data [4].

Epidemiological surveys of Aeromonas-associated diarrhoea in different parts of the world have demonstrated substantial geographic variation with regard to frequency of isolation of Aeromonas spp. and the strength of association with disease [5]. On the basis of case-controlled studies, enterotoxin production and in vitro phenotypic markers associated with enteropathogenicity, individual species of Aeromonas have been implicated as major or potential enteric pathogens [6]. A previous study from Nigeria [7] has noted the association of aeromonads with human enteritis. Despite these reports, the role of Aeromonas spp. in gastrointestinal infection especially in developing countries remains controversial.

A case-control study of Aeromonas-associated diarrhoea has not been previously reported for Nigeria. Between September 1991 and August 1992, we used a controlled study of the frequency of faecal isolation and toxigenesis of aeromonads from healthy and diarrhoeic Nigerian school children to evaluate the significance of these organisms in childhood enteritis. We also compared the frequency of virulence markers of Aeromonas isolates from healthy children with those from diarrhoeic children in order to identify phenotypic markers associated with virulence.

Materials and methods

Climate. Calabar is located along the Calabar River Estuary in the humid rain forest belt of South Eastern Nigeria. Monthly mean temperatures vary from 20 to 26 °C. Heavy rains occur primarily during the months of April to September and make up the area's total precipitation which was 2.8 cm during the study period. In this region, peak prevalence of diarrhoea occurs during the dry months of October to March [8].

Study design and enrollment. For this study, one
nursery and nine primary schools were selected from different locations in the Calabar metropolis. From each school population, every fourth child was enlisted until at least 60 healthy pupils were randomly selected without regard to sex and age. Children with a recent history of diarrhoea or antibiotic usage (prior 7 days) were excluded while those with minor illnesses not referable to the gastrointestinal tract were considered for study. Two stool samples or rectal swabs (in Cary-Blair transport medium) were collected two weeks apart from each enlisted child and delivered to the University of Calabar Teaching Hospital (UCTH) Microbiology Laboratory. All samples were processed within 2 hours of collection.

Index cases were 296 randomly selected diarrhoeic children admitted at the UCTH from whom stools or rectal swabs were collected during the study period. The study concentrated on but was not limited to children 5 years old or younger who had not received antibiotics during the 7 days prior to enrollment. Potential cases were identified at the time of admission, following clinical evaluation and primary treatment by the attending physician. Mothers of infants were interviewed and clinical as well as epidemiological information was recorded. The same questionnaires were used for cases and controls. All stool specimens were examined microscopically for cysts, ova and larvae of parasites. Rotavirus testing was not performed.

**Isolation and identification of Aeromonas strains.** For isolation of aeromonads, faecal samples were plated directly on ampicillin (10 µg/ml)-sheep blood agar (ABA). Subcultures were also made of ABA after overnight enrichment in alkaline peptone water (APW, pH 8.5) at 37 °C. Plates were incubated at 37 °C for 48 hours.

Aeromonads were presumptively identified on the basis of a positive oxidase test and resistance to the vibriostatic agent 0/129. Oxidase-positive colonies morphologically resembling *Aeromonas* spp. were confirmed as members of the genus *Aeromonas* by conventional biochemical tests [9], and identified to the species level according to the schemes of Janda [10] and Carnahan [11]. All test were performed at 37 °C and results read daily for up to 5 days. Isolates were tested for haemolytic activity on sheep and human blood agar and strains producing zones of beta-haemolysis with diameters of 2 mm or more were considered positive.

**Detection of other enteropathogens.** All stool samples and enrichment broths were also plated on MacConkey, salmonella-shigella, and thiosulfate-citrate-bile salt-sucrose, agar (Oxoid). Plates were incubated overnight at 37 °C. Bacterial isolates were characterized by standard procedures [12]. Enteropathogenic *Escherichia coli* (EPEC), *Salmonella*, and *Shigella* isolates were confirmed by agglutination with polyvalent and specific antisera (Wellcome Diagnostics, UK). Comparison of results for *Aeromonas*-positive diarrhoeic and healthy groups of children was done statistically using the chi-square test.

**Exotoxin and haemolysin assays.** Aeromonads and *E. coli* strains were tested for enterotoxigenicity by the infant mouse test using cell-free culture filtrates. Also, culture filtrates of aeromonads in phosphate buffered saline (pH 7.4) were assayed for haemolysin using 1% freshly washed rabbit erythrocytes. Details of these methods have been previously described [13].

**Results**

A total of 1232 stools was collected from the 616 healthy school children (mean age, 6.1 year) during the study. The sex distribution (male/female) was 1.1:1. Of these, 453 (73.5%) had formed stools, while stools form 163 (26.5%) were soft. Of the 296 diarrhoeic children (mean age, 6.4 year), 13.5% had haematochezia, 25.7% were febrile (37.5 °C), 11 were dehydrated, 18.2% had nausea and or vomiting while 1.7% were under shock. All children aged 2–5 years with stools yielding *Aeromonas* spp. were symptomatic and had watery diarrhoea of 3 to 8 days duration; the number of bowel openings ranging from 8 to 12 per day. Both sexes were affected equally in all age groups. All patients who were hospitalized recovered uneventfully after parenteral and or oral rehydration.

Overall, enteropathogens were detected from the stools of 36 of 296 (12.2%) diarrhoeic and 15 (2.4%) of 616 healthy children (Table 1). Twelve (1.9%) of the asymptomatic children had faecal carriage of *Aeromonas* compared to 9 (3.0%) diarrhoeic cases, associated with five *A. hydrophilia*, and four *A. veronii* (two each of biotypes sobria and veronii); all of which were recovered from children ≤ 5 years of age. Other bacterial enteropathogens isolated from diarrhoeic stools included 48 EPEC, 6 ETEC, 5 Salmonella spp., 2 Shigella spp. and 4 *Vibrio parahaemolyticus*. One Aeromonas-positive diarrhoeic child was co-infected with ETEC. All the Aeromonas isolates from diarrhoeic stools were detected in large numbers on ABA on primary isolation. There were no additional isolates following overnight enrichment in APW. On the other hand, 4 (33%) of the isolates from stools of healthy children would have been missed if APW enrichment was not used. Repeat stool cultures of patients two weeks after primary isolation were negative for *Aeromonas* spp. In contrast, however, only one asymptomatic child presumably lost his faecal carriage of *Aeromonas* during the two-week sampling interval. The 11