



Speciation, clinical profile & antibiotic resistance in *Aeromonas* species isolated from cholera-like illnesses in a tertiary care hospital in north India

Balvinder Mohan, Nandini Sethuraman, Ritu Verma & Neelam Taneja

Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh, India

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Background & objectives: *Aeromonas* species have been reported to cause various illnesses in humans such as wound infections, septicaemia, peritonitis and pneumonia. Their role in causation of cholera-like illness is also being increasingly recognized. This retrospective study was done to know the presence of *Aeromonas* as a cause of acute diarrhoea in a tertiary care hospital and to find the common species of *Aeromonas* causing diarrhoea and their antibiotic susceptibility patterns.

Methods: Fifty isolates of *Aeromonas* were obtained over a period of 15 yr from 2000 to 2014 from patients of suspected acute gastroenteritis resembling cholera. Biotyping was done for 35 of these isolates available in culture collection, based on a panel of 13 biochemical reactions. Antibiogram was put up for all of these isolates by disk diffusion methods and interpreted according to the Clinical and Laboratory Standards Institute guidelines.

Results: Of the 50 patients of *Aeromonas*-related acute gastroenteritis, 13 (26%) had typical features of cholera with rice water stools and severe dehydration. Eight patients (16%) had dysentery-like picture. One patient died of severe dehydration and septicaemia. The most common species were found to be *Aeromonas caviae* (34%) followed by *Aeromonas veronii* biovar *veronii* (29%), *Aeromonas veronii* biovar *sobria* (26%) and *Aeromonas hydrophila* (9%). All tested isolates were uniformly susceptible to cefepime, amikacin, azithromycin and meropenem; 14 per cent were susceptible to amoxicillin, 32 per cent to nalidixic acid, 60 per cent to co-trimoxazole, 54 per cent to ciprofloxacin, 60 per cent to ofloxacin, 74 per cent to chloramphenicol, 76 per cent to ceftriaxone, 74 per cent to cefotaxime, 88 per cent to gentamicin and 86 per cent to furuxone.

Interpretation & conclusions: *Aeromonas* is an important, often neglected pathogen capable of causing a variety of gastrointestinal tract symptoms such as acute diarrhoea and dysentery and may even mimic cholera. It is, therefore, pertinent to recognize this pathogen as an important agent in the causation of severe diarrhoea.

Key words Acute gastroenteritis - *Aeromonas* - cholera-like illness

Diarrhoea is an important cause of morbidity and mortality, especially in the developing world. It is estimated that each year, 1.67 billion episodes of diarrhoea occur in children under five years of age and 430 million episodes occur in adults >16 yr of age, the burden being heaviest in Asian and African countries¹. The pathogens causing diarrhoea are varied and numerous. According to the Global Enteric Multicenter Study (GEMS) study, the largest contributors to childhood diarrhoea are *Rotavirus*, *Cryptosporidium*, enterotoxigenic *Escherichia coli* and *Shigella*, other common agents being adenovirus 40/41, *Aeromonas* and *Campylobacter*². *Aeromonas* was among the top five enteropathogens causing childhood diarrhoea in Asia, especially in Pakistan and Bangladesh². Of the more dangerous forms of diarrhoea is cholera, caused by *Vibrio cholerae*, which leads to severe dehydration and high mortality if left untreated. Globally, it leads to about 120,000 deaths annually³. It is highly endemic in Southern Asia and India where it causes seasonal outbreaks as well as sporadic cases⁴.

Aeromonas, on the other hand, is a less well-characterized pathogen. It is a Gram-negative rod, facultatively anaerobic and is oxidase positive⁵. It normally inhabits aquatic environments, not only fresh water but also salty estuarine waters, causing soft tissue infections in aquatic animals. It is responsible mainly for three clinical syndromes *i.e.* septicemia, wound infections and acute gastroenteritis. The acquisition of this infection can usually be traced back to ingestion of seafood or exposure to seawater or estuarine environment, in which the organism is seen to be present often causing disease in aquatic animals⁶. We describe here the results of a retrospective descriptive study of a period of 15 yr *i.e.* 2000-2014, of the cases of acute gastroenteritis and acute watery diarrhoea resembling cholera, due to *Aeromonas*, at a tertiary care hospital in north India.

Material & Methods

Diarrhoeal stool samples from 1595 patients suspected to have cholera were collected over a period of 15 yr between 2000 and 2014, irrespective of age group from both inpatient and outpatient facilities at Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. The samples were transported to the Enteric Microbiology Laboratory in Cary-Blair medium or in sterile containers and processed within two hours of receipt of samples in the laboratory. Patients' clinicodemographic details

were noted wherever available. These included age, sex, ward/outpatient department, presenting clinical features and underlying illness.

Bacteriological isolation: Conventional culture technique for isolation of stool pathogens was followed. In addition, samples were also inoculated onto 5 per cent sheep blood agar plate without ampicillin. Apart from direct plating, enrichment broths such as selenite-F and alkaline peptone water were used. Colonies showing typical characters of *Aeromonas* with haemolysis and oxidase positivity were sub-cultured onto 5 per cent sheep blood agar for further characterization. Species identification was done based on conventional biotyping according to the Aerokey II system with slight modifications following a panel of 13 biochemical reactions^{7,8}. Isolates were stored in brain-heart infusion broth with 10 per cent glycerol at -80°C .

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the *Aeromonas* isolates was carried out by the disk diffusion method of Kirby-Bauer⁹. Mueller-Hinton agar was used for susceptibility testing. Commercially available disks (HiMedia Laboratories Pvt. Ltd., Mumbai) of amikacin (30 μg), amoxicillin (10 μg), azithromycin (15 μg), cefepime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cotrimoxazole (25 μg), furoxone (30 μg), gentamicin (10 μg), meropenem (10 μg), nalidixic acid (30 μg), ofloxacin (5 μg) and tetracycline (30 μg) were used. Standard strain of *E. coli* ATCC 25922 susceptible to all the drugs was used for quality control of disks. Results of zone diameters were interpreted as sensitive, intermediate resistant or resistant according to the Clinical and Laboratory Standards Institute criteria¹⁰.

Results

Aeromonas was isolated from 50 of the 1595 samples from patients suspected of cholera from 2000 to 2014. Age range of cases is depicted in Table I. Maximum number of patients (n=18) were seen during the months of July and August. Males outnumbered females by a ratio of 2:1. Of the 50 patients, 13 (26%) had severe dehydration and rice water stools typical of cholera. Eight patients (16%) had features of dysentery with bloody stools. One patient, from whom *Aeromonas caviae* was isolated, died after developing severe dehydration following consumption of seafood and alcohol at a party. Two patients had dual bacterial infections with *Shigella sonnei* and

Table I. Age ranges of patients studied

Age range (yr)	Number of patients (per cent of total) (n=50)	Number of patients (per cent of total) (n=44)*
0-5	18 (36)	14 (32)
6-15	4 (8)	2 (4.5)
16-25	12 (24)	12 (27.3)
26-35	5 (10)	5 (11.4)
36-50	6 (12)	6 (13.6)
>50	5 (10)	5 (11.4)

Source: Ref 13 *Age distribution after removing the six cases of nosocomial diarrhoea previously published

of antimicrobial agents as proposed by the Centers for Disease Prevention and Control (CDC) and European CDC¹¹. It was found that 24 (48%) isolates were multidrug resistant.

Discussion

In a country like India where there is high morbidity due to diarrhoeal diseases, it is pertinent to identify the true cause of diarrhoea and target the particular organism for treatment. *Aeromonas* is present ubiquitously in the environment and presents itself to multiple opportunities for human contact. Before it was established as an aetiologic agent in

Table II. Susceptibility of *Aeromonas* isolates to antibiotics tested

Antibiotic	Number of susceptible isolates (%)	Number of isolates with intermediate susceptibility (%)	Number of resistant isolates (%)
Amoxicillin	7 (14)	2 (4)	41 (82)
Nalidixic acid	16 (32)	0	34 (68)
Ciprofloxacin	27 (54)	6 (12)	17 (34)
Ofloxacin	30 (60)	9 (18)	11 (22)
Co-trimoxazole	30 (60)	0	20 (40)
Cefotaxime	37 (74)	1 (1)	12 (24)
Ceftriaxone	38 (76)	0	12 (24)
Cefepime	50 (100)	0	0
Gentamicin	44 (88)	2 (4)	4 (8)
Amikacin	50 (100)	0	0
Furoxone	43 (86)	0	7 (14)
Chloramphenicol	37 (74)	1 (2)	12 (24)
Tetracycline	36 (72)	6 (12)	8 (16)
Azithromycin	50 (100)	0	0
Meropenem	50 (100)	0	0

Salmonella Senftenberg, respectively. All others were mono-infections.

Of the 35 strains for which biochemical speciation was done, the most common species was found to be *A. caviae* (34%), followed by *Aeromonas veronii* biovar *veronii* (29%), *Aeromonas veronii* biovar *sobria* (26%) and *Aeromonas hydrophila* (9%). A single isolate of *Aeromonas jandaei* was obtained.

Antibiotic susceptibility testing was done for all 50 isolates (Table II). All tested isolates were uniformly susceptible to cefepime, amikacin, azithromycin and meropenem. Multidrug resistance was defined as non-susceptibility to ≥ 1 drug belonging to ≥ 3 classes

human infections, it was well known as a cause of soft tissue infections and septicemia in aquatic animals. *Aeromonas* as an enteropathogen has received a lot of controversies. According to Janda and Abbott⁵, for an organism to be labelled as enteropathogenic, it should have led to clonal outbreaks and a source of outbreak should be established. *Aeromonas* is a normal habitant of aquatic environments, fresh as well as salty estuarine waters⁵. Multiple species may also exist in a single environment, making it difficult to establish the common source. Infections are believed to be acquired through contaminated water or food items indirectly on contact with contaminated irrigation water or seafood due to their filter feeding mechanism⁵. Only

a few outbreaks of *Aeromonas*-related gastroenteritis are reported¹². From our centre, a unique outbreak of hospital-acquired diarrhoea due to *Aeromonas sobria* was reported in 2004 affecting six children in a haemato-oncology unit¹³. These isolates had similar biotypes and antibiograms; however, molecular typing could not be performed. In a review of *Aeromonas*-associated diarrhoea by von Graevenitz¹², a causal role of *Aeromonas* in gastroenteritis could not be established due to high degree of co-infections reported and lack of clonality in outbreak clinical and environmental isolates. It is, therefore, important that more evidence is available to establish causation. In the present study, over 15 yr, 1595 cases were clinically suspected of cholera, of whom 50 (3.14%) were found to be due to *Aeromonas* species. This may be an underestimation of the overall incidence of *Aeromonas*-related gastroenteritis as we studied its role in causation of cholera-like illness only, in the setting of ongoing cholera outbreaks. In a prospective study over two years conducted in two tertiary care centres in Kolkata, India, 3.1 and 6.5 per cent of their total diarrhoea cases had *Aeromonas* isolated from them, amounting to a total of 164 cases¹⁴. About 42 per cent of these cases had co-infections with other pathogens. In our study, only in two cases, there were other pathogens identified *i.e.* *S. sonnei* and *Salmonella enterica* serotype Senftenberg. The estimates of asymptomatic carriage range between 1 and 4 per cent overall and <1 per cent in industrialized countries^{5,15-17}. *Aeromonas* is known to infect immunocompetent and immunocompromised individuals of all age groups⁵. However, some studies specify adults as a vulnerable population more than children^{12,18}. In our study, maximum infection was found in children aged under-five (36% of total) followed by 16-25 age group (24%). This pattern did not change even after excluding the six cases of hospital-acquired diarrhoea previously reported¹³. According to the GEMS study also, *Aeromonas* ranks among the top five most common enteropathogens causing childhood diarrhoea in developing countries². We also observed that the isolation rates were maximal in the warmer months of the year that is July-August, similar to other studies^{12,18}.

Ability of *Aeromonas* to cause cholera-like disease with typical rice water stools has been rarely reported and is limited to a handful of case reports^{5,19,20}. These cases range in age from two year old to 67 yr old patients. However, in our study, there were 13 patients

with typical rice water stools and severe dehydration. In the Kolkata study, 44.9 per cent of 78 patients were reported to have severe dehydration although the typical stool character was not described. In our study, one of the patients died shortly following consumption of seafood at a party, which was likely the source of *Aeromonas* isolated from his stool. He had severe dehydration followed by hypotension, shock and death. More prospective studies focussed on this grave presentation may further support this important finding.

The identification of the organism often requires a high index of suspicion with the inclusion of selective medium in the routine identification scheme for stool pathogens. We introduced 5 per cent sheep blood agar into the conventional bacterial identification scheme for stool samples to isolate *Aeromonas* to increase the sensitivity of isolation of even ampicillin susceptible strains. It was found that 14 per cent of our isolates, none of which were *Aeromonas trota*, were susceptible to amoxicillin. This has been reported in other studies also confirming that some isolates are missed using ampicillin blood agar as a medium for selective isolation of *Aeromonas*^{21,22}. Moreover, 35 per cent of *A. caviae* has been reported to be susceptible to ampicillin⁸. The species frequency isolated from diarrhoea cases is similar to other studies wherein *A. caviae* and *A. veronii* remain the most common species isolated^{23,24}. *A. hydrophila*, which is the most common species reported in clinical cases overall, more often causes septicaemia and wound infections^{25,26}. There is a certain difference in the species distribution according to the geographical area also. In Australia and Thailand, *A. hydrophila* was the most common species whereas, in Europe and Americas, *A. caviae* is most common²⁷. In the Kolkata study also, *A. caviae* was found to be the most common species of *Aeromonas* causing gastroenteritis¹⁴.

It is important to recognize the presence of multidrug resistance in this organism. In our study 48 per cent of isolates were multidrug resistant, similar to the Kolkata study and in contrast to a study from Western Australia^{14,21}. In the current study, a high-level resistance to quinolones and co-trimoxazole was observed. In a previous report from our centre also, 36 per cent ciprofloxacin resistance was reported in 14 *Aeromonas* isolates²⁸. In Western countries such as Spain and Australia, fluoroquinolone resistance was much less seen although nalidixic acid resistance of 41 per cent was reported in a Spanish study in clinical isolates of *Aeromonas*²⁹. In the Kolkata study,

12-22 per cent resistance to ciprofloxacin was reported and the mechanism involved was shown to be mutations in the quinolone resistance-determining region of *gyrA* and *parC*^{14,30}. Resistance to third-generation cephalosporins was 24 per cent which was similar to the previous report in 2004 from our centre, but in contrast to Western countries, where no or low-level resistance was seen to third- and fourth-generation cephalosporins^{21,28,29}. Macrolides such as azithromycin are viable treatment options to which the organism was seen to be uniformly susceptible. Cholera being one of the most common causes of bacterial diarrhoea, cases with classical symptoms of acute watery stools are often assumed to be cholera and treated with ciprofloxacin to which they are more or less uniformly susceptible although, in the last decade, fluoroquinolone resistance is emerging in cholera^{31,32}.

The importance of *Aeromonas* as a gastrointestinal pathogen must be recognized, and therefore, it must be actively sought in a diarrhoea case.

Conflicts of Interest: None.

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Reprint requests: Dr Neelam Taneja, Department of Medical Microbiology, Research Block A, Postgraduate Institute of Medical Education & Research, Sector 12, Chandigarh 160 012, India
e-mail: drneelampgi@yahoo.com