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Isolation and antibiotic sensitivity of *Aeromonas* from children with diarrhea

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ABSTRACT

Aeromonas species are gram-negative, motile, facultative anaerobic, rod shaped, oxidase positive bacteria of the recently assigned family *Aeromonadaceae*. The significance of *Aeromonas* species as causative agent of human diarrhoea has recently been established. The aim of the present study was to investigate the distribution, and antibiotic sensitivity of *Aeromonas* in nonhospitalized children with diarrhea.One hundred and seventeen rectal swabs from children with diarhhea were cultured for isolation of *Aeromonas* organisms as the etiological agents. In addition to *Aeromonas*, other enteric pathogens were also isolated. Overall, the isolates of enteric pathogens amounted to 36.8%, consisting of *Salmonella*, *Shigella*, *Aeromonas*, and *Vibrio*. *Aeromonas* was only found in 5.1% of cultures, with a ratio of *A. caviae* and *A. hydrophila* of 2:1, while *Salmonella* made up the majority of causative organisms with an isolation frequency of 18.8%, followed by *Shigella* with 11.1%. In this study no isolates of *Vibrio cholerae* O1 were found as etiological agents of diarrhea; however, *V. cholerae* non-O1 and *V. parahaemolyticus* were found in small numbers (<1%). All isolates of *Aeromonas* were resistant to ampicillin and tetracycline, but sensitive to ciprofloxacin and ceftriaxone, as were the other enteric pathogens. Although the frequency of isolation of these enteric pathogens was higher than for *Vibrio* spp., their role in infective diarrhea was less clearcut in comparison with *Salmonella* and *Shigella*.

Keywords: Diarrhea, *Aeromonas*, resistance, children

INTRODUCTION

There are approximately 40 species of diarrhea-causing pathogens, and around half of them were only described within the last three decades. All these pathogens are relatively important in some age groups and/or geographical localities, both because of the chronicity of the involved pathogens as well as the duration of the disease, or because of progress of the disease from the acute to the chronic form.(1) To date many cases of diarrhea are considered to be caused by viruses, because of the difficulties and failures in isolation and identification of these pathogenic bacteria.(1) Among these bacteria is the species *Aeromonas*, which is one of the important groups of enteropathogenic microorganisms whose etiological role in diarrhea is subject to controversy, such that it has received scant attention and is frequently ignored.(2) The species *Aeromonas* consists of motile mesophilic Gram-negative rods. As a result of various studies(3-6) it is recognized that the species *Aeromonas* is universally distributed in freshwater and other environments and may be isolated from clinical material, potable water, foods and beverages. In these environments the bacteria are capable of reproduction even at low temperatures.(7) Among the bacteria causing diarrhea the role of *Aeromonas* as an enteric pathogen is increasingly acknowledged and there is definite evidence that the organism is associated with gastroenteritis in children, adults, and the elderly(6,7) and with cases of 'traveler's diarrhea'.(7) Although healthy individuals may become carriers of this bacterium, it is reported that *Aeromonas* is a significant cause of diarrhea.(8,9)

The three phenotypes of *Aeromonas* that are considered to be the main pathogens and to be associated with gastrointestinal infectious disease are *A.hydrophila*, *A.caviae*, and *A. veronii* biovar *sobria*, but the most frequently associated with diarrheal infection is *A. hydrophila*,(5) as reported by Juan et al.(10) In addition these organisms produce virulence factors that are useful as markers for differentiating between pathogenic and nonpathogenic strains.(11)

In Indonesia, data on the presence of *Aeromonas* in connection with diarrheal disease are difficult to obtain, because *Aeromonas* is not among the bacteria that are routinely reported as etiological agents of diarrhea, as is the case with *Shigella*, *Salmonella* or *Vibrio cholerae*. Actually this bacterium may cause various disorders, either intestinal (diarrhea) or extraintestinal,(11) making it necessary to pay attention to its presence. The aim of the present study was to investigate the distribution, and antibiotic sensitivity of *Aeromonas* in nonhospitalized children with diarrhea.

METHODS

Research subjects and location

The study was performed in infants and children aged <14 years with diarrhea, who were visiting the Tebet and Mampang Health Centers in South Jakarta. The study was conducted from December 2008 until November 2009.

Media

Xylose-lysine-deoxycholate (XLD), MacConkey (MAC), *Salmonella-Shigella* (SS), and thiosulfate citrate bile salts sucrose agar (TCBS) (DIFCO, Becton Dickinson, Sparks, MD), were used and prepared according to standard methods.

Sampling and culture

Rectal swabs were collected from the study participants after informed consent had been obtained from their parents or caregivers. Personal data and data related to the diarrhea were obtained from the parents or caregivers and recorded on standard forms by special personnel. Collection of rectal swabs was done irrespective of degree of diarrhea (mild, moderate or severe). Rectal swabs were taken at the time of admission to the Health Center and before administration of antibiotics. The swabs were placed in Cary Blair transport medium and stored in a refrigerator, pending transportation to the laboratory. The samples were transported in thermos flasks to the Microbiological Laboratory, Medical Faculty, Trisakti University. The rectal swabs were plated on MAC, SS, XLD and TCBS agar and the agar plates were incubated under aerobic conditions at 37^0C for 18-20 hours. Non-lactose fermenting colonies on MAC, SS and XLD agar, and suspected colonies of *Vibrio* on TCBS agar, were selected and characterized in biochemical test media, namely Kligler's iron agar (KIA), mannitol motility ornithine (MIO), and sucrose semi-solid (SSS).(12) Preliminary screening for colonies of *Aeromonas* was performed by means of the oxidase test. Oxidase negative, ornithine positive (MIO positive) colonies were considered to be *Aeromonas* and were subjected to further testing for species determination. Confirmatory serological testing was performed using specific antisera (Difco laboratories, Detroit, MI). The bacterial isolates were subsequently tested for susceptibility to a variety of antibiotics by the disk diffusion method as described in the manual of the National Committee for Clinical Laboratory Standards (NCCLS).(13,14) The antibiotics used in these sensitivity tests were ampicillin, chloramphenicol, tetracycline, cotrimoxazole, ciprofloxacin, and ceftriaxone (Becton Dickinson and Company, Cockeysville, MA, USA). *Escherichia coli* ATCC 25922 was included in the tests as control strain.

Data analysis

Percentage analysis was done to describe the distribution and antibiotic sensitivity of *Aeromonas*.

RESULTS

Bacterial culture of 117 rectal swab samples collected from infants and children aged <14 years with diarrhea resulted in the isolation from 43 (36.8%) subjects of the following enteropathogenic bacteria in descending order of frequency: *Salmonella* 22 (18.8%), *Shigella* 13 (11.1%), *Aeromonas* 6 (5.1%) and *Vibrio* 2 (1.7%). The number of *Aeromonas caviae* isolates was higher than that of *A.hydrophila* (3.4% vs 1.7% or 2:1). Isolates of *Salmonella* belonged to serogroup B (36.5%), C (22.7%), D (18.1%), and E (22.7%), while isolates of *Shigella* comprised *S.flexneri* (53.8%) and *S.sonnei* (46.2%). Isolates of *Aeromonas* ranked third among the causative organisms in this study, consisting of *A.hydrophila* (33.3%) and *A.caviae* (66.7%). *Vibrio cholerae* was not found among the children with diarrhea in the locality of the present study, but *V.cholerae* non-O1 and *V.*

Table 1. Distribution of enteric pathogens isolated from children with diarrhea

Organism	**Number of positives**	**Age of patient/number of isolates (%)**		
		0-1 yr	**>1-4 yr**	**>4-14 yr**
Salmonella spp.	22			
Salmonella B	8	3 (37.5)	4 (50)	1 (12.5)
Salmonella C	5	2 (40)	3 (60)	0
Salmonella D	4	1 (25)	3 (75)	0
Salmonella E	5	2 (40)	3 (75)	0
Shigella spp.	13			
S. flexneri	7	1 (14.3)	6 (85.7)	0
S. sonnei	6	4 (66.6)	1 (16.7)	1 (16.7)
Aeromonas spp.	6			
A. hydrophila	2	0	2 (100)	0
A. caviae	4	1 (25)	3 (75)	0
Vibrio spp	2			
V. cholerae non-O1.	1	0	0	1 (100)
V.parahaemolyticus	1	0	0	1 (100)

Table 2. Distribution of antibiotic sensitivity of pathogenic enteric bacteria isolated from children with diarrhea (n=43)

Organism	**Total number of isolates**	**Antibiotic / proportion of resistant bacteria**						
		AM	**C**	**TE**	**SXT**	**CRO**	**CIP**	**NA**
Salmonella spp.								
Salmonella B	8	5 (62.5)	1 (12.5)	5 (62.5)	0	0	0	0
Salmonella C	5	1 (20)	0	1 (20)	1 (20)	0	0	1 (20)
Salmonella D	4	1 (25)	0	1 (25)	0	0	0	0
Salmonella E	5	1 (20)	0	1 (20)	0	0	0	0
Shigella spp.								
S. flexneri	7	4 (57.1)	4 (57.1)	4 (57.1)	6 (85.7)	0	0	0
S. sonnei	6	0	0	6 (100)	6 (100)	0	0	0
Aeromonas spp.								
A. hydrophila	2	2 (100)	0	2 (100)	2 (100)	0	0	0
A. caviae	4	4 (100)	0	4 (100)	0	0	0	0
Vibrio spp								
V. cholerae non-O1.	1	1 (100)	0	0	0	0	0	0
V.parahaemolyticus	1	1 (100)	0	0	0	0	0	0

AM = ampicillin; C = chloramphenicol; TE = tetracycline; SXT = cotrimoxazole; CRO = ceftriaxone; CIP = ciprofloxacin; NA = nalidixic acid

parahaemolyticus were obtained in small numbers (<1%) (Table 1).

The results of the antibiotic sensitivity testing indicated that both *A.hydrophila* and *A. caviae* were resistant to ampicillin and tetracycline, but that *A.hydrophila* was also resistant to co-trimoxazole (Table 2). *Shigella flexneri* was resistant to various antibiotics, such as ampicillin, chloramphenicol, tetracycline and cotrimoxazole, followed by *Salmonella* serogroup B, which was resistant to ampicillin, chloramphenicol and tetracycline. All enteropathogenic organisms were still sensitive to ceftriaxone and ciprofloxacin.

DISCUSSION

There are still doubts as to the role of *Aeromonas* in the etiology of diarrheal infections in humans, which is presumably because *Aeromonas* is a heterogenous group of microorganisms, with only some subgroups being pathogenic, while there are still problems in subgrouping of these microorganisms.(11,15) However, within the last two or three decades the role of *Aeromonas* in various human diseases has been proven, particularly as a cause of gastroenteritis.(8,10,11,15)

Although clinically it may be obvious that *Aeromonas* causes diarrhea, this organism is not looked for routinely by means of fecal culture or rectal swab, such that very little is known about the clinical evaluation of this bacterium. The isolation of *Aeromonas hydrophila* as a cause of diarrhea has been reported from Taiwan,(10) India,(11)and other localities.(15-17) Juan et al.(10) reported that *Aeromonas hydrophila* is more prevalent in the summer, around August and September, with an isolation frequency of 24-30%. The number of affected males and females is almost equal, and is highest between the age of 1-3 years.

From various localities it has been reported that the main causative organisms of diarrhea are *Salmonella* and *Shigella*,(18,19) followed by others such as *Campylobacter* and *Aeromonas* *hydrophila*.(10,20) In the present study the isolation of *A. hydrophila* ranks after that of *Salmonella* and *Shigella* in order of frequency. Their numbers are much smaller than those reported by Juan et al.,(10) but their order of frequency is similar to that reported by Yates(18) in his discussion on the etiologic agents of traveler's diarrhea. The order of presentation of the most frequently isolated organisms may differ from one locality to another, due to several factors, such as differences in application of microbial identification techniques, seasonal variation and geographic location.(21) The frequency of isolation of *Aeromonas* obtained in the present study is less than the 9.7% reported by Subashkumar et al.(21) and the 17.7% reported by Sinha et al.,(11) but almost identical to the frequency reported by other investigators.(7, 23,24) The isolation frequency of *Aeromonas* might have been higher, had the study been conducted in a hospital, because patients with severe diarrhea are generally hospitalized.

It has been reported that gastroenteritis due to *Aeromonas*, particularly *A.hydrophila*, is more commonly found in children and the elderly(10,17) and in tourists (traveler's diarrhea).(6,7,18,25) According to Vila(7) *A.caviae* is isolated with the highest frequency in traveler's diarrhea, whereas *A.hydrophila* is predominant in the local population.(9-11,26)

Although diarrhea due to *Aeromonas* is a self-limiting disease,(5) occasionally it may become severe and around 50% of patients with diarrhea due to this organism may develop persistent diarrhea(7) such that antibiotic therapy is indicated. The drugs of choice for diarrhea due to *Aeromonas* are the quinolones (ciprofloxacin). In our study, all *Aeromonas* species, both *A.hidrophila* and *A.caviae*, were still sensitive to ciprofloxacin. However, resistance to quinolones should receive more attention, because within the last years the emergence of quinolone-resistant *Aeromonas* strains have been reported in industrialized countries.(25) As to the â-lactam antibiotics, the majority of *Aeromonas* strains have been reported to be resistant to ampicillin, but to be still sensitive to third-generation cephalosporins such as ceftriaxone,(7,10) as is evident from the results of the present study. Resistance to chloramphenicol, tetracycline and cotrimoxazole is due to extensive usage of these antibiotics, particularly in developing countries.(7)

The limitation of this study lies in the relatively small sample size that is inadequate for ascertaining that *Aeromonas* spp. plays an important role as an etiological agent of diarhhea in children. However, the presence of *Aeromonas* itself in feces is indeed less recognized and has received less attention, such that the organism is seldom looked for in patients with diarrhea.(10)

Although the isolation frequency of *Aeromonas* is higher than that of *Vibrio* spp., its role in diarrheal infection is less obvious in comparison to *Salmonella* and *Shigella*. From the aspect of antibiotic therapy, the emergence of multiresistant *Aeromonas* strains should receive attention and antibiotic administration should be performed with care.

CONCLUSIONS

The main etiological agents of diarrhea are *Salmonella*, *Shigella*, *Aeromonas* and *Vibrio*, although the role of *Aeromonas spp* has to be put under scrutiny. All *Aeromonas* isolates are resistant to ampicillin and tetracycline, but are still sensitive to ciprofloxacin and ceftriaxone.

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