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The Role of *Aeromonas hydrophila* in Diarrhea

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Abstract

Aeromonas hydrophila is a causative agent of a number of human infections. Aeromonads have been isolated from patients with diarrhea. In spite of a number of virulence factors produced by *Aeromonas* species, their association with diarrheal diseases has not been clearly linked. In current study, 1546 fecal samples of a randomly selected population were screened for presence of *A. hydrophila.* Out of the total number of cases, 20 were suffering from diarrhoea and the rest were asymptomatic healthy individuals. The result showed that 3.4% of the samples were positive for *Aeromonas* spp. *A. hydrophila* was isolated as the sole enteropathogen from 80% diarrheal and 20% asymptomatic cases. A significant association was found between the *A. hydrophila* and diarrhoeal diseases in rural and urban areas (P<0.05), while no difference was revealed between genders. The highest rates of *A.hydrophila* (3.9%) was detected in children <6 years of age. A significant association was also found between *A. hydrophila* and different age groups (P< 0.05). In conclusion, our epidemiological study showed that *Aeromonas* spp. as a sole enteropathogen could be responsible for diarrhea.

Keywords: Aeromonas hydrophila, *Diarrhea, Aeromonads, Epidemiology*, *Iran*

Introduction

Aeromonas spp comprise mesophilic motile and psychrophilic nonmotile gram negative ubiquitous bacteria. Worldwide studies have demonstrated that *Aeromonas* spp are universally distributed and commonly isolated from clinical (1), environmental (1, 2) and food samples (3, 4). The role of these bacteria in enteric infections of immunologically normal adults and children (5) has been the subject of a number of studies. The illnesses reported in the literature range from mild to dysentery-like diarrhea (6). In some studies, *Aeromonas* spp were isolated more frequently from diarrheal stools than from normal control groups (6), while in the others the isolation rates from the two groups were almost similar (6). It is estimated that aeromonads may cause up to 13% of the reported gastroenteritis cases in the United States (3). *Aeromonas* spp. are considered as opportunistic infectious and enterotoxigenic pathogens (7-9). Among various *Aeromonas* spp., *A. hydrophila* is commonly involved in causing human infections such as septicemia and gastroenteritis (10). Isolation of *A. hydrophila* from water and food sources, and the increasing resistance of this organism to antibiotics and chlorination in water, presents a significant threat to public health (10-12). *Aeromonas* spp are commonly isolated from a wide range of aquatic systems including lakes, rivers and drinking water as well as a variety of foods (1). Although *Aeromonas*-induced gasteroenteritis is the most common in young children, the organism is being isolated lately with high frequency from patients with traveler's diarrhea (12). There is little information on the incidence of infections due to *Aeromonas* spp in general population and its role in diarrhea. Therefore, it might be of any help to investigate the prevalence of the organism in different geographic areas. The present study covered the Ilam province at winter months to elucidate the prevalence and role of

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Aeromonas spp and *A.hydrophila* in particular in diarrehal infections in this region.

Materials and Methods

Area and population Ilam province is located in the south-west of Iran. It has an area of 19,044 km^2 comprising 7 cities and 830 villages. According to the latest Iranian census (1996), the total population of Ilam was estimated 481,854, of which 245,565 (50.9%) reside in urban and 236,389(49.1%) in rural areas (13). Ilam was selected as the field of the current study because diarrheal diseases are one of the major public health problems in this region, and the weather condition and temperature in winter are suitable for *Aeromonas* spp growth in nature. Moreover, seafoods consupmption including fish in the winter is more common. A two-stage random sampling was employed for sample collection. The whole population was divided into 500 blocks, of those 50 (27 urban and 23 rural) were selected in the first stage. In the second stage, lists of all families in the blocks were obtained, and 8 families (average family size~ 4 persons) were randomly selected in each block. A total of 1731 individuals were chosen with a precision of 0.5% and confidence limit of 95%.

Data collection and fecal sampling A questionnaire containing different clinical and personal data, i.e. clinical symptoms, antibiotic usages and source of drinking water was completed for each person. The diarrhoea was defined on the basis of the number of defection per 24 h and the form of stool. Three times or more watery or soft defecations were considered as diarrhea. Sampling was performed during the winter months of 1998-99 using Cary and Blairs transport medium.

Culture methods Stool specimens were processed for *Salmonella* and *Shigella* with standard methods (14). In brief, stool samples were cultured on Maconkey agar, *Salmonella-Shigella* (ss) agar and slenite F broth for *Salmonella* and *Shigella.* Stool specimens were plated on TCBS and Maconkey agar for Vibrio and *Aeromonas spp*. (15). After 24 to 48 h of incubation at 37^{o}C, the suspected colonies for *A. hydrophila* were tested for cytochrom oxidase (Kovac's method) (16). The oxidase positive strains were further evaluated by Triple sugar iron agar slants and API-20E commerical strips (Analytab products, Inc., plainview, N.Y). In addition, all lysin decarboxylase reactions were confirmed by using a macrotube (Difco laboratories, Detroit Mich) and the strains were failed to grow in, 6.5% sodium chloride.

Statistic Analysis The χ^2 test, Fisher's exact test for 2×2 tables and Mantel- Haenszel test were used for statistical analysis. Mean valuse were reported with SD (x± s.d).

Results

Study population In total, 1731 persons were randomly selected for this study, of which 185 refused to provide fecal samples. Therefore 1546 persons, including 776 (50.2%) females and 770 (49.8%) males with mean ages of 20.4 ±15.5 (range 1-80 years) and 22.4 ±17.7 (range 1-85 years), respectively, were included in this study.

Prevalence of* A.hydrophila *and* Aeromonas *spp infection according to sex and age in rural and urban areas Out of 12 *A.hydrophila* isolated from diarrheal cases, 6 (50%) and 6(50%) cases were isolated from males and females respectively. Distribution of A. *hydrophila* isolated among both rural and urban population was found to be independent of sex.

The highest rates of *A.hydrophila* isolation was detected in children <6years of age (Table1).

A significant association was found among *A. hydrophila* isolation and different age groups in rural and urban areas (P<0.05).

In rural areas, 42 (5.5%) persons were positive for *A.hydrophila* or *Aeromonas* spp infection and in the urban community, 11(1.4%) were positive for *A.hydrophila* or other *Aeromonas*

spp with no difference between genders (Table2).

Prevalence of* A. hydrophila *and* Aeromonas. *spp (other than* A.hydrophila *) infection in diarrheal and asyptomatic persons Out of 1546 persons studied, 20 were suffering from diarrhoea and the rest were asymptomatic healthy individuals (Table 2). From diarrheal cases, twelve (60%) samples were positive for *A. hydrophila*, which was isolated as the sole enteropathogen, whereas 3(15%) samples were positive for *Aeromonas* spp. From asymptomatic healthy persons 26(1.7%) samples and 12(0.8) samples were positive for *A.hydrophila* and *Aeromonas* spp, respectively (Table 3). In rural and urban areas a significant association was found between the *A.hydrophila* and diarrhoeal illness (P<0.05) (Table 2).

Table1: Frequency of *Aeromonas* spp infection in different ages

Ages	Positive		Negative	Total
	A.hydrophila No(%)	*A*.spp No(%)	*Aeromonads* No(%)	No(%)
<6 years	6(3.9)	1(0.6)	147(95.5)	154(10)
6-10	5(2)	1(0.4)	239(97.6)	245(15.8)
11-30	16(2.4)	9(1.3)	650(96.3)	675(43.7)
>30	11(2.3)	4(0.9)	457(96.8)	472(30.5)
Total	38(2.4)	15(1)	1493(96.6)	1546(100)

Table 2: Distribution of *Aeromonas* spp isolation in asymptomatic and diarrhoeal person in rural and urban areas

	Regions						Total	
	Rural			Urban				
Symptoms	Positive		Negative	Positive		Negative	positive	negative
	A.hyd	*A*.spp	Aeromonads	*A.hyd*	*A*.spp	Aeromonads	Aeromonads	Aeromonads
	No (%)		No (%)	No (%)		No (%)	No (%)	No (%)
Asymptomatic	20(2.7)	10(1.3)	711(96)	6(0.8)	2(0.3)	777(99)	38(2.5)	1488(97.5)
Diarrhoeal	10(62.5)	2(12.5)	4(25)	2(50)	1(25)	1 (25)	15(75)	5(25)
Total	30(4)	12(1.6)	715(94.5)	8(1)	3(0.4)	778(98.6)	53(3.4)	1493 (96.6)

Table 3: Frequency of *Aeromonas hydrophila* and *Aeromonas*.spp (other than *A.hydrophila*) isolated from asymptomatic and diarrhoeal cases

Symptoms	*A.hydrophila* No(%)	*Aeromonas* spp No(%)	Total No(%)
Diarrhoeal	12(80)	3(20)	5(100)
Asymptomatic	26(68.4)	12(31.6)	8(100)
Total	38(71.7)	15(28.3)	3(100)

Discussion

The role of Aeromonads as significant diarrheal diseases agent is controversial. These organisms have been epidemiologically linked to acute diarrhea in a controlled study (17) but not in another study (18). In an oral-challenge study performed as for *A.hydrophila*, it failed to show significant diarrhea in adult volunteers (19). On the other hand, there are several case reports that support a role for aeromonads in the etiology of diarrheal diseases. Diarrheal diseases in certain individuals have been associated with excretion of organisms as pure or predominant cultures, serlogical responses to the organisms, and resolution of symptoms and pathology with the disappearance of the organisms from the stool (20).

In the current study 3.4% of population was found to be infected with *A.hydrophila* and *Aeromonas*.spp isolates, and it was found that *A.hydrophila* could be isolated from diarrheal as well as asymptomatic persons, whereas for *Aeromonas* spp the corresponding rates were 15% and 0.8% respectively. In a case-control study in children less than 10 years old, *Aeromonas* spp. were isolated from 14 cases (4.5%) out of which one isolate was *A.hydrophila* (21). Our study revealed a significant association

between diarrhea and *A.hydrophila,* which confirmed the findings in the other studies (21-22). Our study showed that *A.hydrophila* is an important cause of gasterointeritis in Ilam. Similar to other studies in Nigeria (23), Saudi Arabia (11) and Oklahoma City (24) *A.hydrophila* was isolated as the sole enteropathogen from 38 (2.4%) cases, indicating that *A.hydrophila* could be responsible for diarrhea in some cases. On the other hand, other studies have showed a high prevalence of mixed infections of *A.hydrophila* with the other pathogens (25). Since aeromonads are present in food samples comprising of fish (12), milk, ice creams and water (22), it can be argued that they are mere passengers in the intestinal tract. The increasing presence of *A. hydrophila* in fish may become a potential human health hazard (26). A direct link between the patient and the incriminated source of infection was suspected in a patient with severe *Aeromonas* infection after seafood shucking, however, the identity of the two isolates involved was not confirmed, but in the other study an *A.hydrophila* indistinguishible from the patient's isolate was found in a shrimp cocktail consumed by the patient (4).

A striking feature of the present study was the seasonality of isolations of *Aeromonas* species with a sharp peak in winter (Data are not shown). This may be related to the climate and warm temperature condition in these areas in winter, as well as to the methods for isolating the organism. Since people in winter uses seafoods including fish in some parts of the province, especially in rural areas, *Aeromonas* spp were isolated most frequently in rural areas during that period. In the United States it has been showed that isolation of *Aeromonas* spp. from feces is more frequent in summer and parallels isolation of *Aeromonas* spp from tap water (27). On the other hand, gasterointestinal infections of *Aeromonas* spp are generally considered waterborne, for this reason *A.hydrophila* has been placed on the USA protection Agency Contaminant Candidate List of emerging pathogens in drinking water (28). In conclusion, our epidemiological study showed that *Aeromonas* spp as a sole enteropathogen could be responsible for diarrhea, however further epidemiological studies from other the regions of Iran are required to establish the exact role of Aeromonads isolates in diarrhea.

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