

Original article

Aeromonas-Associated Diarrhea in Istanbul: A Comparison of Two Different Eras in the Past 16 Years

(İstanbul ilinde *Aeromonas* ilişkili diyare: Son 16 yıl içinde iki farklı dönemin karşılaştırılması)Yavuz FURUNCUOĞLU¹, Melih BAŞAR¹, Emine ER², Hakan GÜVELİ³, Arif YÖNEM⁴,
Alaattin ÖZTÜRK⁵, Recep ÖZTÜRK⁶,¹ Medicalpark Göztepe Hospital Internal Medicine, İSTANBUL² Mihrimah Sultan Medical Center Microbiology, İSTANBUL³ Medicalpark Göztepe Hospital Gastroenterology, İSTANBUL⁴ Gülhane Military Medical Faculty Endocrinology, İSTANBUL⁵ Fatih University Sema Hospital General Surgery, İSTANBUL⁶ İstanbul University Cerrahpaşa Medical Faculty Infectious Disease and Clinical Microbiology, İSTANBUL**ÖZET**

Amaç: 1997'den önce İstanbul'da *Aeromonas* pek çok ishalden sorumlu idi. Bu tarihten sonra bu gram negatif bakteri nadiren görülür oldu. *Aeromonas*'a bağlı ishal sıklığındaki değişiklikleri ve muhtemel sebeplerini araştırdık.

Materyal ve Metot: 1994-1997 ve 2003-2008 tarihleri arasında ishali olan ardışık 553 hasta ve 736 dışkı örneği geriye dönük olarak farklı yaş gruplarına göre *Aeromonas* türleri varlığı açısından incelendi.

Bulgular: 1994-1997 arasında 35 vakada (35/147, %26,5), 2003-2008 arasında 2 vakada (2/205, %0,97) etken bakteri olarak *Aeromonas* türleri izole edildi.

Sonuçlar: *Aeromonas* türlerinin sebep olduğu ishal vakalarındaki bu azalma muhtemelen Sağlık Bakanlığının 1997'de İstanbul bölgesindeki yetersiz hijyenli içme suyu satan açık su satış depolarının kapatılmasının sonucudur.

Anahtar Kelimeler: *Aeromonas*; ishal; içme suyu

ABSTRACT

Background: Before 1997, *Aeromonas* spp. were responsible from many diarrhea cases in Istanbul. After that time, this gram-negative bacteria was encountered in seldom cases. We investigated the change in frequency of *Aeromonas*-associated diarrhea and its possible causes.

Material and Methods: Between 1994 and 1997 and between 2003 and 2008, fecal samples of 553 and 736 consecutive patients with acute diarrhea, respectively, belonging to different age groups were retrospectively investigated for the presence of *Aeromonas* spp.

Results: *Aeromonas* spp. were isolated as the causative bacteria in 35 cases (35/147, 26.5%) from 1994 to 1997 and in 2 cases (2/205, 0.97%) from 2003 to 2008.

Conclusions: We conclude that the decrease in acute diarrhea caused by *Aeromonas* spp. most probably was the result of the Health Minister's ban in 1997 of open drinking water sales depots, which were delivering inadequately sanitized drinking water to residential areas in Istanbul.

Key Words: *Aeromonas*; diarrhea; drinking water

INTRODUCTION

Aeromonas spp. are gram-negative facultative anaerobes that are oxidase-and catalase-positive, mostly motile, and non-sporing¹⁻³. Formerly, they were classified as members of the Vibrionaceae family, but after molecular studies, these microorganisms were accepted as a new family called Aeromonadaceae²⁻⁶. They are 1.1-1.4/0.4-1 µm in diameter, and most are motile with one polar flagellum; *A. salmonicida* and *A. media* (mesophilic spp.) are non-motile. *Aeromonas* spp. produces acid and gases by fermenting glucose and other carbohydrates.

They express different types of hydrolytic enzymes. Some species grow at 10-40 °C, making them virulent to humans, but in general, their optimal growth temperature is 22-28 °C.

Aeromonas spp. can grow in 0-4% NaCl media but can not grow in hypertonic (6%) NaCl medium. They are resistant to 2,4-diamino-6,7-diisopropyl pteridine (0/129)¹⁻⁹.

The natural habitats for *Aeromonas* spp. are aquatic bodies, including stagnant water and streams, sewage, brackish coastal waters, and chlorinated spring water. *Aeromonas* spp. are found in soil, green vegetables, raw milk, preserved meat, and seafood¹⁻⁷. They may infect fish, frogs, and reptiles¹⁻⁵. Some species, such as *A. hydrophila*, *A. sobria*, *A. caviae*, *A. schubertii*, *A. veronii*, *A. jandaei*, and *A. trota*, have been shown to be associated with human infections^{1-6,9}.

Correspondence:

Yavuz FURUNCUOĞLU, M.D.
Medicalpark Göztepe Hastanesi Internal Medicine, İstanbul
e-mail: dryavuzf@yahoo.com
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Aeromonas spp. may cause acute diarrhea; wound and soft tissue infections; bacterial meningitis; sepsis; hepatobiliary system, urogenital tract, eye and ear infections; endocarditis; osteomyelitis; and pneumonia in humans.

Aeromonas spp. express important virulence factors, including several toxins and enzymes such as enterotoxins, cytotoxins, hemagglutinins, invasive factors, surface adhesins, proteases and elastases. *Aeromonas* spp. have been found to be associated with nosocomial infections and outbreaks of diarrhea in infant nurseries^{2-6,10-12}.

MATERIAL AND METHODS

In this study, the stool culture results of patients who presented to our clinics in Istanbul, Turkey, with acute diarrhea were analyzed and retrospectively compared for two different time periods, from 1994 to 1997 and from 2003 to 2008. We recorded the age and sex of the patients and the types of isolated bacteria. Stool samples for 553 acute diarrhea cases between 1994 and 1997 and for 736 acute diarrhea cases between 2003 and 2008 were investigated. For the first time period, we tallied the number of culture positive for each bacterial species, but because we observed a high *Aeromonas* frequency, in the second time period, only *Aeromonas*-positive cultures were taken into consideration and all other cultures positive for bacteria were excluded. The analysis of the stool samples consisted of both macroscopic and microscopic examinations. Investigation for parasites was performed using routine physiologic saline and Lugol's solution. Examination of stool specimens for the presence of leukocytes and erythrocytes was also performed. Fecal samples were prepared for Gram's staining. A sample was prepared with 1 g of stool and 3-4 ml of physiologic saline. The sample was used to inoculate GN Broth Hajna (Biolife) agar, APS agar, APS agar with ampicillin, MacConkey agar, and SS agar. After one night of incubation, colonies obtained from GN Broth Hajna agar were transferred onto SS (Oxoid) agar, MacConkey (Difco) agar and ONOZ (Merck) agar. Colonies from APS agar were transferred onto TCBS (Oxoid) agar, and colonies from APS agar with ampicillin were transferred onto sheep blood agar (Columbia blood agar EH, Difco).

Colonies were filtered through a 0.45 µm membrane and used to inoculate sheep blood agar to isolate *Campylobacter* spp. The sheep blood agar plates were incubated with a *Campylobacter* BR 56 (Oxoid) gas pack, which favors the growth of *Campylobacter* spp. After 24 hours of incubation, pathologic bacteria species were identified by

classical methods.

RESULTS

A total of 147 different pathogenic species were isolated from 553 acute diarrhea cases (26.5%) between 1994 and 1997. *Aeromonas* spp. were responsible for 35 cases (6.3%). Other identified pathogens were *Shigella* (64 cases, 11.5%), *salmonella* (24 cases, 4.3%), *campylobacter* (13 cases, 2.3%), Enteropathogenic *E. Coli* (7 cases, 1.2%), *Vibrio* (3 cases, 0.5%) and *candida* (1 case, 0.1%). Fifteen cases were infants, 12 cases were between 1 and 12 years old, and 8 cases were older than 12 years. In contrast, between 2003 and 2008, among 736 acute diarrhea cases only 2 were positive for *Aeromonas* (0.27%). A total of 205 different pathogenic species were isolated from the 736 acute diarrhea cases (27.8%) between 2003 and 2008. *Aeromonas* spp. were responsible for 2 cases (0.27%). Other identified pathogens included *Shigella* (97 cases, 13.1%), *salmonella* (44 cases, 5.9%), *campylobacter* (28 cases, 3.8%), Enteropathogenic *E.Coli* (26 cases, 3.5%), *Vibrio* (5 cases, 0.67%) and *candida* (3 cases, 0.4%) (Table 1). One patient was an infant, and the other one was over 12 years of age (Table 2). During this period, the total number of *Aeromonas* related cases of acute diarrhea markedly declined in our clinics and in Istanbul.

Table 1. The isolated Pathogenic Species

Years	1994-1997	2003-2008
Acute diarrhea cases no	553 (100)	736 (100)
Isolated bacterias no	147 (26.5%)	205 (27.8%)
<i>Aeromonas</i>	35 (6.3%)	2 (0.27%)
<i>Shigella</i>	64 (11.5%)	97 (13.1%)
<i>Salmonella</i>	24 (4.3%)	44 (5.9%)
<i>Campylobacter</i>	13 (2.3%)	28 (3.8%)
Enteropathogenic <i>E.Coli</i>	7 (1.2%)	26 (3.5%)
<i>Vibrio</i>	3 (0.5%)	5 (0.67%)
<i>Candida</i>	1(0.1%)	3 (0.4%)

Table 2. Age distribution of *Aeromonas*-Associated Diarrhea Cases in İstanbul

Age	1994-1997	2003-2008
<1 years, no.	15	1
Between 1-12 years, no.	12	-
>12 years, no.	8	1

DISCUSSION

Diarrhea associated with *Aeromonas* species often results from inappropriately sanitized drinking water. Acute self-limited diarrhea is common in children. Among elderly individuals, chronic enterocolitis is sometimes observed even if they do not have any predisposing factors. Fever, vomiting, and leukocytes and/or erythrocytes in the

feces are some common features of *Aeromonas*-associated diarrhea. Such cases are sometimes misdiagnosed as ulcerative colitis^{4-6,13-15}.

Aeromonas-associated diarrhea cases, as well as carrier states, have been reported frequently in different parts of the world. According to studies from various parts of the world, *Aeromonas* spp. were found to be responsible for 2.26% of all diarrhea cases in Nigeria, 2.1% of cases in the northern parts of India, 2.4% of cases in Peru, 0.6% of cases in Netherlands and 10% of cases in Australia. *Aeromonas* spp. were found in non-diarrheic control groups at frequencies of 0.4% in Nigeria, 0% in the northern parts of India and 0% in Australia^{4-6,13-16}. *Aeromonas* spp. are isolated in 9-30% of cases with or without diarrhea in Thailand. These microorganisms are isolated in 38-48% of individuals with traveler's diarrhea and in 9-15% of symptom-free individuals in Thailand².

The distribution of *Aeromonas* spp. varies in different geographic regions. *A. hydrophila* and *A. sobria* are common in Australia, Thailand and Bangladesh. *A. caviae* is common in Europe and USA^{3-4,9}. In 1994 in Turkey, Öztürk et al. isolated 578 pathogenic bacteria from 1890 diarrhea cases, of which 51 cases (2.7%) were caused by *Aeromonas* spp¹⁷.

In our study, we retrospectively compared two different time intervals of *Aeromonas*-associated diarrhea in patients visiting our clinics in Istanbul, Turkey. Between 1994 and 1997, while non-

properly sanitized open drinking water sales depots were open and legally selling drinking water to houses, *Aeromonas*-positive stool cultures for diarrhea cases were frequent (6.3%).

In 1997, a ban against open drinking water sales was declared by the Ministry of Health¹⁸.

Only polycarbonate packaged, properly sanitized drinking water was allowed to be sold after that time. During our second time interval, between 2003 and 2008, there were only 2 *Aeromonas*-associated diarrhea cases out of 736 cases of diarrhea seen at our hospital (0.27%). In addition, there was a decrease in the total number of diarrhea cases after the ban.

Aeromonas spp. was the second most often identified micro-organism causing acute diarrhea after *Shigella* spp., and the incidence of *Aeromonas*-associated diarrhea has been affected by sanitation standards and social needs. After the improvement of the standards for drinking water delivery to residential areas, there were almost no *Aeromonas*-associated diarrhea cases. In areas with inadequate drinking water sanitation and delivery, *Aeromonas* spp. must be kept in mind as a causative agent of diarrhea. With proper drinking water sales regulations in all areas, *Aeromonas* will not be an important cause of acute diarrhea.

Conflict of interest statement none declared.

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