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Low Prevalence of *Aeromonas hydrophilain* Infectious Diarrhea Samples of Pediatric Patients in Arak, Iran

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Abstract

Background: *Aeromonashydrophila (A. hydrophila)* is an aquatic bacterium that can cause a spectrum of infectious diseases, including both gastrointestinal and extraintestinal infections. Due to the high rate of diarrheal infections in pediatric patients in central Iran, this study was designed to determine the frequency of *A. hydrophila* in diarrhea samples from children in this region.

Methods: In this descriptive cross-sectional study, diarrheal stool specimens were collected from 200 children admitted between February and October of 2015 to educational and medical centers affiliated with the Arak University of Medical Sciences. The samples were analyzed both phenotypically by culture and genotypically by the polymerase chain reaction (PCR).

Results: *A. hydrophila* was isolated from two of the 200 samples tested (1%). The presence of bacterial genetic markers further confirmed the diagnosis.

Conclusions: Based on this study, *A. hydrophila*is not highly prevalent in children with diarrhea in Arak; however clinical diagnostic laboratory personnel should be aware of the possible presence of *A.hydrophila* in children with diarrhea as it can cause dangerous health problems in both them and young adolescents.

Keywords: *Aeromonas hydrophila*, Diarrhea, Frequency, Iran, Pediatrics

Introduction

Members of the genus *Aeromonas*are Gram-negative, rod-shaped, non-spore-forming facultative anaerobes that are widely distributed in aquatic environments and found in most parts of the world (1). Although *Aeromonas* was initially positioned in the family *Vibrionaceae*, successive phylogenetic analyses found that the genus *Aeromonas* is not closely related to vibrios. Therefore, the family *Aeromonas* was separated from the family *Vibrionaceae*, and was considered as a new family, the *Aeromonadaceae* (2). *Aeromonas hydrophila* causes both gastrointestinal and extra-intestinal infections. These extra-intestinal infections include cellulitis, septicemia, and wound, urinary tract, hepatobiliary, and ear infections (3). Symptoms of gastrointestinal infections range from watery to dysenteric, or bloody, diarrhea (4). Currently, the rate of diarrheal infections in pediatric patients is high in Arak, which is located in central Iran, and most routine stool cultures are not able to identify the causative agent(s). Therefore, this study was designed to determine the frequency of *A. hydrophila* in pediatric diarrhea patients in Arak.

Materials and Methods

Sample Collection

In this descriptive cross-sectional study, infectious diarrheal stool specimens were collected from 200

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children admitted between February and October of 2015 to educational and medical centers affiliated with the Arak University of Medical Sciences (Amir Kabir). None of the children had taken antibiotics for at least one week before entering the study. The study questionnaire was completed after obtaining written consent from the patients or their parents or guardian. The study protocol (no. 2137) was approved by the Ethical Committee of Arak University of Medical Sciences.

Culture and Bacterial identification

The diarrheal stool specimens containing more than four white blood cells (WBCs) in wet mount were directly cultured on MacConkey agar culture medium and sheep blood agar (Merck, Germany) containing 1000 µg/dl of ampicillin (Merck, Germany) after enrichment for one day in alkaline peptone water (Merck, Germany)according to the method of Gracey et. al. (5). After overnight incubation at 37 °C, differential tests including oxidase, triple sugar iron agar (TSI), sulfur indole motility (SIM), citrate, lysine decarboxylase, urea agar, methyl red (MR), Voges-Proskauer (VP), and hemolysis examination (Merck, Germany) and the analytical profile index (API)-20E tests (Biomerieux, France) were conducted on suspected colonies.

A previously identified and sequenced *Aeromonas*isolate, available in the microbiology collection of the Arak University of Medical Sciences, was used as a positive control for phenotypic tests and identification of isolates.

Extracting DNA and PCR

Five ml of each diarrheal specimen were added to phosphate-buffered saline (PBS) culture medium and stored at -20 °C.

DNA was extracted from stool according to the QIAmp DNA stool minikit manual (Qiagen, Valencia, CA) and concentrations of the DNAs extracted from the samples were measured using a NanoDrop 2000 system (Thermo Scientific). The presence of bacterial DNA was confirmed with a universal bacterial *16srRNA* primer.

The aerolysin gene was amplified by the polymerase chain reaction (PCR) using *Aer* primers (Table 1). The presence of aerolysin confirmed the isolates as *Aeromonas*.

Table 1. Primers used in this study

Target gene	Primer	Sequence 5'→3'	Expected product size (bp1)	References
Universal DNA bacterial	16s-rRNA-F 16s-rRNA-R	5-AGGAGGTGATCCAACCGCA-3 5-ACCTGGAGGAAGGTGGGGAT-3	367	(6)
Aerolysin	Aero-F Aero-R	5-TGTCGGSGATGACATGGAYGTG-3 5-CCAGTTCCAGTCCCACCACTTCA-3	720	(7)

The 25 µl final volume of the PCR included 12.5 µl of master mix (1X), 0.5 µl of Taq DNA polymerase (2.5 units), 1 µl of each forward and reverse primers (10 Pm), 2 µl of DNA template (5 ng), and 8 µl of double-distilled water (all these were purchased from YektaTajhiz Company, Iran). The PCR program to amplify the *Aer* gene included an initial denaturation at 94 °C for 2 min, 35 cycles of 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 2.5 min, and a final elongation step at 72 °C for 10 min.

The PCR products were sequenced by PouyaGostar Gene Company, Iran.

Results

Of the 200 samples tested, *A. hydrophila* was isolated from two samples by phenotypic methods based on the API 20E tests and the findings that they were lactose-negative and oxidase-positive. Also, results of the differential tests including beta hemolysis, acid/acid, gas-positive, growth in 6.5% NaCl-negative, citrate-negative, lysine decarboxylase-positive, urea-negative, motion-positive, indole-positive, DNase-positive, MR-negative, VP-positive and O129 disk-resistance, confirmed these isolates were *A. hydrophila*.

PCR products of 720 bp confirmed the presence of *Aeromonas* in the two samples (Fig. 1).

The two *A. hydrophila*-positive samples were negative by culture and PCR for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and pathogenic *E. coli* as negative controls.

One of the samples containing *A. hydrophila* was obtained from a 10-month-old girl who had been on a trip in rural area one week before the onset of symptoms and the other was from a 10-year-old girl who had not traveled. Examination of their stools showed a low concentration of WBCs in both. In addition, abdominal pain, mild dehydration, and intense abdominal cramps were observed in both patients.

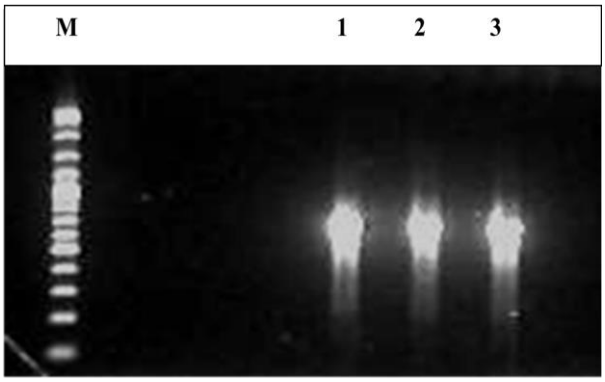

Fig. 1. Electrophoresis of the Polymerase Chain Reaction Product of the Aer Gene. Product size of the *Aer* gene was 720 bp. M: marker 1 kb (Fermentas), 1, 2 and 3 are PCR products of 3 isolates.

Discussion

In the present study, with the aim of determining the frequency of *A. hydrophila* in infectious diarrheal stool specimens of children, *A. hydrophila* was isolated from two of 200 samples using phenotypic and genotypic methods.

Aeromonas-induced diarrhea is a contagious disease seen in both industrialized and developing countries in all age groups (3). Holmberg and Farmer found that *Aeromonas* were present in stool specimens of 0.5-16.9% of patients and 0-10% of non-symptomatic individuals (8). Infections by *Aeromonas* species are more common in those with impaired immune systems, especially infants and the elderly, than in immunocompetent individuals (9). In several studies reported from different parts of the world, *Aeromonas*species were isolated from 0.6-7.2% of infants and children (10). *Aeromonas* has been found in 1-88% and 0-45% of children with diarrhea and healthy controls, respectively (6). Some studies have shown a statistically significant difference between the percentage of *A. hydrophila*-infected diarrheal patients and controls, while in others no difference was seen (6). In fact, studies performed in different parts of the same country have reported different results. However, it should be noted that these studies were performed in different towns and at different times of the year (11).

In our study, the frequency of *A. hydrophila* infections in diarrheal stool specimens from children admitted to the pediatric hospital of Arak University of Medical Sciences was 1%. This frequency is similar to the *Aeromonas* infection rates found in studies conducted in Malaysia (0.62%), Nigeria (1%), and the U.S. (1.1%), and a little less than the rates reported in Spain, Sweden and Israel (2%), and Saudi Arabia (2.5%) (9, 10, 12-16).

In a study by SoltanDallal in Tehran, Iran, the frequency of *Aeromonas* species isolated from children under 10 during a 10-month period was 4.5% (17). In another study by Aslani and Alikhani in Ilam, Iran, 3.4% of samples were *Aeromonas* (hydrophila)-positive (18). In other studies, the frequencies of *Aeromonas* were reported as 11.8% in Venezuela, 15% in Vietnam and Libya, 9% in Kuba, 9.7% in India, 9.2% in Bangladesh, 5.9% in China, 4.8% in Switzerland, 5.6% in Japan, and 88% in Egypt (17-28).

The low frequency of *Aeromonas* in the present study could be due to different geographical factors has been previously investigated by Ljungh et al. (29). The effect of geography on the frequency of *Aeromonas* has been confirmed by Burk et al. (30). As the natural habitat of *Aeromonas* is aquatic environments, organisms may enter the digestive system through consumption of contaminated water and food. This could explain the low percentage of *Aeromonas*infections in this study, which included children mainly from urban areas (12). The number of *Aeromonas* cases associated with gastroenteritis rises during summer, which is related to the number of *Aeromonas* in water systems (31). *Aeromonas* infections occur via primary and secondary routes mainly during the hot seasons and most of the available data show

that most isolates were acquired from contaminated drinks (3).

This study was performed during the hot season in Arak, a time when *Aeromonas* infection rates would be predicted to be high. The low infection rate we found may be due to differences between hosts, geography, weather conditions, culture conditions, and/or transmission factors (32).

Based on the low percentage of *A. hydrophila* infections found in this study, we conclude that *A. hydrophila* plays only a minor role in causing diarrhea in children in Arak. Despite its low infection rate in our study, we encourage diagnostic laboratory personnel to be aware of the possible presence of *A. hydrophila* in children with diarrhea as it can cause dangerous health problems in both them and young adolescents.

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