

## ISOLATION OF AEROMONAS SPECIES FROM DIARRHEAL PATIENTS IN ISMAILIA GOVERNORATE

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من اجل معرفة مدى انتشار انواع الايرومونات كمسبب للاسهال  
بين الاطفال في محافظة الاسماعيلية تم تجميع عينة براز من  
اطفال اقل من سنوات المترددين على الوحدة الصحية بالكيلو  
والذين يعانون من اسهال حاد وذلك في الفترة من مايو  
سبتمبر . كما تم عينة براز من أطفال أصحاء من  
أعمار وأجناس متناظرة كمجموعة ضابطة. وجد أن عينة ايجابية  
لانواع الايرومونات بنسبة % من عدد الحالات. ولم يتم فصل  
الايرومونات في اى عينة من المجموعة الضابطة. كذلك وجد ان معظم  
الحالات الايجابية كانت مصحوبة بارتفاع درجة الحرارة والقيء.  
وأظهرت نتائج اختبارات المضادات الحيوية ان انواع الايرومونات  
المعزولة حساسة لمعظم انواع المضادات الحيوية موضع الاختبار.

*In order to know the prevalence of Aeromonas species as a  
causative agent of diarrhea in Ismailia, Three hundreds and fifty  
stool samples and rectal swabs were collected from infants and  
children under the age of 5 years visiting El Kilo11Clinic suffering  
from acute diarrhea in the period from May 2004 to September  
2004. A stool samples were collected from control group of fifty  
healthy infants and children of matched age and sex. It was found  
that 29 diarrheal samples were positive for Aeromonas species with  
prevalence of 8.28%. None of control samples were positive for  
Aeromonas. In most cases it was found that the infection is water or*

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food borne. *Aeromonas* strains show high degree of antibiotic susceptibility.

## INTRODUCTION

The genus *Aeromonas* is a gram negative oxidase positive micro organism and it was a member of the family Vibrionaceae which included *vibrio* and *Plesiomonas* species.<sup>1</sup> Its separation from the genera *Vibrio* and *Plesiomonas* depends on resistance to the vibriostatic compound O/129, no growth on sodium chloride and absence of Ornithine decarboxylase except in *A. veronii* biovar *veronii*.<sup>1</sup> Now it creates a new family *Aeromonaceae*.

Some strains of *Aeromonas* bacteria (aeromonads) are a significant, yet often underrated, cause of gastroenteritis, particularly in children under 5 years old and older persons in the summer.<sup>2&3</sup> They can also cause life-threatening infections, such as septicemia and meningitis in immunocompromized individuals.<sup>4&5</sup>

*Aeromonas* are widely distributed in nature and can cause diseases in fish, reptiles, and amphibians.<sup>6</sup>

The *Aeromonas* species have been isolated from natural mineral water and well water.<sup>7</sup>

In one study in Chennai, two hundred stool samples were taken from children younger than 10 year old having diarrhea, *Aeromonas* was isolated from 6.5% of samples.<sup>8</sup>

In another study in Bangladesh *Aeromonas* was isolated from 5.5% of cases of acute diarrhea among

children.<sup>9</sup> And of 130 diarrheic patients, *Aeromonas* species were isolated in nine patients (6.9%) in Hong Kong as reported by Siu-Wa and Cheung.<sup>10</sup>

*Aeromonas* species involved in travellers diarrhea as reported by Vila, *et al.*<sup>11</sup> who determined the prevalence of *Aeromonas* species associated with travelers diarrhea and found that *Aeromonas* species were isolated from (2%) of travelers in Spain.

There are various subspecies, including *Aeromonas Salmonicida*, a fish pathogen,<sup>6</sup> and *Aeromonas hydrophila*, a human pathogen.<sup>1</sup> Approximately 85% of diarrhea-associated with *Aeromonas*, isolates belong to the species *Aeromonas hydrophila* (HG1 and HG3), *A. veronii* biovar *sobria* (HG8/10) (formerly *A. sobria*), and *A. caviae* (HG4).<sup>1</sup> However, it is not yet possible to identify virulent strains definitively. The *alt* and *act* genes, which encode heat-labile cytotoxic and cytotoxic enterotoxins respectively, seem to be related strongly to the diarrhea associated with *Aeromonas* and they found in 71.9 and 20.1% of strains respectively, examined in one study.<sup>3</sup> The infection is strongly associated with drinking untreated water.<sup>7</sup>

Patients usually presented with a spectrum of diarrhea from mild to

sever abdominal cramps, vomiting, and fever. Blood in stool can be seen as well.<sup>12</sup> In some cases the symptoms are sever enough to required intravenous fluid therapy and observation and persistant diarrhea is uncommon as reported by Siu-Wa and Cheung.<sup>10</sup>

The highest proportion of *Aeromonas* diarrhea was found in younger children, particularly in those under 3 years of age.<sup>9</sup>

Flouroquinolone are the drug of choice for the treatment of *Aeromonas* induced infection but it should not be used in treating pediatric patients.<sup>13</sup> Alternative therapies include trimethoprim-sulfamethoxazole, aminoglycosides, imipenem, meropenem, parenteral broad spectrum cephalosporins, and tetracyclines.<sup>14&15</sup>

The main objective of this study is to determine the prevalence of *Aeromonas* species as a diarrheal cause among children in Ismailia and their antibiotic susceptibility.

## MATERIALS AND METHODS

### Patients

350 infants and children under the age of 5 years with the following criteria:

- 1- Acute diarrhea (defined as three or more stools a day and lasting less than 14 days).
- 2- Bloody or watery diarrhea.
- 3- Children not received antibiotic 2 days before sample collection.
- 4- May have one of the following symptoms or all: fever, vomiting, and abdominal cramps.

Control group of 50 healthy infants and children of matched age and sex. Control group had not taken antibiotic during the previous 2 weeks of samples collection.

### Microbiological media

- 1- Alkaline peptone water PH 8.6.
- 2- Ampicillin blood agar (30 microgram/liter).
- 3- Cefzolidine-Irgasan-Novobiocine (CIN) agar.
- 4- *Aeromonas* selective agar.
- 5- Muller Hinton agar.

### Microbiological tests

Stool samples were enriched on alkaline peptone water overnight at 37° and were streaked on Ampicillin blood agar, CIN agar and *Aeromonas* selective agar for 24 hour at 37°. Presumptive *Aeromonas* isolates were screened for standard phenotypic traits ( -haemolysis on blood agar, oxidase positive and indol positive) and species identity was determined using API-20E identification system.

### Antibiotic Susceptibility

Antibiotic susceptibility patterns were determined by the disk diffusion method on Muller Hinton agar using this antibiotic which supplied from (**Oxoid**); Ampicillin 10µg (AMP), Ciprofloxacin 5µg (CIP), Ofloxacin 10 µg (OFX), Chloramphenicol 30 µg (C), Ceftriaxone 30 µg (CRO), Cefepime 30 µg (FEP), Sulfamethoxazole / Trimethoprim 25 µg (SXT). The susceptibility of the strains was determined according to the size of inhibition zone.<sup>16</sup>

## RESULTS AND DISCUSSION

### Culture and isolation

Culture for *Aeromonas* species were performed on 350 diarrheic stool specimens. Twenty nine samples were found to be positive for *Aeromonas* species of them 8 strains identified as *A. hydrophila*, 8 strains identified as *A. caviae*, and 13 strains identified as *A. sobria*. None of the control group samples was positive to *Aeromonas* species. The isolation rate in this study was 8.28% among infants and children visiting El Kilo 11 Clinic in Ismailia Governorate as shown in Table 1.

Isolation rates of *Aeromonas* from diarrheal stool vary, ranging from a low rate of 0.7-0.9% in the Netherlands and France, to medium rate of 2.5-7.1% in the United States, 5.5% Among Bangladeshi children, 6.5% in Chennai, 11% in Japan, 24% in Ivory Coast, and to a high rate of 50% in infants in Peru as reported by Komathi *et al.*,<sup>8</sup> Teka *et al.*<sup>9</sup> and Pazzaglia, *et al.*<sup>17</sup>

In agreement with Holeberg *et al.*<sup>2</sup> and Albert *et al.*<sup>3</sup> the median age of the patients' population was 19 months (range from 3 months to 5 years). Of whom 59% females and 41% males.

Most of *Aeromonas* associated gastroenteritis is food or water borne. The clinical symptoms in *Aeromonas*-associated diarrhea were fever in 26 cases out of 29 positive cases (89.6%), vomiting in 18 cases out of 29 positive cases (62%), abdominal cramps in 3 cases out of 29 positive cases (10.3%), and bloody diarrhea

were seen in 2 cases only (6.9%) as shown in Figure 1. Numbers of attacks of diarrhea per day were varied among cases between 2 times to 20 times.

The distribution of *Aeromonas* cases on summer months were as follow: May, 2 cases out of 51 cases (3.9%), June, 5 cases out of 86 cases (5.8%), July, 10 cases out of 94 cases (10.6%), August, 7 cases out of 67 cases (10.4%), and September, 5 cases out of 52 cases (9.6%) as shown in Figure 2. It was noticed that *Aeromonas*-associated diarrhea was most common in the peak of July to September, this may be explained by the peak of hot humid weather.

### Antibiotic susceptibility results

The antibiotic susceptibility of the isolated *Aeromonas* strains to ciprofloxacin and ofloxacin was 96.6%. Susceptibility to cefepime and ceftriaxone was 93.1% and 93.1% respectively as shown in Figure 3.

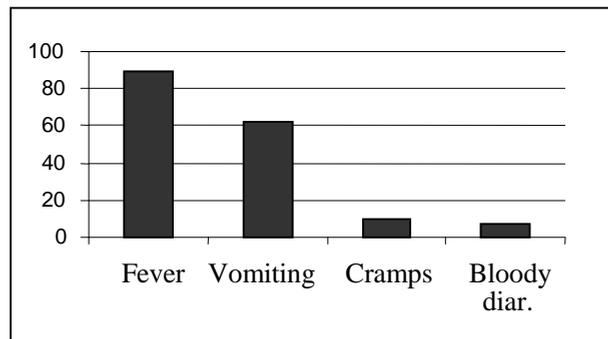
The susceptibility to chloramphenicol and sulfamethoxazole was 63% and 55.5% respectively. One strain of *Aeromonas* was sensitive to ampicillin.

*Aeromonas* showed a high degree of antibiotic susceptibility that agreed with previous reports.<sup>10,13&15</sup>

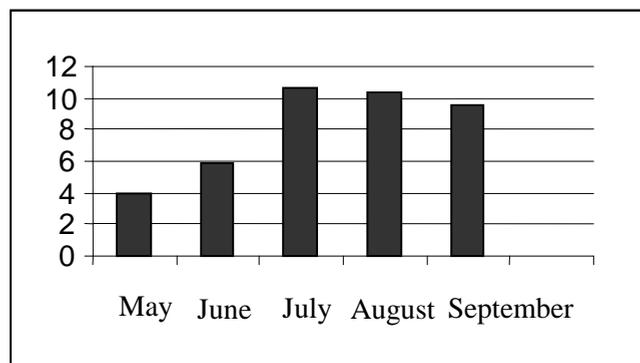
The susceptibility of chloramphenicol and sulfamethoxazole-trimethoprim was 63% and 55.5% that indicate increase the resistance to these antibiotic that agreed with Ko *et al.*<sup>14</sup> Broad spectrum cephalosporins such as cefepime and ceftriaxone

**Table 1:** Prevalence of *Aeromonas* among diarrheal samples and control samples.

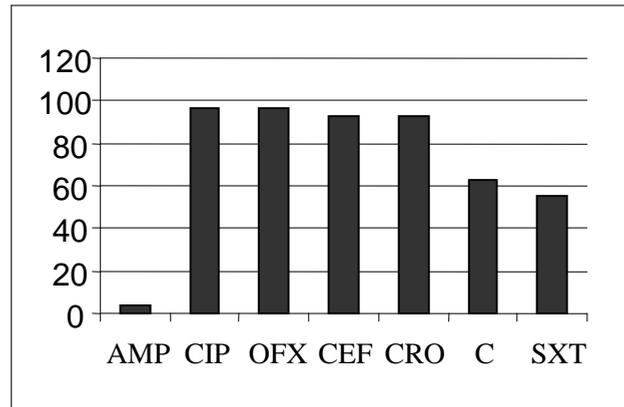
	Positive cases	Percentage	Negative cases	Percentage	Total
Diarrheal samples	29	8.28 %	321	91.72%	350
Control samples	0	0 %	50	100%	50



**Fig. 1:** Clinical symptoms of *Aeromonas* associated diarrhea



**Fig. 2:** The distribution of *Aeromonas* cases on summer months



**Fig. 3:** Antibiotic susceptibility of the isolated *Aeromonas* strains

have been reported to be active against *Aeromonas* strains and it can be used as alternative therapy to quinolones.

The resistance to Ampicillin may be considered as a character to *Aeromonas* species as reported by many authors.<sup>13&14</sup> It was 96.6% in this study.

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