Journal of Al Azhar University-Gaza (Natural Sciences), 2011, 13 : 69-82

Yersinia enterocolitica and Aeromonas hydrophila in Clinical, Food and Environmental Samples in Gaza Strip, PNA

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Received 18/07/2011 Accepted 05/10/2011

Abstract: The interest of the occurrence of *Yersinia enterocolitica* and *Aeromonas hydrophila,* their pathogenicity and antimicrobial resistance is increasing worldwide because both were linked to acute and chronic gastroenteritis, septicemia and wound infections. Though reports on the occurrence of both pathogens among human are available all over the world, no published data are available from Gaza strip. Moreover, there is no routine testing for the detection of *Yersinia* and *Aeromonas* in clinical or environmental samples. This study was conducted to investigate the occurrence, sources of both *Y. enterocolitica* and *A. hydrophila* in clinical, food and environmental samples.

This study examined 300 diarrheal stools to investigate the presence of *Y. enterocolitica* and *A. hydrophila* in clinical samples. 95 food samples and 84 environmental samples were also tested. Conventional isolation technique was employed. All suspected *Y. enterocolitica* and *A. hydrophila* were identified using conventional microbiological techniques. Of the 479 tested samples, 30 (6.2%) were positive for *Y. enterocolitica* and 179 (37.4%) for *A. hydrophila.* The overall incidence of *Y. enterocolitica* and *A. hydrophila* in clinical samples was 4.7% and 34.3% respectively, with high frequency of both pathogens in AL-Dorrah and AL-Nasser hospitals.

Yersinia species were isolated from all sampled sources except seawater. The highest incidence was from sewage (19.1%) followed by animal excreta (11.5%), while, clinical samples showed the lowest percentage (4.7%). *A. hydrophila* was also isolated from all sampled sources, whereas meat and water showed the highest incidence (48.9% and 46.9% respectively).

Keywords: *Y. enterocolitica, A. hydrophila, KOH treatment, Gaza strip, Palestine*

Introduction

Yersinia enterocolitica and *Aeromonas hydrophila* are Gram-negative, facultative anaerobic bacteria that can be isolated from many sources, such as food, drinking water, sewage, environmental water and human clinical samples with a world-wide distribution. These bacteria can

develop in refrigeration temperatures and are responsible for food and water-borne diseases, that can cause a wide range of human diseases that vary in severity from a self-limiting gastroenteritis to potentially fatal septicemia **[1,2]**.

The genus *Yersinia* comprises an important group of bacterial pathogens, with *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* representing the species of interest. *Y. enterocolitica* is the most common agent of this genus that are associated with a spectrum of clinical syndromes in man, with gastroenteritis as the most frequently encountered manifestation. The organism has been isolated most frequently in temperate areas of the world; the majority of cases being reported from the cooler regions of Europe and North America **[3]**. Most cases are sporadic or occur in small clusters, but large outbreaks have been reported worldwide in families, schools, hospitals, and in association with community gatherings **[4]**.

Although *Y. enterocolitica* has been isolated from a number of environmental, food, and water sources, there have been relatively few documented outbreaks of human illness where food was proved by culture to be the source of infection. According to Ackers *et al.,* (200), the three well-documented outbreaks, contaminated chocolate milk, raw milk, and tofu were the vehicles of transmission. Pasteurized milk was implicated epidemiologically in another outbreak **[5]**.

The genus Aeromonas includes at least 13 species, among which is the motile, mesophilic *A. hydrophila* **[6]**. Seasonal variations in isolation of *Aeromonas* from stools have also been reported, with highest recovery during the warmer months. The mesophilic species have been associated with a wide range of infections in humans, that have been isolated from freshwater, salt water, ground waters, drinking water (chlorinated and unchlorinated drinking water), and have been frequently isolated from various food products, and from patients with diarrhea. Drinking water and food are reservoirs of *A. hydrophila* and therefore may be important sources of human infections, leading to intestinal and non-intestinal diseases. Epidemiological studies implicated *Aeromonas* species in causing water and food-borne outbreaks and traveler's diarrhea **[7]**.

Y. enterocolitica and *A. hydrophila* are important human pathogens

that are increasingly recognized by researchers as a cause of various clinical syndromes **[8,9]**. The presence of *Y. enterocolitica* and *A. hydrophila* in food products is of a special concern since those organisms are capable of growth at refrigerator temperatures. In several countries, *Y. enterocolitica* has eclipsed *Shigella* species and approaches *Salmonella* species and *Campylobacter* species as the cause of acute bacterial gastroenteritis **[3]**.

In the present study, we attempted to isolate *Yersinia* and *Aeromonas* from clinical, food and environmental samples using direct plating on Cefsulodin-irgasan-novobiocin (CIN) agar after KOH treatment.

MATERIALS AND METHODS

Abbreviations: The following abbreviations are used in this paper: PBS, phosphate-buffered saline; TSB, Trypticase soy-broth; CIN, cefsulodin-irgasan-novobiocin; KIA, Kligler iron agar; BHIA, Brain heart infusion agar; BHIB, Brain heart infusion broth; SS, Salmonella Shigella agar; HE, Hektoen enteric; XLD, Xylose lysine deoxycholate agar; MCA, MacConkey agar; KOH, potassium hydroxide.

Media: BHIA, CIN, SS, HE, XLD, TSB, PBS, Blood and MacConkey agar were prepared according to instructions of the manufacturer (Himedia, India).

Samples: (i) **Fecal samples:** Three hundreds diarrheal stools were collected from different hospitals (Kamal oudwan, Al-Shifa, Al-Nasser, Al-Dora, Al-Aqsa, Nasser, Gaza-European and Al-Najar) in sterile containers, (ii) **Sewage samples:** Twenty-six sewage samples were collected from six sources (Biet-lahia and Shiek Ejleen wastewater treatment plants, El-shifa hospital, Gaza European hospital, Al-Aqsa hospital, and Al-Nasser hospital) in sterile 50 ml plastic bottles, (iii) **Animal excreta Samples:** Twenty-six samples were collected from slaughterhouses and houses using sterile bottles, (iv) **Food samples:** Fifty samples from the following materials; meat, turkey, chicken, sausage, ice-cream, cheese and milk samples were purchased from local supermarkets and houses, (v) **Water samples:** Two-liter samples of different water types (tap and well water) were collected in sterile bottles. Tap water was obtained from municipal distribution system in various localities all over Gaza strip. Seawater was taken at a depth of 1.5–2 m near the sewage discharge point of

Gaza wastewater treatment Plant (GWWTP).

Enrichment media: Standard enrichment media which have been described and used previously **[9]**: (i) PBS (pH. 7.6), incubated at 4°C for 14 days; and (ii) TSB, incubated at 25, 30, 32 °C for 24 h. PBS was used for pre-enrichment, and TSB was used for a secondary selective enrichment. All pre-enrichment media were inoculated at a ratio of 25 g of food sample to 100 ml of broth, 25-30 ml of sewage sample to 10-fold volume of broth, 5 g of animal excreta to 20 ml of broth, 1 g of fecal samples to 10-fold volume of broth **[3,9,10]**. Selective enrichments were inoculated with 5.0 ml of pre-enrichment medium per 20 ml of broth.

Isolation Media: All enrichments were streaked onto HE, XLD, CIN agar, incubated at 32°C for about 18 h, SS agar, was incubated at 30°C for about 24 h, while MCA agar was incubated at 25°C for 24 h. 0.5 ml of the PBS enrichment was removed, treated with 4.5 ml KOH, and then streaked onto CIN agar only. The remainder of the PBS homogenate was refrigerated at 4°C and subcultured after 1, 4, 7 and 14 days. 0.1 ml was spread onto CIN agar and the plates were incubated at 32°C for 18 h. In addition, 0.5 ml of the PBS enrichment was removed, treated with KOH, and then streaked onto CIN **[4,8]**.

Identification: Each colonial type present was selected with no fewer than two nor more than four colonies per plate used to individually inoculate KIA slants, Simmon's citrate agar, Christensen's urea agar and were incubated overnight at 28°C. Isolates exhibiting typical reactions "Citrate –ve, urease +ve and (K/A-)" were subjected to 22 additional biochemical tests (API 20E system (BioMerieux) to confirm identification as *Y. enterocolitica* and *A. hydrophila* **[8]**.

RESULTS

Distribution of *Yersinia and Aeromonas* isolates

Yersinia species were isolated from all sampled sources except seawater samples. The highest incidence was from sewage (19.1%) followed by animal excreta (11.5%), while, clinical samples showed the lowest percentage (4.7%). With regard to *A. hydrophila*, meat and water showed the highest incidence (48.9% and 46.9% respectively). The overall frequency of *Yersinia* and *Aeromonas* isolates was 6.3%

and 38.1% respectively (Table 1).

Table (1): Distribution of *Yersinia and Aeromonas* isolates according to sample type.

Sample type	No.	*Yersinia*		*Aeromonas*	
		No.	%	No.	%
Clinical samples	300	14	4.7	103	34.3
Animal excreta	26	3	11.5	10	38.5
Meat	45	3	6.7	22	48.9
Milk	50	3	6	18	36
Sewage	26	5	19.1	11	42.3
Water	32	2	6.25	15	46.9
Total	479	30	6.2	179	37.4

***Yersinia and Aeromonas* from clinical samples:**

Cultures of *Y. enterocolitica* and *A. hydrophila* were performed on 300 diarrheic stool samples. The median age of the patient population was 3.6 years (range 40 days to 47 years) (Table 2).

Table (2): *Yersinia* and *Aeromonas* isolates distributed according to age (N=300)

Age group	Frequency	Percent	*Yersinia*		*Aeromonas*	
			No	%	No.	%
Below 2 years	126	42	5	4.0	31	24.6
2 - less than 6 years	132	44	7	5.3	61	46.2
6 - less than 15 years	28	9.3	1	3.6	8	28.6
Over 15	14	4.7	1	7.1	3	21.4
Total	300	100	14	4.7	103	34.3

From table 2, it can be observed that there are no notable differences among the various age groups with regard to the frequency of *Yersinia* isolates, while, the age group 2-6 showed marked increased frequency of *Aeromonas* isolation when compared to other age groups.

***Y. enterocolitica* and *A. hydrophila* from food samples**

Milk and milk product samples

Of the 50 different milk samples analyzed, 3 were positive for *Y. enterocolitica*, (6.0%) and 18 showed *A. hydrophila* growth (36%). *Y. enterocolitica* was isolated only from cow milk (3 isolates, 16.7%), and the occurrence of *A. hydrophila* was slightly higher in cheese (75%) than in goat milk (66.7%) but rather it is clearly higher than in cow milk (22.2%). There was a significant relationship between number of both isolates and type of dairy product (P< 0.05) (Table 3).

Table (3): Isolation of *Y. enterocolitica* and *A. hydrophila* from milk and milk product

Milk and milk product	Frequency			Isolates	
	No.	%		*Yersinia*	*Aeromonas*
Cow milk	18	36.0	No	3	4
			%	16.7	22.2
Goat milk	6	12.0	No	0	4
			%	0	66.7
Pasteurized milk	5	10.0	No	0	0
			%	0	0
Powdered milk	2	4.0	No	0	0
			%	0	0.0
Ice cream	15	30.0	No	0	7
			%	0	46.7
Cheese	4	8.0	No	0	3
			%	0.0	75
Total	50	100	No	3	18
			%	6	36

P = 0.002

Meat samples

A total of 3 (6.7%) *Y. enterocolitica* isolates were obtained from 45 meat samples. One *Y. enterocolitica* was isolated from 20 cow meat sample and 2 isolates from 7 turkey samples, with no additional isolates from sausage, hamburger, chicken and packed meat samples. On the other hand, 22 *A. hydrophila* isolates were recovered from the same food samples. One *A. hydrophila* was isolated from 6 chicken

samples, 7 from 20 cow samples, 5 from 5 hamburger samples, 2 from 2 packed samples, 2 from 7 turkey samples and 5 from 5 sausage samples. We were able to isolate two *Salmonella* species from these samples (Table 4).

Table (4): Number and percent of *Y. enterocolitica* and *A. hydrophila* recovered from various meat samples

Meat type	Frequency			Isolates	
	No.	%		*Yersinia*	*Aeromonas*
Chicken	6	13.3	NO.	0	1
			%	0.0	16. 7
Cow	20	44.4	NO.	1	7
			%	5.0	35
Hamburger	5	11.1	NO.	0	5
			%	0.0	100
Packed	2	4.4	NO.	0	2
			%	0.0	100
Turkey	7	15.6	NO.	2	2
			%	28.6	28.6
Sausage	5	11.1	NO.	0	5
			%	0.0	100
Total	45	100	NO.	3	22
			%	6. 7	48. 9

Recovery of *Y. enterocolitica* and *A. hydrophila* from environmental samples

Water samples

A total of 2 isolates of *Y. enterocolitica* were recovered from a total of 32 water samples, one from tap water and the other from well water with no isolate from seawater. On the other hand, 15 isolates of *A. hydrophila* were recovered from different types of water, 2 isolates from seawater, 6 isolates from tap water and 7 isolates from well water were obtained; with higher incidence of both organisms in tap and well water (Table 5).

Table (5): Number and percentage of *Y. enterocolitica* and *A. hydrophila* isolated from various water sources.

Water type	No.	%		*Yersinia*	*Aeromonas*

Sea water	5	15.6	NO.	0	2
			%	0.0	40
Tap water	13	40.6	NO.	1	6
			%	7.7	46.2
Wells water	14	43.8	NO.	1	7
			%	7.1	50.0
Total	32	100	NO.	2	15
			%	6.25	46.9

Sewage samples

Five *Yersinia* spp. were recovered from 26 sewage samples. *Y. enterocolitica* was the most frequently isolated *Yersinia* spp. It was found in 3 (11.5%) of 26 samples. The other two isolates were identified as *Yersinia kristensenii* (7.7%). Eleven *A. hydrophila* (42.3%) were recovered from the same samples (Figure 1).

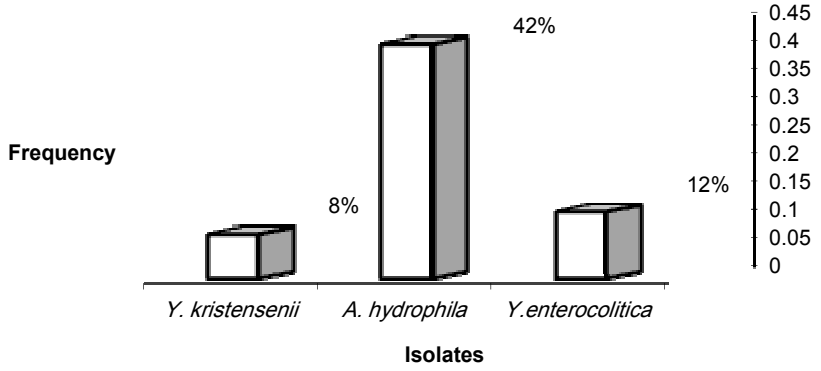

Figure (1): *Y. enterocolitica* and *A. hydrophila* isolated from sewage samples

Animal excreta samples

A total of 3 (11.5%) *Y. enterocolitica* and 10 (38.5%) *A. hydrophila* were isolated from 26 animal excreta samples. The highest number of *Y. enterocolitica* was recovered from cow's excreta (28.6%) and *A. hydrophila* from goat's excreta (62.5%) (Table 6). There is no

statistically significant difference between occurrence of both pathogens and type of animal excreta (P > 0.05).

Table (6): The number and percentage of *Y. enterocolitica* and *A. hydrophila* isolated from animal excreta samples.

Animal excreta			Isolates		
	No.	**%**		***Y. enterocolitica***	***A. hydrophila***
Chicken	4	15.4	NO.	0	0
			%	0.0	0.0
Cow	7	26.9	NO.	2	3
			%	28.6	42.9
Goat	8	30.8	NO.	0	5
			%	0.0	62.5
Turkey	7	26.9	NO.	1	2
			%	14.3	28.6
Total	26	100	NO.	3	10
			%	11.5	38.5

DISCUSSION:

The primary goal of the present study is to investigate the occurrence of *Y. enterocolitica* and *A. hydrophila* in clinical, food and environmental samples in Gaza strip. *Y. enterocolitica* was isolated from all types of samples except seawater while *A. hydrophila* was isolated from all sampling sources.

Of the 300 diarrheal stool samples tested, 14 (4.7%) were positive for *Y. enterocolitica* and 103 (34.3%) were positive for *A. hydrophila*. *Y. enterocolitica* and *A. hydrophila* were isolated either from stool samples from children or from older persons with diarrheal infections, with high incidence from patients below 6 years. We found a frequency of 4.7% for this organism, which is lower than some parts of the world especially northern European countries with a frequency up to 13% **[3]** and higher than other parts, 2.8% in Montreal, Canada, 2.1% from the Oneida County outbreak **[11]** and 1.04% were isolated from 7,290 black Atlanta children during the Thanksgiving-Christmas holidays in 1988 **[12]**.

The differences between the findings of various authors and those of this study might be due to several factors such as; isolation methods, number of analyzed samples, sources of samples, season, and

geographical location. These factors may cause an increase or decrease in the incidence of the *Yersinia* spp. For instance, the present study was carried out in Gaza strip, where the weather is generally warm and humidity is high. It is known that the isolation ratio of *Y. enterocolitica* is higher in colder climates.

With regard to *A. hydrophila,* our results were higher than the findings of some researchers **[13,14]**. In recent years, the number of studies on the prevalence of *Y. enterocolitica* and *A. hydrophila* in food products from various geographical regions has increased significantly **[15,16]**. The frequency of *Yersinia* in milk, milk products and meat samples was lower than previously reported in several countries, including United States and southern Ontario **[17]**. Several studies have been conducted to isolate *Yersinia* spp. in ground beef and the isolation rate was reported to be 9-99.2%. Among these studies, some generated higher isolation rates than the results of this study. In the present study, *Y. enterocolitica* was isolated from 6.7% of meat samples **[18]**.

Ibrahim and **MacRae** reported that *Aeromonas* was present in 60, 58, 74 and 26% of investigated beef, lamb, pork and milk samples, respectively, whereas **Krovacek *et al.,*** found aeromonads in 42% of the food samples originating from a random selection of retail outlets in Sweden. *Aeromonas* were also found in fish and fresh salads, freshly dressed lamb carcasses, oysters, cheese and raw cow's milk **[15]**. In the present study, *Y. enterocolitica* and *A. hydrophila* were isolated from 6%, 36% of the milk samples and 6.7%, 48.9% of meat samples respectively. Because of the obvious differences in sampling period, geographical location, the origin of the samples and methodology for analysis, it is difficult to compare the level of *Y. enterocolitica* and *A. hydrophila* incidence published by different authors. However, the present data clearly confirm the widespread distribution of *Y. enterocolitica* and *A. hydrophila* in retail foods.

Y. enterocolitica and *A. hydrophila* isolates recovered from different water samples, including chlorinated and non chlorinated, fresh water, well water, seawater, wastewater and natural mineral water. Most of these microorganisms were found to be nonpathogenic **[3,10,19]**.

In our study, water isolates represented 6.3% of a total of 32 water samples and (12%) of 26 sewage samples which is lower than those

reported in other parts of the world and higher in the other parts **[11,17,20]**. In another study *Yersinia* spp. were detected in 90.6% out of 32 raw wastewater samples **[21]**.

On the other hand; *A. hydrophila* was isolated (46.9%) from different water samples and 11 (42%) were recovered from sewage samples. High frequency of *Aeromonas* in water supplies were reported by several authors **[21,22]**. **Ormen *et al.*, and Burke *et al.*,** investigated the occurrence of *Aeromonas* spp. in natural and unchlorinated domestic water sources. 42% of the total isolates were identified as *A. hydrophila* which is lower than our result **[23,24]**.

Animals, especially domestic animals, have been suspected as transmitters of *Y. enterocolitica* and *A. hydrophila* to humans **[3,25]**. In various studies *Y. enterocolitica* was isolated from one or more sheep in 78 (17%) of 449 flocks **[26]**. These results are in agreement with our results, where we found that 11.5% of the studied animal excreta were positive for *Y. enterocolitica*. The highest prevalence of *Yersinia* (39, 64, 67, 70%) was observed in Germany, Denmark, Sweden and Norway **[17]**. These results are higher than our results and may be due to the fact that raw pork was main reservoir of *Y. enterocolitica* and transmitted to the other animals. **Gray** isolated *A. hydrophila* from feces of normal horses (6.4%), pigs (9.6%), sheep (9.0%), and cows (21.1%). The total fecal carriage rate in animals is slightly higher than the fecal carriage rate of normal humans, which is < 1 to 7% for most studies, although some studies report higher rates **[27]**. In this study, isolates of *Y. enterocolitica* and *A. hydrophila* were found in human, animal and environmental sources. This suggests the possibility of transmission from environment or animals to humans.

In conclusion; both *Y. enterocolitica* and *A. hydrophila* were isolated from clinical, environmental and food samples collected from the Gaza strip, therefore, it is recommended that public health and clinical laboratory should include the tests for these organisms as part of their services.

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