

Antimicrobial Resistance of Enteric Pathogens Isolated from Acute Gastroenteritis Patients in Gaza strip, Palestine

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Background: Acute gastroenteritis is a common infection among the children of Gaza. The emergence of antibiotic resistance in enteropathogenic bacteria has profound clinical implications on the acute gastroenteritis disease. This study is a matched case-control and aims to determine the incidence of enteropathogenic bacteria, antibiotic resistance and associated-risk factors in diarrheal patients in Gaza Strip.

Methods: A total of 132 patients with acute diarrhea were investigated along with data collected from 132 healthy controls having the same demographic characteristics of patients except they didn't suffer from diarrhea within the last three months. The same data were collected through completing a questionnaire form for the cases and healthy controls. Stool samples were collected from six Primary Health Care Clinics and the samples were inoculated, isolated and identified using standard bacteriological methods in the Remal clinic- Microbiology laboratory.

Results: A total of 12 (9.1%) enteropathogenic bacteria spp. were isolated from 132 stool samples. *Salmonella*, *Campylobacter coli/jejuni*, and *Aeromonas hydrophilia* were isolated in equal numbers from samples (3/12; 25% each), *Shigella* 2/12 (16.7%), and *Yersinia enterocolytica* 1/12 (8.3%). The two *Shigella* isolates were *Shigella boydii*. The antimicrobial profile of all isolated enteropathogenic bacteria showed high resistance rates against the tested antimicrobials. *Campylobacter coli/jejuni* (61.1%), followed by *Y. enterocolytica* (57.1.7%), *A. hydrophilia* (54.7.1%), *Shigella* (28.5%) and *Salmonella* spp. (9.5%). The highest antimicrobial resistance rates were found against erythromycin (75%) and Amoxicillin (65%).

Conclusions: Most enteropathogenic bacteria isolates showed high resistance rate to several antimicrobials. This study demonstrated that *C. coli/jejuni*, *A. hydrophilia*, and *Y. Enterocolytica* are detected as causative agents of diarrhea in Gaza Strip.

Keywords: Gastroenteritis, Diarrhea, Enteropathogenic bacteria, Antibiotic resistance, Gaza strip.



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Introduction

Acute gastroenteritis is a common infection among children in Gaza, and it is known to cause high morbidity and mortality among them if they have not received proper treatment on time (1). The antibiotic resistance of enteric bacteria has profound clinical features on children because it causes severe dehydration and threatens their life (2).

It is well established that antibiotic resistance increased with uncontrolled use of antibiotics in any community, especially when antibiotics are used by population without prescription, and this trend has become a major concern in Gaza (3). Moreover, there is few data about the spread of antibiotic resistance in enteropathogenic bacteria causing diarrhea in children of Gaza. The results of this investigative work would provide useful data for Gaza health authorities in order to control the incidence of infectious diarrhea among children.

Material and methods

A total of 132 stool samples were collected from patients with acute diarrhea during the period from Jan 2010 to Feb 2010. These patients were attended the Primary Health Care Clinics (PHCC) at Ministry of Health (MOH) in Gaza Strip because they have acute diarrhea (defined as the passage of 3 or more loose or liquid stools per day). The age of patients was between one month to 60 years old. In addition, personal data were collected from 132 healthy controls visited the same PHCC at the same period of study. Healthy controls had the same demographic characteristics of patients such as age, gender, except the fact they don't suffer from diarrhea within the last three months.

A questionnaire was administered and data was collected through personal interview of subjects or their guardians. The questionnaire included personal, socioeconomic and past history of diseases. An ethical approval from health authority in Gaza was obtained and informed consent from each participant was also obtained.

Isolation procedures of enteropathogenic bacteria

Each stool sample was directly inoculated onto Xylose Lysine Deoxycolate agar (XLD) agar, *Salmonella Shigella* (SS) agar, Hektoen enteric (HE) agar, *Campylobacter* Blood-Free Selective Agar Base (Modified CCDA) and *Yersinia* selective agar. Approximately 1 g of each sample inoculated into 10 ml of Selenite F broth, alkaline peptone water, and phosphate buf-

fer saline (PBS). The cold enrichment method was used to isolate *Yersinia enterocolitica* (4-6).

Identification procedures of enteropathogenic bacteria

Suspected colonies of enteropathogenic bacteria were identified by colony morphology, Gram stain and biochemical tests including oxidase, catalase, API20E and API campy systems. Confirmation of *Salmonella* and identification of *Shigella* strains carried out by polyvalent antisera (7, 8).

Antimicrobial susceptibility for the bacterial isolates

Antimicrobial susceptibility for the isolated enteropathogenic bacteria was done by disk diffusion method on Muller-Hinton agar. The procedures and zone of inhibition interpretation was done according to Clinical Laboratory Standards Institute (CLSI, 2011) (9). For *Campylobacter*, all strains were suspended in phosphate buffer saline (PBS) pH 7.4 and inoculated on Muller-Hinton agar supplemented with 5% of whole blood according the method described by Miranda et al. (10).

Results

This study included (56%) female and (44%) males. A total of 75% of diarrhea cases and controls were aged less than 5 years. It was found that (15.2%) of cases live in houses with < 5 household members, while (19.7%) of controls live in houses with < 5 household members ($P = 0.209$). An equal number of enteropathogenic bacteria were isolated from both male and female. From male: *Salmonella* and *A. hydrophilia* account for (1.5%), whereas *Shigella* and *Campylobacter coli/jejuni* account for (0.76%). However, for female the percentage was (0.76%) for *Salmonella*, *Shigella*, *A. hydrophilia* and *Y. enterocolytica*, and (1.5%) for *C. coli/jejuni*, ($P = 0.736$) (figure 1).

The antimicrobial profile of all isolated enteropathogenic bacteria showed high resistance rates for *Campylobacter coli/jejuni* (61.1%), followed by *Y. enterocolytica* (57.1.7%), *A. hydrophilia* (54.7.1%), *Shigella* (28.5%) and *Salmonella* spp. (9.5%) as shown in Table 1.

Table 2 shows the relation between diarrhea cases and presence of poultry in houses, and table 3 show the distribution of diarrhea cases in association of available drinking water in houses.

Table 1. Antimicrobial susceptibility of isolated enteropathogenic bacteria.

Enteropathogenic bacteria (n=12)	Antibiotics								Total Percentage of resistance %
	AM	E	CU	GM	TE	SXT	NA	CIP	
Salmonella spp.	0	-	0	0	33.3	0	33.3	0	9.5
Shigella spp.	50	-	0	0	50	50	50	0	28.5
Campylobacter coli/jejuni	75	75	-	-	-	-	-	33.3	61.1
Aeromonas hydrophilia	100	-	75	0	75	100	33.3	0	54.7
Yersinia enterocolytica	100	-	0	0	100	100	100	0	57.1
Total Percentage of resistance	65	75	18.7	0	64.5	62.5	54.1	6.6	40.1

Amoxicillin (AM), Erythromycin (E), Cefuroxime (CU), Gentamicin (GM), Tetracycline (TE), Trimethoprim/sulfamethoxazole (SXT), Nalidixic Acid (NA), Ciprofloxacin (CIP),.

Table 2. Domestic animals in the house associated with diarrhea cases.

P value	Control(n=132)		Case (n=132)		(n=264)	
	%	NO.	%	NO.		
0.001*	11.4	15	26.5	35	Yes	Poultry
	88.6	117	73.5	97	No	
0.111	9.1	12	4.5	6	Yes	Rabbit
	90.9	120	95.5	126	No	
0.086	5.3	7	10.6	14	Yes	Sheep
	94.7	125	89.4	118	No	
0.006*	8.3	11	19.7	26	Yes	Pigeon
	91.7	121	80.3	106	No	
0.500	9.1	12	8.3	11	Yes	Donkey
	90.9	120	91.7	121	No	

*Significance P < 0.05

Table 3. Presence of drinking water in the houses of investigated cases

P value	Control(n=132)		Case(n=132)		Total(n=264)	
	%	NO.	%	NO.		
0.421	9.8	13	11.4	15	Yes	Tap water
	90.2	119	88.6	117	No	
0.500	89.4	118	88.6	117	Yes	Filtered water
	10.6	14	11.4	15	No	
0.142	6.8	9	11.4	15	Yes	Boiled water
	93.2	123	88.6	117	No	
0.286	13.6	18	10.6	14	Yes	Bottle water
	86.4	114	89.4	118	No	
0.042*	2.3	3	7.6	10	Yes	Domestic wells
	97.7	129	92.4	122	No	
0.247	25.8	34	30.3	40	Yes	Water access changes
	74.2	98	69.7	92	No	

Significance P < 0.05 *

Isolated enteropathogenic bacteria

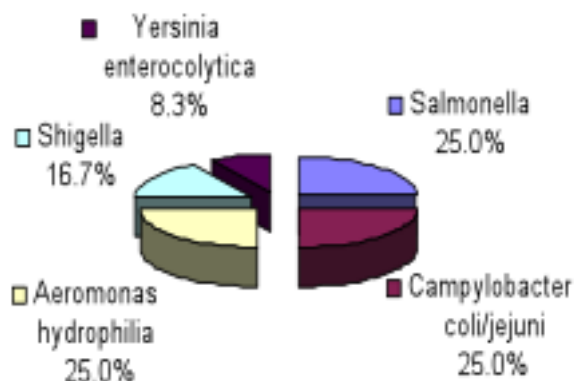


Figure 1. Enteropathogenic bacteria isolated from the stool samples.

Note: An equal number of enteropathogenic bacteria were isolated from both male and female. From male: Salmonella and *A. hydrophilia* account for (1.5%), whereas Shigella and *Campylobacter coli/jejuni* account for (0.76%). However, for female the percentage was (0.76%) for Salmonella, Shigella, *A. hydrophilia* and *Y. enterocolytica*, and (1.5%) for *C. coli/jejuni*

Discussion

This study shows that children aged less than 5 years old are significantly more susceptible (75%) to develop infectious diarrhea than older children, and enteropathogenic bacteria were isolated with a higher frequency (66.7%) from this age group. However, this study can't exclude that many other diarrheal cases might have been caused by enteric viruses or pathogenic intestinal parasites. According to recent reports of WHO, diarrheal diseases caused by viruses, parasites and enteropathogenic bacteria are a leading cause of morbidity and mortality in children; with 1.5–2.5 million deaths estimated to occur annually among children aged < 5 years and mostly in developing countries (11).

The results of this study is limited only to bacterial causative agents of diarrhea and these are nearly similar to previous study conducted in Gaza by Abu Elamreen *et al.*, (2007)

where they have reported that (10%) of their stool samples had enteropathogenic bacteria using conventional culture method (12). Other studies from our Arab countries reported higher percentage of enteropathogenic bacteria (17%) in Egypt (13) and (40%) in Jordan (14). In addition, our findings showed that rate of detection of enteropathogenic bacteria is higher than in a local study in El-Naser Hospital carried during the period 1999 to 2006, which has showed that the isolation frequency of *Salmonella* and *Shigella* was 1.8% and 0.8%, respectively (15).

This study suspected that contamination of groundwater in Gaza Strip with sewage water may be an important source of diarrhea caused by *A. hydrophila*, especially in Khan Yunis area, where *A. hydrophila* is frequently present in its sewage (16, 17). Nearly 10% of the population of the Gaza Strip (over 100,000 people) had no proper tap water supply in February 2009, following the last Israeli military attacks on Gaza (18, 19).

Multidrug resistance was commonly observed among enteropathogenic bacteria isolates, especially in *Campylobacter coli/jejuni* (75% resistant to erythromycin and ampicillin). Studies from neighbouring Arab countries (Jordan, Egypt, Lebanon) showed also wide spread of multidrug resistance among enteric bacteria isolated from human and water sources (14, 20, 21, 22). There was statistically significant correlation between the presence of poultry in the houses and occurrence of diarrhea cases ($P < 0.05$). This is probably because those who live in rural areas have more close contact with livestock and have a greater risk of *Campylobacter* infection (23).

In Conclusion, this study demonstrates a relatively low percentage of enteropathogenic bacteria detected among diarrheal patients, and most of these bacteria isolates were multidrug resistance.

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References

1. WHO. Diarrhea: Why children are still dying and what can be done. (NLM classification: WS 312), Geneva.
2. Georgopapadakou N, Antibiotic Resistance in *Enterobacteria*. Wax R. Bacterial Resistance to Antimicrobials, 2nd ed., Taylor & Francis Group, Boca Raton London New York. 2007. p. 343.
3. Al Jarousha A, El Jadba A, Al Afifi A, et al. Nosocomial multidrug-resistant *Acinetobacter baumannii* in the neonatal intensive care unit in Gaza City, Palestine. *Int J Infect Dis* 2009; 13 (5): 623-8.
4. Vandepitte J., Engaek K., Rohner P., et al., Basic laboratory procedure in clinical bacteriology. WHO Library, 2nd ed. Geneva. 2003. pp. 42-44.
5. Goldman H, Green L, Practical handbook of microbiology. Taylor Francis Group, 2nd ed. USA. 2009. pp. 566-569.
6. Aslani M, Alikhani M. The Role of *Aeromonas hydrophila* in Diarrhea. *Iranian J Publ Heal* 2004; 33 (3): 54-59.
7. WHO. Global Salm-Surv-Laboratory Protocols: Identification of thermo-tolerant *Campylobacter*. 2003. p. 6.
8. WHO. A global salmonella surveillance and laboratory support project of world health organization. Serotyping of *Salmonella enterica* O and H antigen. 2004. p. 2.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: CLSI. 2011.
10. Miranda K, Lage A. Antimicrobial susceptibility of *Campylobacter* sp strains isolated from calves with and without diarrhea in Minas Gerais state, Brasil *Braz J Microbiol* 2007; 38: 357-362.
11. Kosek M, Bern C, Guerrant R. The global burden of diarrheal disease as estimated from studies published between 1992 and 2000. *Bull WHO* 2003; 81(3): 197-204.
12. Abu Elamreen F, Abed A, Sharif F. Detection and Identification of Bacterial Enteropathogens by Polymerase Chain Reaction (PCR) and Conventional Techniques in Childhood Acute Gastroenteritis in Gaza, Palestine. *Int J Infect Dis* 2007; 11: 501-507.
13. Wierzbza T, Abdel-Messih I, Abu-Elyazeed R, et al. Clinic-based surveillance for bacterial- and rotavirus-associated diarrhea in Egyptian children. *Am J Trop Med Hyg* 2006; 74 (1): 148-153.
14. Nimri L, Meqdam M. Enteropathogens associated with cases of gastroenteritis in a rural population in Jordan. *Clin Microbiol Infect* 2004; 10 (7): 634-639.
15. Abu Elamreen F, Sharif F, Deeb J, Isolation and antibiotic susceptibility of *Salmonella* and *Shigella* strains isolated from children in Gaza, Palestine from 1999 to 2006. *J Gastroent Hepatol* 2008; 23 (8): 330-333.
16. Afifi S. Wastewater reuse status in the Gaza strip, Palestine. *Int J Environ Poll* 2006; 28 (1): 76-86.
17. Abulhamd A. Characterization of *Aeromonas hydrophila* Isolated from Aquatic Environments Using Phenotypic and Genotyping Methods. *Res J Agric. & Biol Sci* 2009; 5 (9): 923-931.
18. EWASH. Water quality in Gaza strip, EWASH Advocacy Task Force 2009. pp. 1-4.
19. WHO. Health conditions in the occupied Palestinian territory, including east Jerusalem, and in the occupied Syrian Golan. 2010. A63/28.
20. Wasfy M, Oyofu B, David J, et al.. Isolation and antibiotic susceptibility of *Salmonella*, *Shigella*, and *Campylobacter* from acute enteric infections in Egypt. *J Heal Popul and Nut* 2000; 18 (1): 33-8
21. Araj G, Uwaydah M, Alami S, Antimicrobial susceptibility patterns of bacterial isolates at the American university medical center in Lebanon. *D. Microbiol Infect Dis* 1994; 20 (3): 152-158.
22. Shehabi AA, Odeh JF and Fayyad M. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from Jordanian Human Stools and Drinking Water Sources. *J Chemother* 2006; 18: 468-472.
23. Ellis-Iversen J, Pritchard G, Wooldridge M, et al. Risk factors for *Campylobacter jejuni* and *Campylobacter coli* in young cattle on English And Welsh farms. *Prev Vet Med* 2009; 88 (1): 42-48.

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