

Incidence Rate of Acute Diarrheal Disease in Child Residents of Al- Shula

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ABSTRACT

Stool samples from forty children patients with diarrhea and other forty samples from children without diarrhea (control) (aged 1 month - 2 years) of both sexes were collected during the summer season of 2010. Tap water samples were also collected from houses of Al-Shula region of Baghdad. These samples were screened for the enteropathogenic bacteria. The percentage of bacterial isolates from children with diarrhea was 45% for *E. coli*, 21% for *Klebsiella pneumoniae*, 15% for *Citrobacter freundii* and 11% for *Pseudomonas aeruginosa* and with less frequency *Pseudomonas fluorescens*, *Serratia plymuthica* and *Serratia ficaria*, were also found. Whereas, *Klebsiella terrigena* and *Enterobacter aerugena* were more frequently isolated from control children than from diarrheic children. A similar prevalence of isolates was obtained from tap water samples. These results clearly indicate that contamination and/or poor purification procedures of water supplied to the general public might probably be one of the main causes for diarrhea in Baghdad city. Moreover, bacterial counts from tap water were highest during mid-summer indicating a need for better control of water quality during that time.

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Introduction

Diarrhea is a leading cause of morbidity and mortality among children in developing countries (Guerrant, *et al.*, 1990). The bacterial pathogen most commonly associated with endemic forms of childhood diarrhea is *Escherichia coli* (Huilan, *et al.*, 1991). There are six categories of diarrheagenic *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli*, enteroaggregative *E. coli* and diffusely adherent *E. coli* (Johnson and Lior, 1988) and (Nataro and Kaper, 1998). The epidemiological significance of each *E. coli* category in childhood diarrhea varies with geographical locations (Ogunsanya, *et al.*, 1994) and (Yatsuyanagi, *et al.*, 2002).

Several studies have investigated the microbiology of acute childhood diarrhea in Iraq, unfortunately, none of these studies investigated the etiology of food-borne or water-borne diarrheal disease. Most of these studies were not designed to

detect potentially pathogenic *E. coli*, but rather other bacterial causes of diarrhea such as *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Escherichia coli* and *Yersinia* spp. Most of these diarrheal agents are attributed to a contaminated water supply and contaminated environmental conditions. Al-Bayatti and Al-Taei (Al-Bayatti and Al-Taei, 2010) reported the isolation of different species of bacteria from the stool of patients with severe diarrhea in the primary and junior high school at Al-Rahmania region (at Al-Karkh side) of Baghdad city. The study revealed high prevalence rates of *E. coli*, *Klebsiella* spp. and *Salmonella* spp. which typically are acquired from the consumption of contaminated food and water. Therefore, the findings of this study indicates an association between drinking of tap water and the occurrence of chronic gastroenteritis in adult and acute gastroenteritis in children due to enteropathogenic bacteria and especially *E. coli*.

The goal of this study was to identify pathogenic bacteria from children with diarrhea and from healthy

controls living in Al-Shula region at Al-karkh side of Baghdad city. Furthermore, the efficiency of water purification by the Al-karama purification station which supplies that part of the city with tap water will be determined by measuring the degree of contamination of tap water with coliforms.

Materials and Methods

Patient and Control Population and Sample Collection

Stool samples from 80 children aged 1 month to - 2 years of both sexes were collected. Of this number, 40 samples were obtained from patients with diarrhea who attend Al-Hakeem hospital out-clinic which is situated in Al-Shula region of Baghdad during the summer season (June, September, 2010), and another 40 samples were obtained from healthy children without diarrhea (controls). The diarrheal patients were diagnosed by a physician on the bases of frequent watery stools (usually more than three times daily) lasting for less than 2 weeks. A large proportion of patients also had fever, but none had been treated with antibiotics before stool sampling. Most of the patients were from low-income families that are living in areas in close proximity to Al-Hakeem hospital and were consuming locally provided tap water. Furthermore, and at the same time, 40 samples of tap water were taken directly from houses of Al-Shula region for the evaluation of their coliform content.

Sample Processing and Culture Media

Stool samples from diarrheic and control children and tap water samples were collected in clean sterile screw-capped jars of 250 ml capacity and promptly submitted to the microbiology laboratory (within few hours) for processing. As described previously [(Alabi and Odugbem, 1990) and (Al-Izee, 2008)] the following media types were used for the primary isolation and culture of the diarrheagenic agents: blood agar (BA), MacConkey agar (MCA), xylose deoxycholate citrate agar (XDCA), eosin methylene blue agar (EMB) and thiosulfate citrate bile sucrose agar (TCBS).

Bacterial Culture and Identification

For the isolation of enteropathogens, the methods described by (Ogunsanya et al. 1994) were employed. In brief, inoculations of fecal specimens were done on appropriate media such as MCA and EMB agar (Oxoid, England), XDCA and TCBS agar (Difco-laboratories, Detroit, Michigan, USA). Specimens were also inoculated into enrichment broths such as selenite F-broth to enhance the isolation of *Salmonella* spp. and *Shigella* spp., whereas alkaline peptone water (pH 8.6) was employed for enrichment of *Vibrio cholera*. Next, selenite broth was subcultured on deoxycholate citrate agar and S-S agar whereas peptone water broth was subcultured onto TCBS agar and XDCA. All inoculated media (enrichment and subculture) were incubated at 37°C for 24 hrs. Biochemical tests were employed for the definitive identification of enteropathogenic *E. coli*, and *Klebsiella* spp. as previously described [(Yatsuyanagi *et al.*, 2002) and (Alabi and Odugbemi, 1990)]. For the isolation of *Proteus* spp. the method described by (Al-Izee, 2008) was employed, in which fecal samples were inoculated on MacConkey agar and blood agar and incubated at 37°C for 24 hrs. In addition, the API_{20E} system (Biomérieux) (Prescotts, *et al.*, 1990) was also used for confirmation of the identity of some of the *Enterobacteriaceae* and *Pseudomonadaceae* isolates obtained from fecal and tap water samples.

Statistical Methods

Data of patients and control stool samples are represented as means of two replicates (e.g. two separate samples were taken from each patient and control stool samples and processed separately then the mean numbers of bacteria from the two samples were measured). The differences in proportions of the various types of bacteria isolated from stool were compared using the z-test. The differences in proportions of various types of bacteria from different sources were compared using the Mann-Whitney U test. Results were considered highly significant when P value was less than 0.01 and were considered significant when p value was less than 0.05.

Results

Stool samples from 40 children with diarrhea and another 40 stool samples from control without diarrhea were analyzed to compare the types and numbers of bacterial species present in both populations. Table (1) shows the frequency of pathogenic bacterial species isolated from children with and without diarrhea. Rates of isolation were higher for all the diarrheagenic agents from patients with diarrhea than from controls. For patients with diarrhea *E. coli* and *Klebsiella pneumoniae* were significantly more frequently isolated than from control children. In contrast, *Klebsiella terrigena* and *Enterobacter aerugena* were significantly more frequently isolated from control children than from patients. Table 2 shows the percentages of isolation of seven bacterial species from patients with diarrhea. Of which, the dominants were *E. coli* at 45%, *Klebsiella pneumoniae* at 21% *Citrobacter freundii* at 15% and *Pseudomonas aeruginosa* at 11% and with less frequency *Pseudomonas fluorescens*, *Serratia plymuthica* and *Serratia ficaria*. Interestingly, almost the same species of bacterial isolates were also isolated from the tap water from houses in Al-Shula region. The percentages were almost similar and statistically non significant with the exception of *E. coli*. Also the prevalence rate of *K. pneumoniae* and *P. aeruginosa* are actually higher in tap water samples than in patients with diarrhea. For control non-diarrheal children, bacteria isolated from fecal samples had the following prevalence rates: 3% for *E. coli*, 15.2% for *Citrobacter freundii*, 48.5% for *Klebsiella terrigena* and 33.3% for *Enterobacter aerugena*.

Next, the presence of *E. coli* and other coliforms in drinking water obtained from Al-shula houses was determined. Figure 1 and 2 show that during the summer of 2010, the month of August corresponded to the highest bacterial counts whereas, June had the least counts.

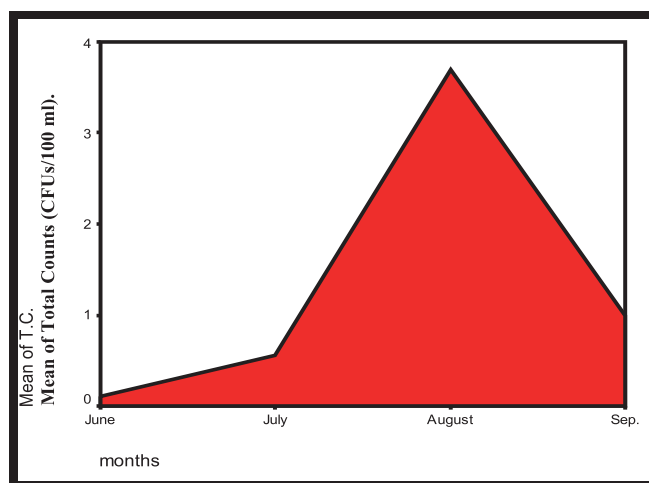


Figure 1: Mean number of *total coliform* counts from Al-Shula houses tap- water during Summer, 2010

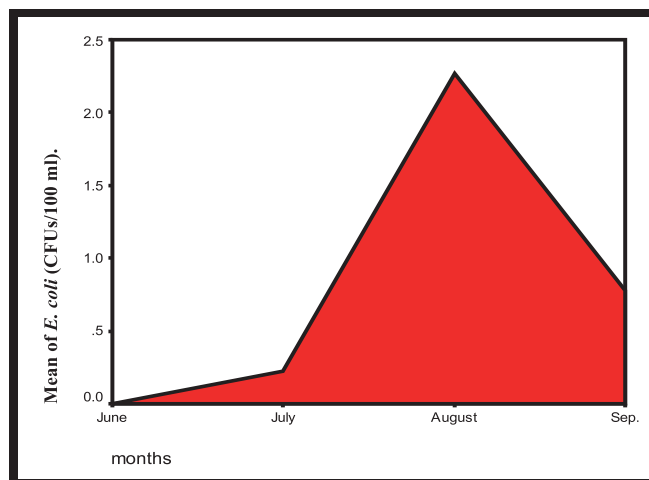


Figure 2: Mean number of *E. coli* counts from Al-Shula houses tap- water during Summer, 2010

Discussion

This paper is a part of study designed to illustrate the efficiency of three different purification stations located along the Tigris River in Baghdad city. One of these stations is Al-Karama purification station which provides Al-Shula region at Al-Karkh side of Baghdad city with drinking water. The purpose of this study is 1) to isolates pathogenic bacteria from children with diarrhea (aged 1 month to - 2 years) and from healthy children of the same age group (as controls) living in the Al-Shula region and who have attended Al-Hakeem hospital out-clinic and 2) to compare these isolates with bacterial isolates from the tap water from Al-Shula houses. The samples for this study were fecal samples from forty children with diarrhea, forty fecal samples

from healthy children, and forty samples of tap water from houses of Al-Shula region.

The presence of *E. coli* and *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* might be an indicator of water pollution from human and other animal sources (WHO, 2003). (Youssef, *et al.*, 2002) studied the diarrheagenic agents in 285 children with acute diarrhea in Jordan. They found that *E. coli* was prevalent in 30.2% of patients. Other studies (Torres, *et al.*, 2001) and (Haque, *et al.*, 2003) showed that enterotoxigenic *E. coli* and the other types of *E. coli* were the main causative agents of diarrhea and were prevalent in 9% and 62.8% of cases, respectively. Furthermore, these studies concluded that the remaining cases of diarrhea were caused by viruses and parasites. Al-Bayatti and Al-Taei (Al-Bayatti and Al-Taei, 2010) studied the enteropathogenic bacteria from 582 children with diarrhea. They showed that the percentage of bacterial isolates recovered were *E. coli* 58.22%, 91.66% and 58.70% during 2001, 2002 and 2003, respectively, followed by *Klebsiella* spp. at 16.66% and 20.63% during 2002 and 2003, respectively, and to a lesser frequency other enteropathogenic bacteria, *Proteus* spp. *Salmonella* spp. and *Vibrio* spp. Lastly, Obi *et al.* (Obi *et al.*, 1997) recorded the prevalence rates for the following bacterial isolates in cases of diarrhea in an urban area of Nigeria; *Campylobacter* spp. at 28% followed by enteropathogenic *E. coli* at 22%, *Salmonella* spp. at 17%, *Shigella* spp. at 14%, *Aeromonas* spp. at 5% and *Yersinia enterocolitica* at 4%.

This current study indicates that *E. coli* is the most frequently encountered enteropathogen associated with diarrhea. This result is in total agreement with previous studies. Furthermore, the percentages of bacterial species isolated from patients with diarrhea are well consistent with the types and percentages of bacterial species isolated from water of purification stations (Al-Bayatti, *et al.*, 2012).

On the other hand, the mean number of total coliform counts and *E. coli* counts from Al-Shula tap water had their highest values during August 2010 and the lowest values during June 2010. These months are the hottest months of the year where the consumption of water increases, which in turn increases the likelihood of infection with

the diarrheagenic agents (Haque, *et al.*, 2003). These results highlight possible differences between the prevalence of enteric bacteria in children with diarrhea compared to healthy controls, and reveal a strong association between *E. coli* and *Klebsiella pneumoniae* with diarrhea in children. Moreover statically our results were highly significant and fell within the 99.99% confidence limit. It was interesting to note that the isolation rate of all the pathogens encountered in this study were independent of sex in both patients with diarrhea and in healthy controls. On the other hand, the differences in the isolation rates, and in the species of bacteria isolated may not be truly biological, as there might be other factors which could have influenced the likelihood of isolation of one species over another, for example, some species may show poor growth after a somewhat prolonged transportation time. We therefore conclude that the types and numbers of Gram-negative potential pathogens, which cause diarrhea varies according to the seasons of the year and the nature of pollutants and waste products discharged from hundreds of industrial plants along the Tigris River. Therefore, we recommend further studies using different types of media (selective and enrichment) for isolation of bacteria to determine the extent of involvement of other possible potential diarrheic pathogens other than what were found in this study and had not been previously reported in Baghdad and in Iraq as a whole.

Conclusions

This study concludes, that diarrhea in children of Al-Shula region and possibly in Baghdad city is caused by Gram-negative bacteria. This diarrhea might be related to environmentally acquired isolates due to consumption of contaminated water. Furthermore, there might also be a direct association between drinking of tap water and the occurrence of acute gastroenteritis in children due to enteropathogenic bacteria and especially *E. coli*. We also conclude that the purification procedures in Al-Karama purification station which supplies residents of Al-Shula region of Baghdad city with drinking water are not satisfactory/efficient.

Table 1: Types & frequency of bacterial species isolated from patients with Diarrhea & controls without Diarrhea

Type of microorganism	Number of microorganisms isolated from		Z test (*)	C.S.
	Patients with diarrhea	Control without diarrhea		
<i>E. coli</i> ***	21	1	4.107	HS
<i>Serratia plymuthica</i>	2	0	1.200	NS
<i>Klebsiella ornitholytica</i>	0	0	-	-
<i>Klebsiella pneumoniae</i>	10	0	2.882	HS
<i>Klebsiella terrigena</i>	0	16	5.337	HS
<i>Pseudomonas aeruginosa</i>	5	0	1.935	NS
<i>Pseudomonas fluorescens</i>	1	0	0.843	NS
<i>Citrobacter freundii</i>	7	5	0.032	NS
<i>Pseudomonas paucimobilis</i>	0	0	-	-
<i>Sphingomonas paucimobilis</i>	0	0	-	-
<i>Pantoea spp.</i>	0	0	-	-
<i>Rahnella aquatilis</i>	0	0	-	-
<i>Enterobacter sakazakii</i>	0	0	-	-
<i>Enterobacter cloacae</i>	0	0	-	-
<i>Enterobacter aerugena</i>	0	11	4.262	HS
<i>Proteus mirabilis</i>	0	0	-	-
<i>Aeromonas hydrophila</i>	0	0	-	-
<i>Vibrio cholera</i>	0	0	-	-
<i>Vibrio fluvialis</i>	0	0	-	-
<i>Serratia ficaria</i>	1	0	0.843	NS
<i>Salmonella orizonae</i>	0	0	-	-
Total	47	33	0.944**	NS

*z-test ($\alpha = 0.05$) = 1.96 (Significant at $P < 0.05$); ($\alpha = 0.01$) = 2.58 (Highly Significant (HS) at $P < 0.01$); NS indicates Non-significant, ** Mann-Whitney U test recorded (highly Significant at $p < 0.01$), *** *E. coli* species include both *E. coli* and enteropathogenic *E. coli*.

Table 2: Types and percentage of bacterial species isolated from patients with and without Diarrhea and from tap-water obtained from Al-Shula houses.

Types of bacterial species	Percentage of bacterial isolates from		
	Patients with diarrhea	Tap water	Control (without diarrhea)
<i>E. coli</i>	45	27	3
<i>Serratia plymuthica</i>	4	3	0
<i>Klebsiella pneumoniae</i>	21	30	0
<i>Klebsiella terrigena</i>	0	0	48.5
<i>Pseudomonas aeruginosa</i>	11	23	0
<i>Pseudomonas fluorescens</i>	2	3	0
<i>Citrobacter freundii</i>	15	14	15.2
<i>Enterobacter aerugena</i>	0	0	33.3
<i>Serratia ficaria</i>	2	0	0
Total	100	100	100

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