Iraqi Journal of Public Health
Nab'a Al-Hayat foundation for Medical Sciences and Health Care

ORIGINAL RESEARCH ARTICLE

Etiology of bacterial diarrhea in children under five years in Kerbala Province, Iraq

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Abstract

Objective: To determine the bacterial causative causes of diarrhea and related factors in children under 5 years in Kerbala Province, Iraq.

Methods: The study was conducted on 648 children infected with diarrhea under the age of 5 years, visited hospitals and health centers in the province of Karbala for the period from January 2016 to October 2016 and collected information related to the patient by a special form for that.

Results: The results showed that rate of bacterial infection of diarrhea is 244 (37.65%) of the total diseased samples of 648 cases. Rural characterized areas of Kerbala were the highest rate of diarrhea (33) compared with the center of Karbala urban landscape, the distributed highest infection was recorded in the Alhur (43.85%), and the lowest infected rate in the center of the of Karbala (39.75%). Diarrhea increase within May 33 (49.24%) and decrease during January 14 (24.56%)

The isolates bacteria were distributed as follows: About 82 isolates (33.6%) from enteropathogenic *Escherichia coli* (EPEC), followed by 34 isolates (13.9%) *Proteus mirabilis*, 31 isolates (12.7%) *Salmonella Typhimurium,* 26 isolates (10.7%) *Klebsiella pneumonia*, 23 isolates (9.4%) *Pseudomonas aeruginosa*, 21 isolates (8.6%) *Shigella spp*, 16 isolates (8.6%) *Aeromonas hydrophila*, 6 isolates (2.5%) *Staphylococcus aureus*, four isolates (1.6%) *Enterobacter areogenosa*, and one isolates (0.4%) *Vibrio cholera*. There was no significant difference between gender in infected diarrhea patients. Bacterial isolates showed *various reactions* to the antibiotic used in the study.

Conclusions: Enteropathogenic *E. coli* considered as a most common etiology of bacterial diarrhea in children under 5 years in Kerbala Province, Iraq, especially, aged 12–23 months. All isolates bacteria showed sensitivity to the Norfloxacin and resistant against Trimethoprim, Amoxicillin. Antibiotics should be used wisely after antibiotic susceptibility test.

Keywords: diarrhea, under 5 years, breastfeeding, susceptibility, bacterial diarrhea

Introduction

In 2015, diarrhea caused nearly 688 million cases worldwide, 499,000 cases and deaths among children under the age of 5 years especially in developing countries[1]. Diarrhea in children is one of the most important diseases that health centers focus on, because it can lead to loss of life for children if they do not receive the required health care and fastest action[2]. It causes high morbidity and mortality among children under the age of 5 years[2,3]. With a serious rate of death among children with diarrhea in Africa[4]. Diarrhea is defined as abnormal loose watery stools more than three times within 24 hours. Diarrhea causes loss of a large amount of body fluids, salts, electrolytes, and other nutrients. Dehydration is the main cause of death, and also causes anorexia of the patient, as well as reduced intestinal ability to absorb water and nutrients[5]. Children, especially under the age of 5 years, are vulnerable to diarrhea in communities with low economic and social conditions and malnutrition. The causes of diarrhea can be transmitted either directly through the fecal–oral route, water, food contaminated with diarrhea, causing bacteria directly or indirectly by some vectors[6,7]. Pathogen of enteric infections transmitted directly or indirectly through consumption of tap water, contaminated food, flies, and frequent in rural areas[8].

In an epidemiological study, the etiology of infectious diarrhea in young children in many different parts of the world, the disease may be caused by viruses, bacteria, and

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Submitted: 10 October 2017 | Revised version received: 28 November 2017 | Accepted: 16 December 2017
Published online: 22 March 2018

parasites. Diarrhea may occur due to one or more common pathogens (coinfections). Different pathogens vary from one region to another in the world[9]. Diarrhea caused by coinfections may be more severe than diarrhea due to a single pathogen alone (monoinfection)[10]. There is a wide spectrum of bacteria that cause diarrhea in children, such as Enterobacteriaceae as coliform bacteria *E. coli*, *Proteus* spp., *Salmonella* spp., *Shigella* spp. *Citrobacter* spp., *Yersinia enterocolitica* and *Klebsiella* spp[11]. Diarrhea associated with *Vibrio cholera* is characterized by severe watery diarrhea[12].

Rotavirus and adenovirus are major causes of viral gastroenteritis infection and associated with severe diarrhea among children under the 5 years[13]. Rotavirus infection diarrhea among children under 5 years in Baghdad (23.33%, 2.23%)[14] and Sulaimani (22%, 3%), respectively[15].

A study in the Thi-Qar province referred to intestinal parasites are one of the most important causes of diarrhea (30.5%) under 6 years in the province of Thi-Qar/Iraq and the most important parasite were detected *Entamoeba histolytica* (17.5%), *Giardia lamblia* (7.5), *Entamoeba coli* (2%), *Hymenolepis nana* (2.5%), and *Enterobius vermicularis* (1.0%) (2) (Al-Mosawi, 2016). Protozoan is a common parasitic cause of diarrhea under 5 years children in Ethiopia[4].

First, the management of diarrhea typically involves rehydration therapy that is the continued feeding in addition to zinc, which proved to be very effective against rotavirus, but less effective against the bacteria that causes diarrhea, such as Shigella and ETEC[16].

Materials and Methods

Patients: The study included 648 children (372 males, 276 females), ranging in the age from 1 week to 5 years of age. Children with diarrhea visited to hospitals in the holy governorate of Kerbala, for the period between the beginning of January 2016 and the end of October 2016. Patient-related information was obtained through a special form through which information was recorded.

Sampling: Examinations were obtained from young children and infants suffering from diarrhea within 1–7 days or more of the date of infection based on hospital records. Children were suffering from frequent diarrhea (more than three times per day), abdominal pain, high fever, loss of appetite, and dehydration, and vomiting. Feeding broth was used to transfer samples from the hospital to the laboratory[17].

Isolation and Identification of isolates: The samples were examined with the naked eye to observe the color, density, and presence of blood or mucus. Samples examined by microscopy for the presence of red blood cells, parasitic ova, and cysts, The samples were taken by a sterile cotton swab and placed in the peptone water broth and transferred within an hour to the laboratory after all the patient's information was recorded. The samples were cultured on MacConkey agar, Sorbitol MacConkey agar, Xylose lysine deoxycholate agar (XLD), Eosin methylene blue agar (EMB). *Selenite* broth, *Salmonella–shigella* agar (SSA), thiosulphate citrate bile salt sucrose (TCBS), blood agar, all agar incubated at 37°C for 18–24/ 8 h period. The developing colonies were examined macroscopically and microscopically, the bacterial isolates were identified by using suitable biochemical tests, and use API 2OE system to confirmed diagnoses, the slide agglutination test with polyvalent and monovalent sera used to diagnose serotyping patterns of *Salmonella* species, *Shigella* species and *Vibrio cholera* and *E. coli* isolates[18,19].

Antibiotic susceptibility test: Antibacterial susceptibility tests were carried out by Kirby–Bauer technique using Mueller–Hinton agar[20]. The results were expressed as susceptible\resistant according to the criteria developed by National Committee for Clinical Laboratory standards[21].

Results

During the study period, which included 10 months of January 2016 for gluten October, 648 stool samples (males 348, females 300) were collected from children with diarrhea. Bacterial growth was obtained from 244 samples while 404 samples did not produce bacterial growth as shown in Table 1.

Table 2 shows the distribution of children with diarrhea as a result of bacteria among the different areas of Karbala Governorate, where the highest percentage of infection was recorded in Al Hur (46.3%) of the total number of cases 54, and followed an AL tamer 45 cases (43.85%), Al Handieya 47 cases (43.11%). and Al Hssaneya 30 cases (42.85%). The lowest infected rate in the city center with 97 cases (31.3%) from total number of case 31.

Table 1 The number of samples and the number of positive and negative samples for bacterial growth

All sample	Positive sample %	Negative sample %
648	244 37.65%	404 62.35%

Table 2 Distribution of Diarrhea samples shall be carried out in Kerbala governorate

Region	All Sample	Sample + Ratio %	Sample – Ratio %
Center of Kerbala	310 47.8%	97 31.3%	213 68.7%
An AL Tamer	105 16.2%	45 43.85%	60 57.15%
Al Handieya	109 16.8%	47 43.11%	62 56.89%
All Hssaneya	70 10.8%	30 42.85%	40 57.15%
Al Hur	54 8.4%	25 46.3%	29 53.7%
Total	648	244	404

The results of the study showed that the distribution of infection of bacterial diarrhea was greater in the rural areas of Karbala 147 cases (43.5%) than the urban area was in the city center 97 cases (31.3%) as in the Fig. 1:

The results of this study showed a relationship between the months of the year and the difference in rates of bacterial diarrhea among children under the 5 years, where the high rate of diarrhea were recorded during months (May, July, August, April, and June) and the percentages were respectively (49.24%, 47.88%, 40.54%, 40.35%, 39.78%). The rate of diarrhea infection decreased during the months (September, October, and January) of the study and by (32.39%, 24.59%, 24.56%), respectively, as in Table 3.

The results of the study showed an increased rate bacteria diarrhea among children between (12–18), (6–12), and (18–24) months and recorded ratios (45.96%, 44.23%, 43.56%), respectively. The lowest percentage recorded among the age groups (48–54) months 24.44%, (54–60) months rate 22.85% as shown in Table 4.

During this study, no association was observed between the incidence of diarrhea and the gender of the infected children. It was also noticed that the samples of bacterial diarrhea were 648, samples distributed by males 348 (53.7%), females 300 (46.3%). Of the 348 male children, 142 (40.8%) were positive, compared with 102 (34%) positive case in total female children as shown in Table 5.

During this study, 244 bacterial samples were isolated and diagnosed for positive growth, there were distributed to 10 different types of pathogenic bacteria that causes diarrhea in children under 5 years. From which 82 cases isolates (33.6%) from enteropathogenic *E. coli* (EPEC), followed by 34 isolates (13.9%) *Proteus mirabilis,* 31 isolates (12.7%) *S. Typhimurium,* 26 isolates (10.7%) *Klebsiella pneumonia,* 23 isolates (9.4%) *Pseudomonas aeruginosa,* 21 isolates (8.6%) *Shigella* spp., 16 isolates (8.6%) *Aeromonas hydrophila,* 6 isolates (2.5%) *Staphylococcus aureus*, 4 isolates (1.6%) *Enterobacter areogenosa, 1 isolates* (0.4%) *Vibrio cholera* Table 6.

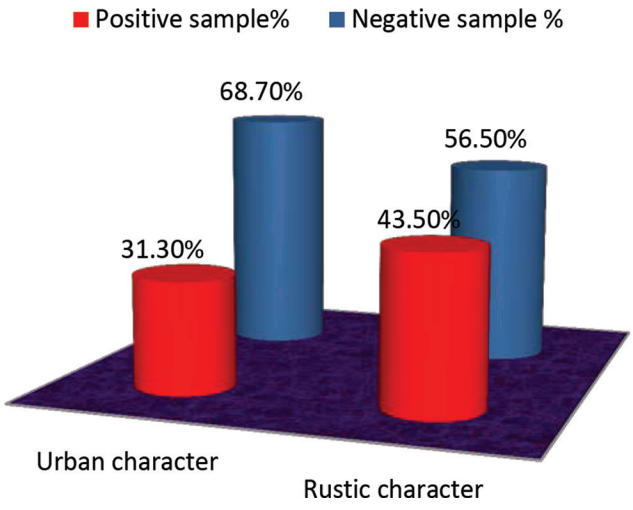

Figure 1 Distribution of diarrheal cases depending on the nature of the area.

This study indicates a relationship between the type of feeding received during the first period of life and the incidence of diarrhea infection. The incidence of diarrhea in

Table 3 Distribution of samples of the study on the months of the year

Months	Positive sample %	Negative sample %	Total
January	14 24.56%	43 75.44%	57
February	17 35.41%	31 64.59%	48
March	18 36.73%	31 63.27%	49
April	23 40.35%	34 59.65%	57
May	33 49.24%	34 50.76%	67
June	37 39.78%	56 60.22%	93
July	34 47.88%	37 52.12%	71
August	30 40.54%	44 59.46%	74
September	23 32.39%	48 67.61%	71
October	15 24.59%	46 75.41%	61
Total	37.65%	62.35%	648

$X^2 = 17.179$, $P < 0.05$.

Table 4 Distribution of children in study according to age and relation to diarrhea

Months	Positive sample %	Negative sample%	Total
1–6	113 28.88%	32 71.12%	45
6–12	46 44.23%	58 55.77%	104
12–18	57 45.96%	67 54.04%	124
18–24	44 43.56%	57 56.44%	101
24–30	25 38.46%	40 61.54%	65
30–36	17 34.69%	32 65.31%	49
36–42	13 30.95%	29 69.05%	42
42–48	10 26.31%	28 73.69%	38
48–54	11 24.44%	34 75.56%	45
54–60	8 22.85%	27 77.15%	35

X^2=18.23, $P < 0.05$.

Table 5 Distribution of study samples in according the gender

Gender	All Sample %	Sample + %	Sample – %
Male	348 53.7%	142 40.8%	206 59.2%
Female	300 46.3%	102 34%	198 66%
Total	648	244	404

$X^2 = 3.177$, $P > 0.05$.

Table 6 Species of bacterial isolated

Bacteria	Bacteria isolates	Ratio
Escherichia coli	82	33.6%
Proteus mirabilis	34	13.9
S. Typhimurium	31	12.7%
Klebsiella pneumonia	26	10.7%
Pseudomonas aeruginosa	23	9.4%
Shigella spp	21	8.6%
Aeromonas hydrophila	16	6.6%
Staphylococcus aureus	6	2.5%
Enterobacter areogenosa	4	1.6%
vibrio cholira	1	0.4%
Total	244	100

Table 7 Relationship between type of feeding and diarrhea

Feeding	All samples	Sample + %	Sample –%
Breastfeeding	101	26 (25.75%)	75 (74.25%)
Mix feeding	128	42 (32.82%)	86 (67.18%)
Bottle feeding	419	176 (42%)	243 (58%)
Total	648	244	404

$X^2 = 10.76$, $P > 0.01$.

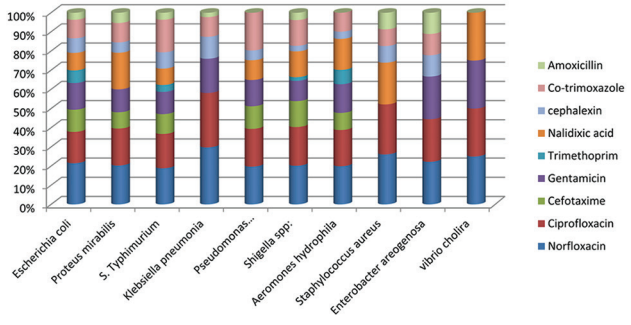

Figure 2 Antibiotic susceptibility of bacterial isolated from diarrhea.

infants who were adopted at the beginning of their lives was limited (25.75%), followed by mixed feeding (32.82%), and the increase the rate of infected diarrhea in children were feed on the bottle (42%), as in Table 7.

Figure shows that bacterial isolates showed different responses to the antibiotic used in the study and demonstrated that when all isolates were sensitive to Norfloxacin followed by Ciprofloxacin, as for Trimethoprim, Amoxicillin. Most isolates were resistant to these antibiotics.

Discussion

The results of the study showed that only 244 samples (37.65%) were positive to bacterial growth and 404 (62.35%) gave negative growth. The rate of bacterial infected diarrhea (60%) in Babylon Province[22] has been increased, because the reason is there are another causes of diarrhea, including viral, parasitic for children under 5 years and also some cases with mixed-infections[16]. Sometimes, the use of antibiotics leads to diarrhea[23]. There are some parasites that cause diarrhea in children[24].

Malnutrition makes children more susceptible to diarrhea and higher risk of morbidity and mortality[16].

The results showed a high incidence of diarrhea in the areas, such as Al Hure, An AL tamer, Al Handieya, and Al Hssaneya. Respectively, of the rural reality and that the lowest proportion was in the city center Kerbala with urban reality. The difference in diarrheal rates between rural and urban areas may be due to several factors, including the low standard of living and economic growth of the family, which increases the incidence of diarrhea[3], the decrease of health awareness among mothers in these areas in how to prevent diarrhea and how to deal with diarrhea complications, most of the cases of death occur due to suffering from severe dehydration[25]. The presence of domestic animals at home increases the chance of diarrhea, especially when dealing with children directly.[26] Contaminated food and water play a role in the transmission of diseases and a conduit for children[27].

Study in Iraq/Baghdad recorded the increase rate of infection diarrhea among males than female and lower level of education for parents lead to increased risk of frequent diarrhea besides the mother's nutrition[28], The peak of the infected of EPEC and rotavirus during the first year of life, and the rate of infection decreases with age while *Salmonella* spp. increases with age, Bacteria EPEC, *Salmonella* spp. and *Campylobacter* spp. were associated with watery diarrhea in children, bloody diarrhea linked with *Shigella* spp[29]. While mixed infections association with virus and other bacteria[30]. Children who were incompletely or not vaccinated at all were increase the chances of risk of infected diarrhea, because absence of vaccination made children more weakly to different diseases like whooping cough, measles, etc. This will directly affect the health adversely, and become susceptible to diarrhea and other diseases[31].

The high rate of diarrhea during the months (May, July, August, April, and June) through the months characterized by increase in the temperatures in Iraq. While moderate heat months or cold climates were characterized by a low rate of bacterial diarrhea[32]. Seasonal distributions of bacteria showed the peak of EPEC in March–June, while the top level of *Salmonella* spp. in October–January[30]. Some the microorganism reach to the top level of infection in winter season like Rotavirus, increase outbreak during cold

months (November, December, January, and February). While bacteria (*E.coli*, *Salmonella*, and *Shigella*) prefer months with high temperatures (May, June, July, and August), diarrhea causes by parasitic infection have been spread throughout the months of the years.[33]

This study showed that the highest infection rate of bacterial diarrhea was recorded among the age groups (12–18) months followed by (6–12), (18–24) months and the lowest infected (54–60) months, his result was similar to what they observed[3]. Diarrhea infections due to bacteria and parasites is more common among the 1–2 years less infected 3–4 years[34]. Studies showed that bacterial pathogens causing diarrhea were different during childhood, there was an increase of ETEC within 6–12 months of age while *Shigella* between 12 and 18 months[35]. The highest incidence of diarrhea was due to *Aeromonas* spp. in age 1–12 and followed by 12–24 months[36]. The increased parasitic infected diarrhea was recorded in the age group 13–24 months[37]. There was a relationship between the highest incidence of *G. lamblia* and *E. histolytica* in the age (19–36) and (12–18) months, respectively[24].

In this study, there was no significant difference between male and female children in the percentage of infected bacteria diarrhea. Similar study in Iraq/Baghdad[28], noticed that males were more likely to have diseases than females at 5 years of age[38,39].

This study showed that the bacteria *E. coli* spp. are more common in bacterial diarrhea infection among children under 5 years. Similar to the study indicated by Ref. 22. Among bacterial etiologies, typical enteropathogenic *E. coli* (EPEC) are associated with a higher risk of mortality in infants 0–11 months of age with moderate-to-severe diarrhea and *Shigella* is the first or second cause of moderate-to-severe diarrhea among children 1–5 years old[40].

Global studies have shown that *E. coli* (ETEC) and *Shigella* are the major important pathogens associated with severe diarrhea in children under 5 years of age in developing countries[35]. The highest incidence of diarrhea was found in bacteria followed by parasites, viruses, and same time present mix diarrhea infection[41], the most important bacterial *E. coli* infected 58.43%, *Klebsiella* spp. 20.12%, *Shigella* spp. 2.30%, and *Pseudomonas* spp. 0.77%, respectively[39]. This is contrary to what is observed by Ref. 42.

The ratio of infection with *Aeromonas* spp. different among region and the other depending factors, such as the environmental and geographical conditions[36].

Breastfeeding the baby during the first few months reduces the incidence of diarrhea and pneumonia[43,44]. Breastfeeding makes the baby get peptide (β-defensing) from milk, which acts as antibacterial activity was against pathogenic bacteria like *Salmonella* spp., *E. coli*, *Serratia marcescens* and *P. aeruginosa*, which causes diarrhea. This peptide is found at very high levels in colostrum[45] Increased risk of death occur in children having infected diarrhea accompanying with metabolic acidosis more often presented with dehydration and hypokalemia[46]. Failure to give sufficient fluids to a child who has diarrhea is a wrong practices that increases complications of diarrhea and may lead to death[47,48].

It has been confirmed that exclusive breastfeeding during the first 6 months of life to be effectively reduce rotavirus infection in children under 5 years[49].

Increasing the number of bacterial isolates resistant to a wide spectrum of antibiotics is the result of the unwise use of these drugs[50]. Giving a child antibiotics during the first 6 months increases the chances of a child having diarrhea later[51]. The study showed that bacterial isolates were sensitive to varying degrees in relation to antibiotics used. Antibiotic-resistant isolates are increasingly present due to misuse, especially in developing countries[52].

Conclusions

The low social, economic, and cultural conditions of the family play an important role in increasing diarrhea rates in children under 5 years. Enteropathogenic *E. coli* is considered the most common etiology of bacterial diarrhea in children under 5 years in Kerbala Province, Iraq, especially aged 12–23 months. Breastfeeding plays a major role in protecting against diarrhea. All isolates bacteria sensitivity to the Norfloxacin and resistant against Trimethoprim, Amoxicillin. Antibiotics should be used thoroughly and carefully after an antibiotic susceptibility test.

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