

Isolation of Some Microbial Agents that cause Acute Gastroenteritis in Children

Basim M. Ibrahim*

BSc, MSc (Microbiology)

Summary:

Background: Childhood diarrhea remains one of the leading causes of preventable mortality and disease burden throughout the developing world. It generated by several pathologic states, most commonly infections. Although the human large intestine ordinarily harbors a huge microbial population, most bacteria, protozoan, and viral agents of diarrhea are not members of this normal gut flora, but are acquired through contaminated food or water.

Objective: This study was conducted to investigate the main microbial causative agent of diarrhea in children below five years of age, and to evaluate the relationship between the incidence of diarrhea with sex and age group of the patients.

Patients and Methods: Total of two hundred twenty three stool samples were collected from children under five years of age suffering from diarrhea, who visit Children Welfare Teaching Hospital at Medical City Complex, Baghdad city during the period from July to the end of September 2010. General stool examination (GSE) was done for all the samples to identify the parasitic and fungal agents, and immunological detection were done to identify the viral causative agent of diarrhea, and the pathogenic strains of bacteria. Also biochemical tests for specimen cultured on specific culture media were done to identify the bacterial causative agents, after ensuring that the patients don't take antibiotics at the time preceding sample collection.

Results: Pathogenic bacteria came first with higher number of microbial isolates. High number of bacterial isolates was related to Enteropathogenic *Escherichia coli* (EPEC). *Entamoeba histolytica* appears as the most frequent parasitic causative agent. Increased rate of infection were falls on the first and second age groups, and also high rate of infection were recorded in males than in females.

Conclusion: bathogenic bacteria appear as the most predominant causative agent of infectious diarrhea in males under two years of age.

Key words: Childhood diarrhea, Infectious diarrhea in children, Acute gastroenteritis in children.

Fac Med Baghdad
2012; Vol. 54, No. 2
Received May 2012
Accepted May. 2012

Introduction:

The most common form of gastrointestinal infection is "Diarrhea" (rapid development of frequent intestinal evacuations of a more or less fluid character, derived from the Greek "DIA" through, and "RHEIN" meaning to flow like a stream). Also it may be defined as an increase in frequency of bowel action. It was found that diarrhea is produced by pathogenic mechanisms which attack the proximal small intestine, the portion of the bowel in which more than 90% of physiologic net fluid absorption occurs [1, 2]. Worldwide, Gastrointestinal diseases are considered as common illnesses, the second most common cause for visits to physicians, and of morbidity and mortality among infants and children under five throughout the developing world. It has been estimated that in Latin America, Africa, and Asia depending on nutritional and socio – economic factors, a child's chance of dying by diarrheal illness before the age of five years can be as high as 50%. In developing

countries, a quarter of infants and childhood mortality is related to diarrhea [1, 2, 3, 4, and 5]. In Iraq, according to the annual report of the Iraqi Ministry of Health (M.O.H), the average of diarrheal patients visiting the medical and health care facilities related to M.O.H for the years 2009 and 2010 is 201 and 212 / 1000 child under five years of age. Also, it is estimated that the percentage of diarrhea cases to the total pathological cases requiring hospitalization in children less than five years of age is 19.1 % for the year 2009, and 24.9 % for the year 2010 [6]. Gastrointestinal infections are classified into three major syndromes: watery diarrhea (which known as gastroenteritis, appears when the infection occurs in the small intestine), dysentery (type of gastrointestinal infections in large intestine represented with small fecal volume with mucus and blood), and enteric fever (invasion of blood stream from the intestine). Also, diarrhea can be classified according to many factors, such as duration of illness (acute vs. chronic), pathophysiology mechanisms (osmotic vs. secretory), severity (small vs.

*Dept. of Microbiology, College of Medicine, University of Baghdad.

large), and stool characteristics (watery, fatty, and bloody). Childhood diarrhea may involve 2 – 4 episodes of watery bowel motions over 24 hours period [1, 2, 5, 4, and 7]. Most infectious diarrhea acquired through fecal – oral route by way of contaminated food (an important vehicle of infectious diarrhea) or water, and also through person to person route. Not all the diseases can occur after an infection, some diseases may occur after ingestion of preformed toxins. It has been found that symptoms of intoxication occurs soon after ingestion of toxins in about (1 – 8 hrs.), while symptoms of an intestinal infection tend to occur much later (24 – 72 hrs.). Many of these cases are self limited (do not require treatment); while in other cases and under certain circumstances, infections can spread to other sites in body and require treatment to prevent complications (e.g., Bacteremia). Many microorganisms are implicated in the pathological mechanism of causing diarrhea, the most common causes are: Viruses (Rotavirus, Caliciviruses, Astrovirus, Enteric adenovirus, Norwalk: Norwalk – like viruses and Norovirus), Pathogenic Bacteria (*Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Vibrio cholerae*, *Campylobacter jejuni*, *Clostridium difficile*, *Yersinia enterocolitica*, *Aeromonas spp.*, *Staphylococcus aureus*, and *Bacillus cereus*), Parasites (*Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Strongyloides stercoralis*), and fungal (*Candida albicans*), [1, 2, 3, 7, 8, 9, 10].

Patients and Methods:

Specimen Collection: Two hundred twenty three stool samples were collected in sterile disposable plastic containers (within no longer than 2 hrs.), from children under five years of age suffering from diarrhea attending the Children Welfare Teaching Hospital, during the period from July to the end of September 2010.

General Stool Examination (GSE): This test involves two steps, macroscopic and microscopic examinations. The macroscopic examination of stool sample is done visually according to [7], for consistency (formed, unformed 'soft', or liquid), color (white, yellow, brown, or black), and presence of any abnormal components (mucus or blood). While the Microscopic examination of stool sample is done to demonstrate RBCs, pus cells, Monilia, bacteria, intestinal protozoa, fatty drops, undigested food, and normally small to moderate epithelial cells. The presence of large number of epithelial cells indicates that the intestinal mucosa is irritated [3].

A Stool Culture: Culture of fresh stool specimens remains the standard for determining an etiologic diagnosis [4]. There are several types of selective culture media used for

primary cultivation of stool samples, which allow the growth of certain type of enteric bacterial pathogens and inhibit the growth of other Enterobacteriaceae and gram-positive (non – pathogenic commensals) organisms [3]. The sample will inoculated on differential culture media (MacConkey's or Eosin Methylene Blue agar media), and on selective culture media (Hektoen Enteric or Salmonella – Shigella or Xylose – Lysine – Desoxycholate agar media), through the using of streaking plate method technique. Final identification is done through using biochemical tests which include the Analytic Profile Index 20E Identification System (API 20 E), [3, 11].

The Immunological tests: Detection for Rotavirus in stool samples was done follows the diagnosis of gastroenteritis to demonstrate the presence of Rotavirus as one of the suspected causative agents of infectious diarrhea in children. The detection procedure involves the using of rapid qualitative immunological test directly on feces against virus group antigen by agglutination of latex particles on a slide [12]. In addition to that, serotyping (agglutination tests) were done to identify the pathogenic strains of *E. coli* which are associated with the infantile gastroenteritis. The procedure involves the using of polyvalent sera, which can be confirmed later by the using of monovalent sera [11].

Statistical analysis: Statistical analysis was performed for data input and analysis by using the ready statistical program 'Statistical Package for Social Sciences' (SPSS). Continuous variables are expressed as means and discrete variables expressed as numbers and percentages.

Results:

Laboratory analysis for 223 stool samples was done in relation to sex and age of the patients. Our results revealed that pathogenic bacteria came first as the most frequent isolate (100 isolates, 44.84%), followed by fungi (65 isolates, 29.15%), then parasites (57 isolates, 25.56%), and finally viruses (1 isolate, 0.45%). High rate of pathogenic bacteria isolates are related to enteropathogenic *Escherichia coli* (EPEC) 47 (21.1%), while high rate of parasite isolates are related to *Entamoeba histolytica* 43 (19.3%). Increased rate of infection were recorded in the first and second age groups with (138) and (44) cases, (61.88%) and (21.52%), respectively. High percentage of cases were demonstrated in males than in females (140) and (83) cases, (62.78%, 37.22%) respectively. The results are shown in the following tables.

Table (1): Number and Percentage of Microbial Isolates.

Pathogenic Causative Agents	Total No. of isolates (%)	Type of isolates	No. (%)
Bacteria	100 (44.84 %)	<i>Enteropathogenic Escherichia coli (EPEC)</i>	47 (21.1 %)
		<i>Klebsiella oxytoca</i>	22 (9.9 %)
		<i>Citrobacter braakii</i>	15 (6.7 %)
		<i>Proteus spp.</i>	10 (4.5 %)
		<i>Salmonella spp.</i>	3 (1.34 %)
		<i>Shigella spp.</i>	2 (0.9 %)
		<i>Pseudomonas aeruginosa</i>	1 (0.45 %)
Fungi	65 (29.15%)	<i>Candida albicans</i>	65 (29.15 %)
Parasite	57 (25.56 %)	<i>Entamoeba histolytica</i>	43 (19.3 %)
		<i>Giardia lamblia</i>	14 (6.3 %)
Virus	1 (0.45%)	Rotavirus	1 (0.45 %)

Table (2): Distribution of Microbial Isolates according to Age groups of the patients.

Pathogenic Causative agents	Total No. of isolates	Age groups / year	No. of cases
Bacteria	100	1 day – 1 year	76
		1.1 – 2 years	15
		2.1 – 3 years	3
		3.1 – 4 years	2
		4.1 – 5 years	4
Fungi	65	1 day – 1 year	34
		1.1 – 2 years	17
		2.1 – 3 years	4
		3.1 – 4 years	3
		4.1 – 5 years	7
Parasites	57	1 day – 1 year	27
		1.1 – 2 years	16
		2.1 – 3 years	5
		3.1 – 4 years	4
		4.1 – 5 years	5
Viruses	1	1 day – 1 year	1

Table (3): Distribution of Diarrheal cases according to Gender of the patients.

Gender	No. of diarrheal cases (%)
Male	140 (62.78 %)
Female	83 (37.22 %)

Discussion:

Diarrhea is an acute syndrome of the intestinal tract in which the volume, fluid content, and frequency of bowel movements increase, it generated by several pathologic states, most commonly infections. Childhood diarrhea remains one of the leading causes of preventable mortality and disease burden throughout the developing world [7, 8, 10]. The results of the present study had demonstrated

that pathogenic bacteria came first with a highest rate of isolates (100 isolates, 44.84%) between other microbial isolates. Between the 100 pathogenic bacteria isolates, 47 (21.1%) isolates are related to enteropathogenic *Escherichia coli* (EPEC). Our findings were in line with other reports in Iraq and other countries indicating that bacterial pathogens are important contributors to ediatric diarrhea, and enteropathogenic strain of *Escherichia coli*

is the most frequently detected pathogen [3, 7, 8, 13, 14, 15]. *Candida species* have been often considered but infrequently documented as a credible cause of diarrhea. Our data analysis demonstrates that *Candida albicans* appears as an important causative agent of diarrhea in children with 65 isolates (29.15%). Our findings were in agreement with the findings of other studies which revealed that there is a relationship between fungi and incidence of diarrhea in children [15, 16]. In children, viral diarrhea is the most common form of infectious diarrhea. Viruses play a predominant role in some seasons, and the Rotavirus may cause about half the cases of acute diarrhea in children under three years on a worldwide basis [9, 17]. Our results show that only one isolate are related to Rotavirus. This may be disagreeing with other outcomes, including studies in Iraq and other countries demonstrating that Rotavirus appears as the most common cause of infectious diarrhea [14, 15, 18, 19, 20]. This may be related to specimen's collection time, most studies demonstrate that there are seasonal peaks (higher prevalence) in winter and in cooler months of the year in temperate countries [1, 3, 9, 11, 19, 21]. In our study, the specimens are collected in hot weather (from July to the end of September), which may be explaining the lowest rate of Rotavirus isolates appears in this study. The results of the present study had demonstrated that parasites appear as important causative agents of diarrhea in children with a rate estimated to 57 isolates (25.56%). *Entamoeba histolytica* appears as the most predominant isolate (43 isolates; 19.3%), then *Giardia lamblia* appears with lower rate of isolates (14 isolates; 6.3%). Our results were in agreement with the finding of other studies indicating that *Entamoeba histolytica* record a higher prevalence in developing countries [9, 13, 18, 22]. While other studies in Iraq show low prevalence [23, 24]. The present data analysis revealed that the highest rate of infections were recorded in the first and second age groups (1 day – 1.0 year), (1.1 year – 2.0 years), with 138 and 48 cases (61.88%) and (21.52%), respectively. Our findings were in agreement with other reports demonstrating that the occurrence of diarrhea varies widely by the age of the child, and the infection was peak in children aged ≤ 2.0 years [4, 7, 8, 18, 25, 26]. The results of the present study also showed that high number of cases were recorded in males than in females (140, 83) cases, (62.78%, 37.22%) respectively. This result was in agreement with other studies in Iraq and other countries [13, 18, 19, 27].

References:

1. Ryan K.J, and Ray C.G: *Sherris Medical Microbiology An Introduction to Infectious Diseases: Enteric Infections and Food Poisoning*. 4th (ed.). McGraw Hill, 2004: 609, 857, 860 – 861.
2. Chamberlain N.R: *Medical Microbiology The Big Picture: Gastrointestinal Tract and Liver*. McGraw Hill Lange, 2009: 169 – 237.
3. Gillespie S, and Bamford K: *Medical Microbiology and Infection at a Glance: Bacterial Diarrhoeal Disease*. Blackwell Sciences Ltd., 2000: 98 – 99.
4. Bruyn G.D, and Bouckennooghe A: *Evidence – Based Infectious Diseases: Diarrhea*. 2nd (ed.). Blackwell Publishing Ltd., 2009: 98 – 114.
5. Manning S.D: *Deadly Diseases and Epidemics: Escherichia coli Infections*. 2nd (ed.). Chelsea House Publishers, 2010: 29.
6. Ali J.L, Baqir H.H, Abdul – Wahab I.A, Majeed S.S, and Abd – Ali F.M: *The Annual report of the Iraqi Ministry of Health: Diarrhea*. Iraqi Ministry of Health, 2010: 47, 67, 138.
7. Larson C.P, Henning L, Luby S, and Faruque A.S.G.: *Modern Infectious Disease Epidemiology Concepts, Methods, Mathematical Models, and Public Health: Infectious Childhood Diarrhea in Developing Countries*. Springer Science + Business Media, 2010: 291 – 308.
8. Talaro K.P, and Talaro A: *Talaro – Foundation in Microbiology: Medical Microfile 20.2 Diarrheal Disease*. 4th (ed.). McGraw Hill Ltd., 2002: 609 – 627; Appen.: D, A – 9, 10.
9. Southwick F: *Infectious Diseases A Clinical Short Course: Gastrointestinal and Hepatobiliary Infections*. 2nd (ed.). McGraw – Hill Lange, 2007: 190 – 209.
10. Morris S, Black R.E, and Tomaskovic L: *Predicting the Distribution of under-five Deaths by cause in Countries without Adequate Vital Registration Systems*. *Int. J. Epidemiol.*, 2003; 32:1041–1051.
11. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, and Heuck C.C: *Basic Laboratory Procedures in Clinical Bacteriology: Stool*. 2nd (ed.). World Health Organization – Geneva, 2003: 37 – 59.
12. Patel M.M, Tate J.E, and Selvarangan R: *Routine Laboratory Testing Data for Surveillance of Rotavirus Hospitalization to Evaluate the Impact of Vaccination*. *Pedia. Infect. Dis. J.*, 2007; 26 (10): 914 – 919.
13. Mahdi L.K: *Infantile Diarrhea in Relation to the type of Feeding*. PhD Thesis, Saddam College of Medicine, Saddam University, Iraq; 1998.
14. Guerrant R.L, Hughes J.M, Lima N.L, and Crane J: *Diarrhea in Developed and Developing Countries: Magnitude, Special settings, and Etiologies*. *Clin. Infect. Dis.*, 1990; 12 (1): S41 – S50.
15. Kozinn P.J, and Taschdjian C.L: *Enteric Candidiasis:*

- Diagnosis and Clinical considerations. Amer. Acad. Pediatr. J.*, 1962; 30 (1): 71 – 85.
16. Evans A.S, Brachman P.S, and Abrutyn E: *Bacterial Infections of Humans: Epidemiology and Control: Epidemiological Concepts*. 4th (ed.). Springer Science + Business Media, 2009: 31.
17. Al-Rawazq H.S: *Infantile Gastroenteritis Multifactorial Disease*. M.Sc. Thesis, College of Medicine, University of Baghdad, Iraq; 2009.
18. Hyeng-II C, Shin-Hyeong C, Jin-Hee L, Yi-Young L, Ji-Hye J, and Jae-Ran Y: *Infection Status of Hospitalized Diarrheal Patients with Gastrointestinal Protozoa, Bacteria, and Viruses in the Republic of Korea*. *Korean J. Parasitol.*, 2010; 48 (2): 113 – 120.
19. Sherchand J.B, Yokoo M, Sherchand O, Pant A.R, and Nakagomi O: *Burden of Enteropathogens Associated Diarrheal Diseases in Children Hospital, Nepal*. *Scientific World*, 2009; 7 (7): 71 – 75.
20. Mahy B.W.J: *The Dictionary of Virology*. 4th (ed.). Elsevier Ltd., 2009: 232.
21. Singh U, Petri J, and William A: *Principle and Practice of Clinical Parasitology: Amebas*. John Wiley and Sons Ltd., 2001: 197 – 218.
22. Mahdi N.K, Al-Sadoon L.A, and Mohammed A.T: *First Report of Cryptosporidiosis among Iraqi Children*. *East Mediteran Health J.*, 1996; (2): 115 – 120.
23. Fahema J: *A study on Diarrhea in Relation to Malnutrition in Children under two years in Baghdad*. M.Sc. Thesis, College of Medicine, Al – Mustansiriyah University, Iraq; 2000.
24. Tanaka G, Faruque A.S.G, and Luby S.P: *Deaths from Rotavirus Disease in Bangladeshi Children: Estimates from Hospital-Based Surveillance*. *Pediatr. Inf. Dis. J.*, 2007; 26: 1014 – 1018.
25. Laupland K.B, and Church D.L: *Population based Laboratory surveillance for Giardia spp. and Cryptosporidium spp. infections in a Large Canadian Health Region*. *BMC Infect. Dis.*, 2005:
26. Samir R.S: *Serotyping and Plasmid Content (as Epidemiological Markers) of Enteropathogenic E. coli causing Diarrhea in Iraqi Children*. M.Sc. Thesis, College of Medicine, University of Baghdad, Iraq; 2006.
27. Rahouma A, Klena J.D, Krema Z, Abobker A.A, and Treesh K: *Enteric pathogens associated with childhood diarrhea in Tripoli – Libya*. *Am. J. trop. Med. Hyg.*, 2011; 84 (6): 886 – 891.