



Etiology of Bacterial and Parasitic Enteropathogens among Patients with Acute Diarrhea in Babylon Province

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Abstract: Infectious gastroenteritis is one of the most common diseases in most people . To clarify the infectious etiology of diarrhea in some patient aged from 8-30 years, we collected in clean suitable container stool samples from 78 patients with diarrhea from general teaching Hilla Hospital from September \2014 to April\2015.

From 78 diarrheal cases, only 38 (48.71%) cases are caused by bacteria and 27 (34.6 %) cases are caused by parasites , also there are 13 (16.66%) cases have mixed infection between them. Gram negative bacteria formed 76.31% (29 cases) represented by *E. coli* 16 cases (55.17%), *Salmonella spp.* 6 cases (20.68%), *Shigella* 3 cases (10.34%), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* 2 cases (6.89%) for each one. While gram positive bacteria consists 23,68% (9ases) represented by *Clostridium spp.* 4 cases (44.44%), *Bacillus spp.* 3cases (33.33%) and *Staphylococcus aureus* 2cases (22.22%), while parasitic infection were *Giardia lamblia* appears as the most frequent parasitic causative agent 11cases (40.74 %) followed by *Entamoeba histolytica* 9 (33.33%) , *Entrobilus vermicularis* 4 (14.81%) , *Hymenolips nana* 3 (11.11%). Increased rate of infection were falls on the first and second age groups, and also high rate of infection were recorded in females than in males.

Key words:-*E.coli*, *salmonella spp.*, *klebsiella*, *pseudomonas*, *Entrobilus*.

Introduction

Gastroenteritis is defined as an inflammation of the stomach, large and small intestines (1). It is one of the most common illnesses in humans worldwide (2). Although it can affect individuals of any age, it presents a significant health risk to those at extremes of age, the very young and the very old (3). It is the second most common cause of death among adults, and the leading cause of childhood death worldwide (4). The major symptoms of gastroenteritis are nausea, vomiting, diarrhoea, loss of appetite, fever, weakness, abdominal

cramps, and severe cases of the disease can lead to dehydration which in some cases is fatal (3,5). Infectious causes of pediatric gastroenteritis can be classified into three broad categories: bacterial, viral, and parasitic agents. Many species of protozoan parasites live in the gastrointestinal tract, infecting about 3.5 billion individuals worldwide. Three species are of particular importance: *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum*.(6)

There are many studies conducted in Iraq for incidence of intestinal parasites, the study of (7) show that the

prevalence of intestinal parasites between children in Ninevah Governorate was 64.16% which (8) indicate that the highest ratio of the intestinal parasites was *Entamoeba histolytica* (20.31%) followed by *Giardia lamblia* and *Enterobius vermicularis* (15.96% and 5.99%).

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programmes. The symptoms of acute bacterial intestinal infection are usually mild to moderate, and spontaneous remission occurs, but in some cases, the disease can cause rapid deterioration of a patient's condition (9). Bacteria that cause gastroenteritis include; *Salmonella* and *Shigella* (the two most common pathogens), *Campylobacter jejuni*, *Escherichia coli*, *Clostridium difficile* and *Vibrio cholerae* (2). Phagocytosis represent one of non specific host defenses against most pathogens. Leukocytes specially polymorphonuclear cells (PMNs) have ability to engulf foreign microbes, inflammatory mediators that secreted with bacterial infection elicit phagocytic activity (10).

A current study was to determine the prevalence of bacterial, parasitic and among patients with acute diarrhea in Babylon province .

Materials and Methods

Stool Samples and Questionnaire

Specimen Collection: 78 stool samples were collected in sterile disposable plastic containers, from patients with diarrhea from Babylon maternity and children hospital from August\2007 to

January\2008. The selection criteria for inclusion of patients with diarrhea was having at least 3 or more soft, semisolid or liquid bloody faeces within 24 hours. Also, selection was made on the basis of a questionnaire filled up by all subjects with assistance of a relative over 18 years old, providing information regarding other gastrointestinal disorders, frequency of diarrheal episodes, age.

Parasitological Stool Examination

a- Macroscopical Stool Examination:

Each fresh stool specimen was examined visually to look for consistency, color , presence or absence of mucus , blood , worms or any other abnormalities .

b- Microscopical Stool Examination:

This includes:

1-Direct Normal Saline Preparation:

A small portion of stool was mixed with one drop of normal saline on a glass slide then covered with glass cover slip . Each slide was examined by microscope under low power (X 10) and then under high power (X 40) to detect presence of helminthes ova and cyst, trophozoites of protozoa (11).

2-Lugol's iodine preparation method:

This was done by mixing a small portion of stool with one drop of Lugols' iodine on a glass slide , then covered with cover slip, examined by microscope using low power (X10) and high power (X40) (11).

3-Zinc Sulphate Centrifugal

Flotation: This procedure allowed the detection of the parasite (cyst, ova) that might be missed by using only the direct examination (11).

Bacteriology

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 (12).

Stool samples were streaked on the surface of MacConkey and Nutrient agar (Himedia, India) for obtaining *E. coli*, *Pseudomonas aeruginosa*, *Acinobacter Klebsiella pneumoniae*, isolates and on S-S agar (Himedia, India) for the selection of *Shigella* and *Salmonella*, also using Blood agar for gram positive bacteria, Mannitol salt agar for *Staphylococcus aureus* isolates, incubated overnight at 37°C for 24-48 hours.

All samples were tested for *Shigella* by using colony morphology, biochemical properties (Serobac, BioRad) (13). Using specific biochemical tests to identified the type of causative bacteria such as, Indole production test, Oxidase, Catalase, Methyl red, Vogas proskauer test, TSI test, Hydrogen sulfide production test, Urease production, Gelatin hydrolysis test, Motility test, Citrate utilize test and Gram stain. All Biochemical Test for Identification of Bacteria are followed as in (14).

Immunological Assay

Immunological assay were done using phagocytosis. 5ml of blood were collected from patients with diarrhea, 0.1 ml of fresh blood were mixed with equal volume of nitro blue tetrazolum stain (NBT), the mixture incubated then examined searching for PMNs positive for NBT (15)

Statistical Analysis

Use the U.S. Census (spss 11) to perform statistical analysis, as analyzed the results using the design random full-scale analysis of variance and adopted the test less significant differences Least significant difference test (LSD) and table analysis of variance (ANOVA Table) below the level of significance 0.05 (16).

Results and Discussion

The study revealed that pathogenic bacteria came first as the most frequent isolate (38 isolates, 48.71%), while (27 isolates, 34.6 %) had parasite infection , also there are 13 (16.66%) cases have mixed infection between them , High rate of pathogenic bacteria isolates are related to Gram negative bacteria formed 76.31% (29 cases) represented by *E. coli*.

16 cases (55.17%) in 13-17 age group, *Salmonella spp.* 6 cases (20.68%), *Shigella* 3 cases (10.34%), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* 2 cases (6.89%) for each one , While gram positive bacteria consists 23,68% (9ases) represented by *Clostridium spp.* 4 cases (44.44%), *Bacillus spp.* 3cases (33.33%) and *Staphylococcus aureus* 2cases (22.22%) shape (1). while high rate of parasite are related to *Giardia lamblia* 11cases (40.74 %) followed by *Entamoeba histolytica* 9 (33.33%), *Entrobilus vermicularis* 4 (14.81%) , *Hymenolips nana* 3 (11.11%) table (1). Increased rate of infection were recorded in the first and second age groups table (2). High percentage of cases were demonstrated in females than in males (46) and(32) cases, (58.97%,41%) respectively table (3). Immune response induced in gastroenteritis, phagocytosis

investigation reveals increase in PMNs cells positive to MBT, percent of PMNs

positive to MBT varied with age group table (4) .

Table (1) : Bacterial and parasitic etiology of diarrhea in 78 patient

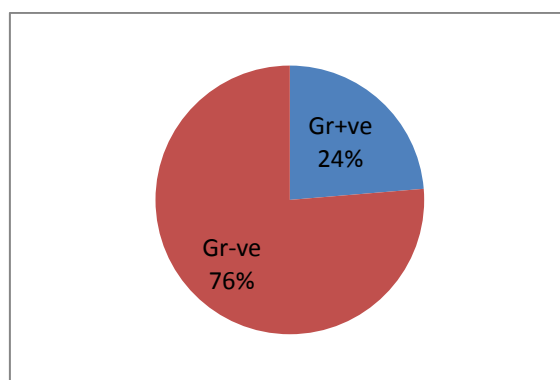
organism	species	NO. of patient	%
Gram negative Bacteria	<i>E. coli</i>	16	55.17
	<i>Salmonella spp.</i>	6	20.68
	<i>Shigella</i>	3	10.34
	<i>Pseudomonas aeruginosa</i>	2	6.89
	<i>Klebsiella pneumoniae</i>	2	6.89
Gram positive Bacteria	<i>Clostridium spp.</i>	4	44.44
	<i>Bacillus spp.</i>	3	33.33
	<i>Staphylococcus aureus</i>	2	22.22
	Total	38	48.71
Parasite	<i>Giardia lamblia</i>	11	40.74
	<i>Entamoeba histolytica</i>	9	33.33
	<i>Entrobilus vermicularis</i>	4	14.81
	<i>Hymenolips nana</i>	3	11.11
	Total	27	34.61
Mixed infection		13	16.66%

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

Pathogenic Causative agents	Total No. of isolates	Age groups / year	No. of VE+ for Pathogenic Causative agents	%
Bacteria	38	8 -12 years	22	57.89
		13 -17 years	8	21.1
		18 -22 years	6	15.78
		23 -27 years	2	5.26
Parasite	27	8 -12 years	9	33.33
		13 -17 years	8	29.62
		18 -22 years	4	14.81
		23 -27 years	6	22.22
Mixed infection	13	8 -12 years	8	61.53
		13 -17 years	3	23.07
		18 -22 years	2	15.38
		23 -27 years	0	0

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

Gender	No. of patient (%)
Female	46 (58.97%)
Male	32 (41%)



Shape (1): Rate of Gram positive to Gram negative bacteria isolates from diarrhea sample

Table (4) Percentage of polymorphnuclear(PMNs) cells positive to NBT.

Age period	% of positive PMNs(M ± SD)
8 -12 years	13.633 ± 1.02
13 -17 years	14.714 ± 3.38
18 -22 years	12.9 ± 1.97
23 -27 years	14.33 ± 3.08

The result of this study concluded that pathogenic bacteria came first with a highest rate ,Between the 38 pathogenic bacteria isolates, 29 cases (76.31%) are related to Gram negative bacteria represented by *E. coli* 16 cases (55.17%), *Salmonella spp.* 6 cases (20.68%), *Shigella* 3 cases (10.34%), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* 2 cases (6.89%) for each one , While gram positive bacteria consists 23,68% (9ases) represented by *Clostridium spp.* 4 cases (44.44%), *Bacillus spp.* 3cases (33.33%) and *Staphylococcus aureus* 2cases (22.22%), Our findings were agreed with other reports in Iraq and other countries indicating that bacterial pathogens are important contributors to pediatric diarrhea, and enteropathogenic strain of *Escherichia coli* is the most frequently detected pathogen (11,12,17,18,19). Other studies have the opposit to the current study, as it indicated that the *Salmonella* and *Shigella* are The two most frequency of

other bacterial species (20). The prevalence and the profiles of intestinal parasites infections vary, we found that *G. lamblia* were the most common parasitic agents in the study population with infection rates 40.74% followed by *Entamoeba histolytica* 9 (33.33%), *Entrobilus vermicularis* 4 (14.81%), *Hymenolips nana* 3 (11.11%), the high prevalence of organisms may be the result of failing to follow health guidelines or their use of water from contaminated sources , the rate of parasite in present study are in general agreement with some investigators Which indicated a number of protozoan can cause gastroenteritis most commonly *G. lamblia* and *Entamoeba histolytica* and another type of parasite have also been implicated(21) but much higher than others (22,23) .The rate of *Entamoeba histolytica* in this study agreed with (24) while the onother evealed both *Giardia lamblia* and *Entamoeba histolytica* were detected in 2% only of

the stool samples examined (25). In addition, the results of the current study differed from the terms of the types of parasites that have emerged in the study samples and their rates with other studies.(26,27). These variations might be due to several reasons such as: the different locations and times of these studies , numbers of the study samples , differences in socioeconomic states of the individuals,as well as some nonhygienic habits, together with lack of sanitary precautions of the community. Distribution of positive samples according to age group differences is shown in (table-2). The majority of pediatric patients in this study were reported in the first and second age groups, this result was similar to which reported in several studies(8,28,29). The results of the present study also showed that high number of cases were recorded in females than in males , This result was in agreement with(30) While the study results did not agree about what mentioned in (31,32), The reason of different rate of infection between sexes may be due to the presence of several factors contributing to the difference in incidence between the sexes, including exposure to pollutants and social behavior and the environment (33). Phagocytosis occurs in phagocytosis during microbial infection ,cellular killing enhanced according to leucocytes activity ,PMNs from the major leucocytes participate in pathogens engulfment (34).

References

1. Jones, M.; Harrach, B.; Ganac, R.; Gozum, M.; Dela Cruz, W. and Riedel, B.(2007). New adenovirus species found in a patient presenting with gastroenteritis. *J Virol*; 81: 5978-84.
2. Okitsu-Negishi, S.; Nguyen, T.; Phan, T. and Ushijima, H.(2004).Molecular epidemiology of viral gastroenteritis in Asia. *Pediatr Int*; 46: 245-52.
3. Jones, S. A.(2003) clinical pathway for pediatric gastroenteritis. *Gastroenterol Nurs*; 26: 7-18.
4. Zamir, D.; Weiler, Z.; Kogan, E.; Ben-Valid, E.; Hay, E.; Reitblat T, et al.(2006) Singledose quinolone treatment in acute gastroenteritis. *J Clin Gastroenterol*;40: 186-90.
5. Allen, K. (2007).The vomiting child--what to do and when to consult. *Aust Fam Physician* ; 36: 684-7.
6. Al-Bwardy, A.; Ramia, S.; al-Frayh, R.; Chagla, H.; al-Omair, A.; el-Hazmi, A.; et al. (1988) Bacterial, parasitic and viral enteropathogens associated with diarrhoea in Saudi children. *Ann Trop Paediatr*; 8: 26-30.
7. Ftohe,Z.I. ; Al -Zako, S.S. & Mahfoth, N. (2008). Study on Intestinal Parasites as a Causative of Diarrhea and Some Effectors on Them in Children of Neinavah Governorate.*Rafidain Journal of science* J.19(2):37-50.
8. Algharawy, A.K .(2015)Study of Prevalence of intestinal parasites infection among children in & attending to Karbala teaching hospital for children. *Journal of Karbala university* 13(1): 50-56 .
9. Ternhag,A.;Törner, A.; Svensson, A.; Ekdahl, K. and Giesecke, J.(2008).Short- and long-term effects of bacterial gastrointestinal infections. *Emerg Infect Dis*; 14:143-8.
10. Labruyere,L. ;C. Zimmer, B. ;Galy,T.,C. Olevo, M. and Guillen , N.(2003).a member of the P12 activated kinase family , involved in the control of *Entamoeba histolytica* migration and phagocytosis , *J.cell sci.*, 16(1):61-71.
11. Larson, C.P.; Henning, L.; Luby, S. and Faruque, A.S.G.: (2010). *Modern Infectious Disease Epidemiology Concepts, Methods, Mathematical Models, and Public Health: Infectious Childhood Diarrhea in Developing Countries*. Springer Science + Business Media,: 291 – 308.
12. Gillespie, S. and Bamford, K.(2000). *Medical Microbiology and Infection at a Glance: Bacterial Diarrhoeal Disease*. Blackwell Sciences Ltd.,: 98 – 99.
13. Svenungsson, B.; Lagergren, A.; Ekwall, E.; Evengrd, B. ; Hedlund, K.O. ;Krnell, A.; Lfdahl, S.; Svensson, L. and Weintraub, A.

- (2000). Enteropathogens in adult patients with diarrhoea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. *Clin Infect Dis*. 30:770-778.
14. Macfaddin, J.F. (2000). "Biochemical Test of Identification of medical Bacteria" the Williams and Wilkins Co. USA.
 15. Park, B.H.; Fikring, S.M. and Smith Wick, E.J. (1968). Infection and nitroblue tetrazolium by neutrophils. A diagnostic aid. *Lancet.*, 2:532-534.
 16. Niazi, A.D. (2004). Statistical analysis in Medical Research. 2nd ed. Coll.of Med., Nahrain Univ. Baghdad. P: 73-98.
 17. Talaro, K.P. and Talaro, A. (2002). Talaro – Foundation in Microbiology: Medical Microfile 20.2 Diarrheal Disease. 4th (ed.). McGraw Hill Ltd.: 609 – 627.
 18. Mahdi, L.K.(1998). Infantile Diarrhea in Relation to the type of Feeding. PhD Thesis, Saddam College of Medicine, Saddam University, Iraq.
 19. Guerrant, R.L.; Hughes, J.M.; Lima, N.L.; and Crane, J.(1990). Diarrhea in Developed and Developing Countries: Magnitude, Special settings, and Etiologies. *Clin. Infect. Dis.*, 12 (1): S41 – S50.
 20. Ayman, J; Hani, G; and Aiman ,M.(2010). Frequency of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Saudi Arabia. *J. Pak . Assoc .60(6):456-459.*
 21. Webb, A .and Starr, M. (2005). "Acute gastroenteritis in children." *Australian family physician* 34 (4): 227–31.
 22. Al- Haidari, S. ; Aref, H. ; Findukly and Entowan , F. (2000) A study on cases of diarrhea admitted to univ. Hospital, Iraq j. *Med. Sci ; 1 : 65-67*
 23. Edward , S.; Al-Mukh, J. and Al-Ani , W. (2000). Epidemiology of bloody diarrhea, *Iraqi j. com. med. ;13 (1) : 6-9.*
 24. Samie,A.; Guerrant, R.L.; Barrett, L.; Bessong, P.O.; Igumbor, E.O. and Obi, C.L.(2009). Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarroal human stools from Vhembe district, South Africa. *J Health . Popul . Nutr. Dec;27(6):739-45.*
 25. Na'was, T.E. and Abo-Shehada, M.N.(1991). A study of the bacterial and parasitic causes of acute diarrhoea in northern Jordan. *J Diarrheal . Dis. Res. Dec. ; 9(4):305-9.*
 26. AL- yassaree, H. f.(2004). Isolation and Identification of three protozoa enteroparasites "E. histolytica ; G. lamblia; and Cr. Partm " M.Sc Thesis , coll.Sci.Univ. Kufa: pp 81 .
 27. William,A. and Sodeman, J.R. (2000) .Intestinal protozoa Amoebas, *Medmicro Chapter 79.Short textbook of Physiology. 20th ed. Langu Medical publication, Losm. Aitos,California. USA.*
 28. Al-Bwardy, A.; Ramia, S.; al-Frayh, R.; Chagla, H.; al-Omair, A.; el-Hazmi, A.(1988). Bacterial, parasitic and viral enteropathogens associated with diarrhoea in Saudi children. *Ann Trop Paediatr* 1988; 8: 26-30.
 29. Chen, S.; Chang. Y. ; Lee. Y. ; Chao. H. ; Tsao. K ; Lin. T.(2007). Molecular epidemiology and clinical manifestations of viral gastroenteritis in hospitalized pediatric patients in Northern Taiwan. *J Clin Microbiol*; 45: 2054-7.
 30. Heidari, A. and Rokni, M.B. (2003). Prevalence of intestinal parasites among children in day-care centers in Damghan-Iran. *Iranian J. Publ. Health*, 32: 31-34.
 31. Al-Saeed, A.T. and Issa, S.H. (2006). Frequency of *Giardia lamblia* among children in Dohuk, Norther Iraq. *East. Med. Health J.* 12 (5): 555-561.
 32. Al-Mussawi, H.S. (2012) .Epidemiological study for *Giardia lamblia* parasite in Babylon province and test activity of cold aqueous extract and crude powder of pomegranate peels in experimentay infected cats MsC Thesis, College of Sciences for women University of Babylon .
 33. Klein, S.L. (2004). Hormonal and immunological mechanisms mediating sex difference in parasite infection. *Parasites Immunol.*, 26: 247-264.
 34. Abd,N.J.(2009).isolation of *Nisseria gonoria* from gonococcus patients with determination of the immunological state .*Babi . univ.J . 1(16):464 – 469.*