



## Detection and Identification of Bacterial Enteropathogens from Faecal Specimens in Basrah City

### KEYWORDS

Entropathogens, *Escherichia coli*, Diarrhea

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### ABSTRACT

Acute diarrhea is one of the leading causes of morbidity and mortality worldwide. It is one of the most important causes of death in the developing world. In this study we investigated the main bacterial causative agent of diarrhea in Basra city from different age groups, and evaluated the relationship between the incidence of diarrhea with sex and age groups of the patients. The results showed that the *Escherichia coli* is the main causative agent of diarrhea in Basra city and a higher rate of infection was recorded in males than in females.

### Introduction

Diarrhea can be defined as an increase in the frequency of bowel movements or passage of three or more unformed stools in a 24-hour time period. It is commonly associated with one or more enteric symptoms such as nausea, vomiting, increase in abdominal gas, abdominal pain or cramps and tenesmus (1). Gastrointestinal diseases are considered as an important cause of morbidity and mortality worldwide and as one of the most important causes of death in developing countries (2).

In Iraq, it was 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 (3). Most cases of acute diarrhea are caused by enteric infection by a viral (Rotavirus, Caliciviruses, Astrovirus, Enteric adenovirus, Norwalk: Norwalk – like viruses and Norovirus), 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*) or fungal pathogen (*Candida albicans*), (4, 5, 6, 7). These pathogens can cause potentially serious diseases which may be fatal, especially in children. The most common route of infection by these organisms is the ingestion of contaminated foods and drinks (8).

The aim of this is to detect and identify bacterial enteric pathogens from faecal samples and to assess the distribution percentage of isolated bacteria in Basrah city with the aim of establishing a proper preventive program.

### Patients and Methods

A Total of one hundred and ten stool samples were collected from diarrheal patients of different age and sex in Basra city during the period from July 2014 to the end of April 2015. General stool examination (GSE) was performed macroscopically and microscopically to investigate the consistency, color, presence of any abnormal components (mucus or blood) and to demonstrate RBCs, pus cells, bacteria, intestinal protozoa, fungi, and epithelial cells (9).

**A Stool Culture:** Streaking plate method technique was used to culture stool samples on different types of differ-

ential and selective culture media. The samples were inoculated on differential culture media (MacConkey agar), and on selective culture media (Salmonella – Shigella and Xylose – Lysine – Desoxycholate agar media). Finally, the confirmation and the speciation of pathogenic bacteria was carried out by using biochemical tests including Triple Sugar Iron medium (TSI) and Analytic Profile Index 20E Identification System (API 20 E), (10, 11).

### Results

From a total of 110 diarrheic patients, *Escherichia coli* was the most common bacterial pathogen (39%), followed by *Klebsiella oxytoca* (5%), *Pseudomonas aeruginosa* (3%), *Salmonella spp* (2.0%), *Shigella spp* (2%) and *Proteus spp.* (1%) as shown in (Table 1).

Increased rate of infection were recorded in age groups of over 15 years and 2-15 years with (42) and (40) cases, (38.1%) and (36.4%), respectively (Table 2).

A Higher percentage cases were demonstrated in males than in females (70) and (40), (63.7%, 36.4%) respectively (Table 3).

### Discussion

Diarrhoea remains one of the most common illnesses of all population and one of the major causes of infant and childhood mortality in Iraq. The reduction in diarrheal mortality may be possible by determining the high risk subjects and targeting them for intensive intervention. New knowledge on diarrhoeal agents will help in planning for future studies on various aspects of diarrheal diseases in this population. The aim of this study was to determine the prevalence of bacteria causing diarrhoea in Basra city. The results in this study revealed that *Escherichia coli* is the main causative agent (32%) of diarrhoea in Basrah city whereas, other types of bacteria including *Klebsiella oxytoca*, *Proteus spp.*, *Salmonella spp*, *Shigella spp* and *pseudomonace aeruginosa* were 4.5%, 0.9%, 1.8%, 1.8% and 2.7% respectively. When compared to other studies these percentages are lower than that reported by Ali et al. (2009) in children up to 2 years in Kirkuk province were *E. coli* 67.9% *Proteus spp.* 7.81%, *Salmonella spp.* 4.52%, *Shigella spp.* 3.29% and except for *Klebsiella pneumoniae* 2.05% and *Pseudomonaceas aeruginosa* 0.08% which were lower than our results. However, our results are higher than some results reported by Ibrahim (2012) in Baghdad where

he recorded *Enteropathogenic Escherichia coli* (EPEC) 21.1%.

These variations could be explained by differences in the endemicity of enteropathogens, socioeconomic and specific prevalent among the different population. The identification of enteropathogens reflects the importance of these organisms in the epidemiology of diarrhoea in Basra city and suggests that either these organisms are most common in the environment or that they are associated with more severe presentations which may lead patients to seek medical help. Improvements in techniques and methods used in the detection of enteropathogens may justify the higher rates of detection of infectious agents.

The present data analysis revealed that the highest rate of infections were recorded in the fourth and third age groups (> 15 years), (2-15 years), with 42 and 40 cases (38.1%) and (36.2%), respectively. This may be because of the consumption of contaminated food and drinks. The results of the present study also showed that a higher number of cases were recorded in males than in females (70, 40) cases, (63.6%, 36.4%) respectively. This result was in agreement with other studies in Iraq and other countries (13, 14).

In spite of the relatively small size of samples used in the present study, it can be said that the data obtained in this study provides useful information to identify enteropathogens from faecal samples in Basrah city using conventional techniques and to assess the distribution percentage of isolated bacteria.

The limitations of this study were the relative small number of samples and the use of the conventional culture methods only, without the use of molecular and immunological methods to detect bacteria. To enhance the specificity and to increase the sensitivity in further study; a larger number of samples, molecular methods (e.g. PCR) and immunological methods should be employed to detect other types of bacteria, fungi, viruses and all other diarrhoeal causes.

## Conclusion

The bacterial enteric pathogens (*E.coli*, *Klebsiella oxytoca*, *Proteus spp.*, *Salmonella spp.*, *Shigella spp.* and *Pseudomonas aeruginosa*) are the major cause of diarrhoea in the population of Basrah city. Among the positive cases identified, 39 (32%) isolates are related to *Escherichia coli*.

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

Type of isolate	NO. ( %)
<i>Escherichia coli</i>	9 (32%)
<i>Klebsiella oxytoca</i>	5 (4.5%)
<i>Proteus spp.</i>	1 (0.9%)
<i>Salmonella spp.</i>	2 (1.8%)
<i>Shigella spp.</i>	2 (1.8%)
<i>Pseudomonas aeruginosa</i>	3 (2.7%)
<i>Staphylococcus</i>	1 (0.9%)
Microflora	43 (39%)
Negative growth	14 (12.7%)

**Table (2): Distribution of diarrheal cases according to Age groups of the patients.**

Age group (year)	No. of diarrheal cases (%)
< 1 year	24 (21.8%)
1 – 2 years	4 (3.6%)
2 – 15 years	40 (36.4%)
> 15 years	42 (38.1%)

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

Gender	No. of diarrheal patients (%)
Male	70 (63.6%)
Female	40 (36.4%)

## REFERENCE

- DuPont HL (1997) Guidelines on acute infectious diarrhea in adults. The Practice Parameters Committee of the American College of Gastroenterology. *Am J Gastroenterol* 92(11):1962-1975
- Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ (2006). Global and regional burden of disease and risk factors, systematic analysis of population health data. *Lancet*, 367(9524):1747-1757.
- Abd – Ali FM (2010). The Annual report of the Iraqi Ministry of Health: Diarrhea. *Iraqi Ministry of Health*, 47, 67, 138.
- Ryan K.J, and Ray C.G: *Sheris Medical Microbiology* 1(2004). An Introduction to Infectious Diseases: Enteric Infections and Food Poisoning. 4th (ed.). McGraw Hill, 609, 857, 860 – 861.
- Gillespie S, and Bamford K (2000). *Medical Microbiology and Infection at a Glance: Bacterial Diarrhoeal Disease*. Blackwell Sciences Ltd., 2000: 98 – 99.
- Modern Infectious Disease Epidemiology Concepts, Methods, Mathematical Models, and Public Health (2010). *Infectious Childhood Diarrhea in Developing Countries*. Springer Science + Business Media, 291 – 308.
- Talaro K.P, and Talaro A: Talaro – Foundation in 8. *Microbiology: Medical Microfile 2002 Diarrheal Disease*. 4th (Ed.). McGraw Hill Ltd., 609 – 627; Appen. D, A – 9, 10.
- Kare Molbak, Henrik Jensen, Liselotte Ingholt, and Peter Aaby (1997). Risk factors for diarrheal disease incidence in early childhood: a community cohort study from Guinea-Bissau. *Am J Epidemiol*. Aug 1; 146(3):273-82.
- Heuck C.C: *Basic Laboratory Procedures in Clinical Bacteriology* (2003). Stool. 2nd (ed.). World Health Organization – Geneva, 37 – 59.
- Isenberg, Henry D. (2004). *Clinical Microbiology Procedures Handbook*, American Society for Microbiology, Washington DC. P.3.8.1.1-21.
- Murray P. R. (1978). Standardization of the Analytab Enteric (API 20E) system to increase accuracy and reproducibility of the test for biotype characterization of bacteria. *J Clin Microbiol*. Jul; 8(1): 46-49
- Chatin I. Ali, Ayhan R. Mahmood, Nihad A. Jafar, Sajida Khorsheed (2009). Prevalence of enteropathogenic diarrhea in Children up to 2 years in Kirkuk province. *Tikrit Medical Journal* 15(2):124-131.
- Basim M. Ibrahim (2012). Isolation of Some Microbial Agents that cause Acute Gastroenteritis in Children. *J Fac Med Baghdad*, Vol.54, No.3.
- Ji-Hye J, and Jae-Ran Y (2010) Infection Status of Hospitalized Diarrheal Patients with Gastrointestinal Protozoa, Bacteria, and Viruses in the Republic of Korea. *Korean J. Parasitol*. 48 (2): 113 – 120.