Clinical and epidemiological study of bacterial species that cause diarrhea isolated from children under five years of age and their sensitivity to antibiotics

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A B S T R A C T

Diarrhea is a serious and widespread disease, especially in developing countries. The study included the collection of (265) fecal samples from children with diarrhea attending the Child's Central Teaching Hospital in Baghdad for the period from January to September 2022, whose ages ranged from less than five years, where males constituted 63% and females 37%.The results of bacterial growth showed that (198) stool samples, with a rate of (74.7%), were positive, while the number of samples did not show results was (70) with a rate of(25.3%). The isolates were subjected to standard bacteriological and biochemical tests. Among the bacterial pathogens that appeared at a high rate were *E.coli* 52%, followed by *Salmonellaspp* 17%, *Shigellaspp*10%,*Proteusmirabilis* 7%, *Kilbsiellapneumoniae* 6%,*Compylobacterjejuni* 4 %,*Pseudomonas aeruginosa*2.5% and*Staphlococcusaureus*1.5%. Some of the factors affecting the prevalence of diarrhea in children were studied, such as age, sex, and monthly infections. The highest infection rate appeared in the first age group, less than one year old, with the highest infection rate 62%,while the lowest percentage was in the age of 4 - 5 years which is 3.5%.As for the monthly infections, they reached their peak in August, with a rate of 19%. The sensitivity of bacterial isolates towards antibiotics was tested, all isolates showed high resistance to Ampicillin 100%, while most of the isolates showed high sensitivity to chloramphenicol, Erythromycinand Co-trimoxazol.

Keywords*: Diarrhea, Antibiotic sensitivity, Pathogenicbacteria, Epidemiological factors.*

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INTRODUCTION

Diarrhea is a serious and widespread disease in the world, as children under the age of five years are exposed to this disease, and the infection is concentrated in infants whose ages range from six months to two years (1). Severe cases of diarrhea are one of the main causes of child death in the world. In developing countries, between 700-1000 million cases of diarrhea occur annually, causing the death of 5 million children every year (2). The main cause of death in severe cases of diarrhea is due to dehydration resulting from the body losing the necessary fluids, as diarrhea is an important cause of malnutrition. Therefore, diarrhea and malnutrition are among the main causes of death in many countries (3) The causes of disease are numerous including: Bacterial agents, as in *E.coli*, *Salmonella spp*., *Compylobacterspp*, and *Shigella spp*., including viral agents, as in Rota virus, Corona virus, and Adenovirus, along with parasitic causes, the most important of which are *Entamoebahistolytica*, *Giardia lamblia*, and yeasts such as *Candidaalbicans* (4). Diarrhea comes as a result of the entry of pathogens into the intestinal cavity of children through food, drinks, or hands contaminated with these pathogens, or as a result of the transformation of some members of the normal flora into pathogens when their proportion exceeds the normal limit due to a change in the intestinal environment as a result of taking a certain drug or the child infected with aone of the pathogens, which makes it easier for these microorganisms to cause disease (5). The risk of bacterial resistance to antibiotics in enteric bacterial pathogens is a predominant problem and is associated with the control of diarrhea.

Many researchers have mentioned high rates of bacterial resistance to many antibiotics commonly used in the treatment of inflammatory diarrhea, such as Ampicillin, Tetracycline, and Co-trimoxazol (6). Therefore, there is great importance to determining the type and pattern of antibiotic resistance to various bacterial factors causing diarrhea to make antibiotics given to treat cases of inflammatory diarrhea are more appropriate in the treatment of these children. Hence, the current study aimed to isolate and diagnose some bacterial causes of inflammatory diarrhea in less than five years of age and determine their sensitivity towards some antibiotics used in the treatment of cases of inflammatory diarrhea. Study the effect of some factors such as age, sex and monthly infections on prevalence of diarrhea.

Materials and Methods

1- **Sample collection:** Samples were collected from children attending Child's Central Teaching Hospital in Baghdad, whose ages ranged from less than five years, for the period from January 2022 to September 2022. The samples were collected from children in small, clean and dry plastic containers.

2- **Direct examination of the stool:** notes are recorded about each sample of stool, which includes consistency, color, presence of mucus, blood and pH .

3- **General examination of the stool under a microscope:** A clean glass slide was taken and a swab of the stool sample was placed on it, mixed with a drop of physiological salt solution 0.1 ml. The samples were examined under the microscope to detect the pus cell ,RBC and the polycystic phaseand nutrients for *E.histolytica* and *G. lamblia* and yeast cells .

4- **Culture of samples:** Samples were cultivated as quickly as possible if they were brought to the laboratory "to avoid decomposition or rotting of the sample orbacterial death within a short period, as happens with *Shigella spp*. The stool samples were cultured directly on salmonella - shigella agar medium, MaCconkey agar, EMB agar, blood agar and incubated at 37°C for 24 hours.

5- **Diagnosis of bacterial isolates:** Bacterial isolates were diagnosed according tothe standard bacteriological and biochemical tests (7,8).

6- **Testing the sensitivity of bacteria to antibiotics:** A sensitivity test for antibiotics was carried out by disk diffusion method according to Kirby – Bauer method (9)using antibiotic discs (Erythromycin Ampicillin, Gentamicin Rifampicin, Cefotaxime, Nitrofuration, Co-trimoxazole, Chloramphenicol Tetracycline) to perform a sensitivity test of the bacterial isolates under study.   The results were read by observing the zones of inhibition around the antibioticdiscs, and the results were interpreted according to the standards outlined by the Clinical and Laboratory Standards Institute (CLSI)(10).

Results and Discussion

(265) stool samples were isolated from children with diarrhea whose ages ranged from less than five years. The samples that gave bacterial growth were (198) samples, with a rate of (74.7%), while (67) samples, with a rate of (25.3%), did not give anybacterial growth**,** as shown in Table (1).

Table 1:The results of the primary isolation of children's stool samples and their percentage

Isolation results	Number	Percentage %
Growth	198	74.7
No growth	67	25.3
Total	265	100

►**Bacterial isolates from children with diarrhea:**

The results of the current study showed, as indicated in Table (2), that *Escherichiacoli* are the most common types of bacteria, with a rate of (52%), followed by *Salmonella spp.* (17%), *Shigellaspp* (10%). *Proteus mirabilis*(7%), *Klebsiellapneumoniae* (6%).*Compylobacterjejuni*(4),*Pseudomonasaeruginosa*(2.5%).and*Staphylococcus aureus* (1.5%). The reason for the dominance of *Escherichiacoli* bacteria is due to its possession of many virulence factors, the most important of which are Attachment and (AEF Effacing Factor and Fimbrial Adherence Factor (FAF), which enable it to adhere to the epithelial cells lining the intestine, as well as its ability to produce enterotoxins. As well as being one of the genera of the intestinal family, which it colonizes the human gastrointestinal tract, as the intestine is the main depot for them (11). As for the infection with *Salmonella spp*, it was in the second place, and this is due to the possession of Salmonella bacteria many virulence factors, including the ability to produce endotoxins and its possession of cilia and flagella, as well as the presence of the virulence antigen, which is responsible for the high ability of Salmonella bacteria to invade tissues, which has an important role in causing cases of diarrhea (12).

The result of the current study agreed with what was reached by (13) who isolated *Shigellaspp* bacteria, and it came in the third rank. The reason for this is that it possesses many harmful factors , including the intensity of its invasion of the mucous epithelial cells lining the intestines .It can produce various effective toxins, in addition to the ability of *Shigella*dysentertiae type 1 to produce exotoxin, which is attributed to the high virulence of this type of *Shigella* (14).

Table (2): Bacterial species isolated from children with diarrhea.

| Bacterial species	Number of isolates	Percentage %
Escherichia coli	103	52
Salmonella spp	34	17
Shigellaspp	20	10
Proteus mirabilis	14	7
Klebsiellapneumoniae	11	6
Compylobacterjejuni	8	4
Pseudomonas aeruginosa	5	2.5
Staphylococcus aureus	3	1.5
Total	198	100

►**Epidemiological factors:**

●**The age:** Table (3) shows the distribution of infections according to age groups, as the age group less than one year accounted for the highest rate of 62%, followed by the age group (1 - 2 years) in the rate of 17%, the age group (2 -3 years)with a ratio 11%, the age group (3 - 4 years) constituted 6.5%, and the lowest percentage was in the age group (4-5 years), which is 3.5%, meaning that The incidence of diarrhea decreased with increasing age, and this result agreed with what was reached (3) wherethe highest incidence of diarrhea in the first age group was 53.5%. Perhaps the reason for the emergence of the highest percentage of infection in the first age group is due to the incomplete immune system, weak physical structure, and the difficulty of diagnosis in children at these young ages (15).Possibly, the reason for the decrease in diarrhea infections with increasing age is due to the maturation and development of the children's immune system from switching from breast-feeding to regular food during weaning, and all this avoids the child from the various pollutants that are transmitted it through formula milk (16).

Table (3): Distribution of diarrhea infections by age group.

Age group	Number of infections	Percentage %
Less than year	123	62
1-2years	34	17
2-3years	21	11
3-4years	13	6.5
4-5years	7	3.5
Total	198	100

●**Thegender:**Figure (1) shows that infection is more prevalent in males (63%) than in females (37%) infections. It may be related to genetic factors in children, in addition to the involvement of bad health habits in causing diarrhea in children, which is related to both mother and child. There is difficulty in controlling the cleanliness of children and their hands, with reference to the increased activity of male children over females. Likewise, most of the scientific sources did not mention any logical scientific explanations. A study on the reasons for the difference in the incidence of diarrhea between males and females, and this result agrees with what has been reached (17).
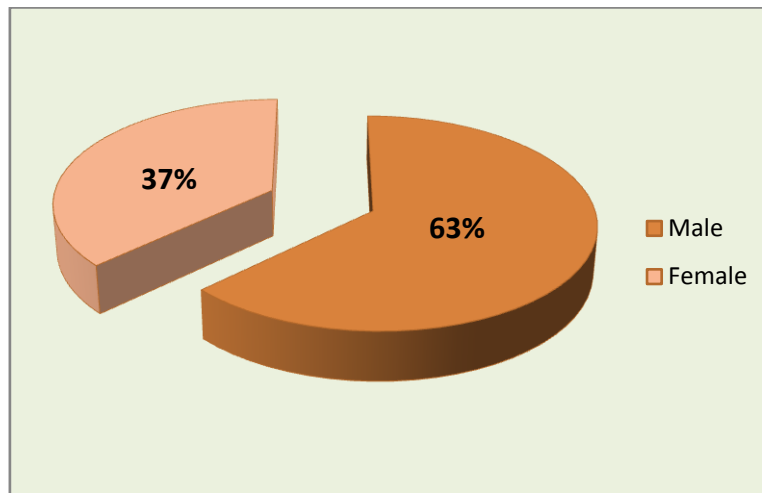
Figure(1): Distribution of diarrhea infections according to gender.

●**Months of the year:** The distribution of diarrhea infections was studied for the period fromJanuary 2022 to September 2022, where the results of the study showed the highest rate of infection (19%) in the month of August followed by July(17%) , June(15%) ,September(13%), May (11%), April(9%) , March(6.5%),February(5.5%) and lowest rate of infection was in the month of January at(4%), as shown in figure(2). Perhaps this is due to the fact that diarrhea rates rise during the summer season, as high temperatures encourage the growth of bacteria, especially if they are close to the optimum levels of growth, which helps to increase the reproduction of insects, which play a distinctive role in the transmission of Disease In addition to that there are many causes of diarrhea, including the low health awareness of the mother, especially with regard to the child and his personal hygiene (18).
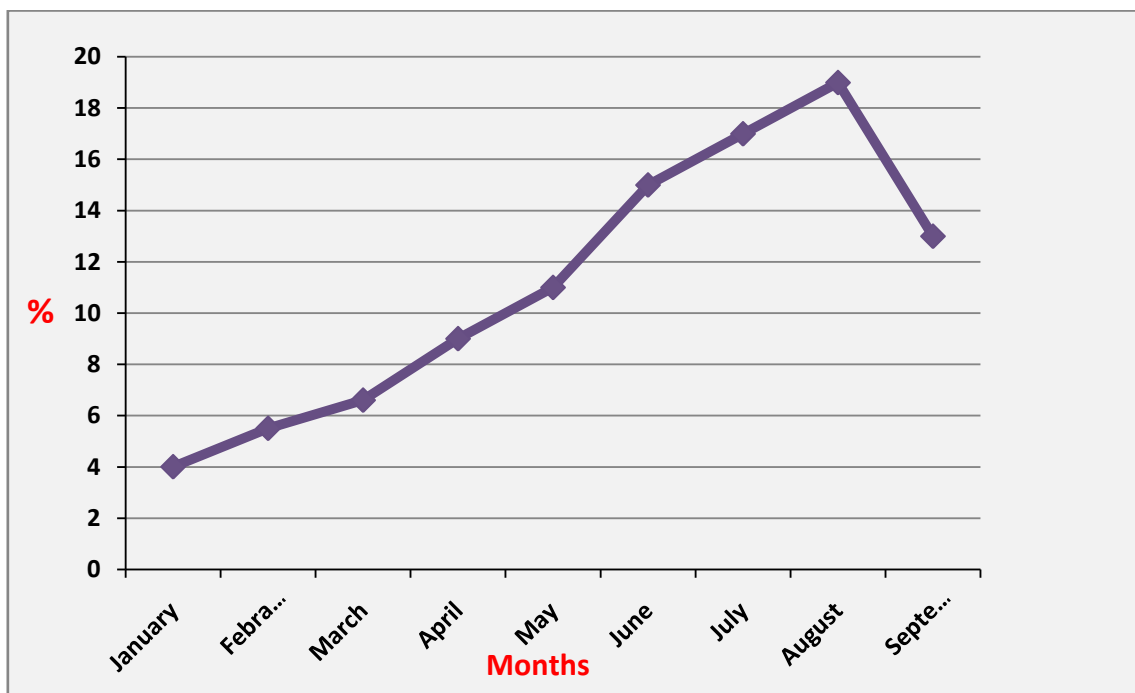
Figure (2): Distribution of diarrhea infections according to months of the year.

►**Resistance of bacterial isolates to antibiotics:**

The results of the current study showed that all bacterial isolates showed high resistance against the used antibiotics in the study figure (3). The highest resistance to Ampicilli0 was 100%, while the lowest percentage of resistance to Chloramphenicol, Erythromycin, and Co-trimoxazol was 21%, 17% and 14% respectively. The rates of resistance to Nitrofurantoin, Cefotaxime, Rifampicin, Tetracycline and Gentamicin were 91%, 85%, 77%, 61% and 56%, respectively. It is clear from the results of the current study that all bacterial isolates had high resistance to Ampicillin, due to the bacteria's ability to produce beta-lactamase enzyme. In addition to the unspecified and indiscriminate use of such treatments, one of the statistics in America indicated that more than 50% of antibiotics are prescribed to patients without conducting investigations for pathogens and testing for sensitivity to these antibiotics (19). Also, the genes responsible for the characteristic of antibiotic resistance can be transmitted by means of transmission of genetic information, and these genes are carried on the R-plasmid resistance plasmid that encodes resistance to many antibiotics (20). The result of the current study was consistent with what was reached (21), where they found that most of the bacterial isolates were sensitive to the antibiotics Co-trimoxazol, Erythromycin, and Chloramphenicol, and they considered these antibiotics the best treatment for diarrhea.
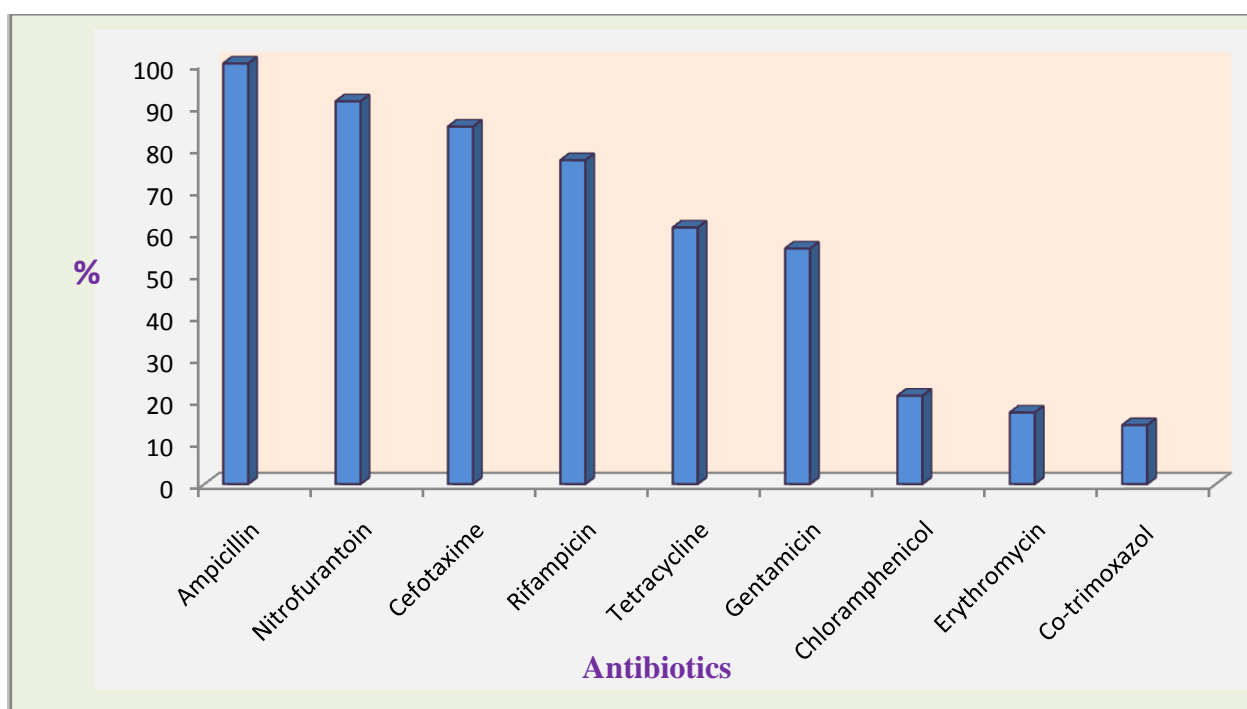

Figure (3): Antibiotic resistance of bacteria isolated from cases of diarrhea.

CONCLUSION

Diarrhea in children is a serious disease that can lead to death. Therefore, it is necessary to know the pathogens and conduct an antibiotic sensitivity test to develop an integrated treatment plan to limit the spread of resistant bacteria. Therefore, we need many future studies to reduce diarrhea infections in children.

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