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Isolation of Bacteria Associated with Acute Diarrhea in Children below Five Years Old and Studying resistance to Some Antibiotics in Basra Governorate

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Abstract

Diarrhea is a significant cause of illness and death in many parts of the world and people of all ages. Fecal-oral transmission and intake of food and water polluted with pathogenic organisms are the major causes of acute infectious diarrhea, based on research. This investigation was carried out to isolate various bacteria that cause diarrhea in children below 5 years, and 80 stool samples were collected. The study included the effect of age and area of residence on the incidence of diarrhea. These samples were planted on selective and differential culture media, which included the media of the MacConkey agar and blood agar, as well as the use of biochemical tests to diagnose these isolates using Vitek compact 2. The results of the study showed that 60 bacteria isolates belonging to seven different genera were obtained, *Escherichia coli*, *Salmonella Typhimurium*, *Klebsiella pneumonia*, *Shigella* spp, *Pseudomonas aeruginosa*, *Enterobacter aeruginosa*, and *Proteus mirabilis*. And at the rates of 23.33%, 20%, 16.66%, 16.66%, 15%, 5%, and 3.33%, respectively. The results showed that the age group (1-2 years) recorded a high percentage of diarrhea. The sensitivity test showed that all the isolates were 100% sensitive to Ciprofloxacin and 100% Amoxicillin resistant, while these isolates varied in their sensitivity to the rest of the antibiotics. Finally, this investigation confirmed that diarrhea in children under the age of five was caused by multiple-drug resistant, gram-negative enterobacteria.

Keyword

Diarrheal Bacteria, Vitek compact 2 system, Antibiotics resistance.



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1.Introduction

Diarrheal diseases are the leading cause of death among kids under the age of five globally, with roughly 2.5 million fatalities a year. The most prominent medical signs of diarrhea are severe stools with water in them that develop unexpectedly and go on for less than 7 or 14 days. It also includes the frequent passing of sloppy or wet feces with or without visible blood. The majority of cases involve vomiting and fever (1). Dehydration is a serious outcome of severe watery diarrhea, which may give rise to blood and tissue acidosis and death. The rotavirus enterotoxigenic *Escherichia coli*, *Shigella*, the bacteria *Campylobacter jejuni*, *Cryptosporidia*, *Vibrio cholerae*, and *Salmonella* are the primary causes of serious diarrhea that is watery among kids (2). *Shigella*, *Campylobacter jejuni*, *Salmonella*, and, in uncommon instances, the bacterium *Entamoeba histolytica* generate diarrhea, a form of diarrhea that includes blood vessels stains in stool, an eating disorder, and rapid loss of weight due to invaders destroying the lining of the intestines (3). Diarrhea is among the most serious medical worry in pediatric hospitals worldwide, and it is the most common sickness in children below the age of five, especially in developing nations, contributing to roughly 63% of the worldwide burden (4).

The environment and changes in the seasons affect the propagation of diarrhea and the proliferation and longevity of causal living things and their means of propagation (5). Bottle feeding for babies (ages 1-6 months) has been associated with hunger, lower immunity, and diarrhea (6). Nonetheless, completely breastfed kids have fewer episodes of diarrhea than partly breastfed babies, while completely weaning kids had the highest incidence (7). Bacteria produce the vast majority of infectious illnesses. The creation of laboratory methods for cultivating these microbes using an appropriate development medium called culture is essential for medical professionals to establish a successful therapy for their patients immediately (8). With increasing reports of resistance to antibiotics amongst bacteria in the gut, managing diarrhea caused by bacteria will be difficult. Antibiotics are found to treat over forty percent of all non-bacterial diarrhea diseases in children. A few investigations showed the function of bacteria diseases in children. A few studies revealed the role of bacteria instances with a 3% frequency along with elevated levels of resistance to routinely used medications (9). The present investigation aims to isolate bacteria that



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cause acute diarrhea in children under the age of five years and study their antibiotic resistance

2. Material and methods

Collection of sample

80 samples of feces were collected from cases of chronic diarrhea for children those arriving at the Al-Fayhaa Hospital and Teaching Hospital. ranging in age from less than five years old. and then transported directly to the laboratory.

Isolation and Identification

The samples were cultivated on MacConkey agar and blood agar, which were all incubated at 37°C for 18 to 24 hours. The forming colonies were examined under a microscope, and bacterial isolates were identified using appropriate biochemical tests and the Vitek 2 compact system to confirm diagnoses. After the isolated colonies developed on the medium, a suspension of bacteria was made in 3 mL of sterile saline (aqueous 0.45% - 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm transparent plastic (polystyrene) test tube, and the clarity of the suspension was determined to a McFarland standard of 0.5 using a VITEK-2 Densi Check instrument (10). The duration between the inoculum production and the issuance of certificates stayed within 30 minutes. The VITEK-2 compact system was identified using a card following the manufacturer's instructions (11). The 64-well plastic card comprises 41 tests, including Eighteen for sugar fermentation, two decarboxylase tests, and three additional tests (tryptophan deaminase, urease, and malonate utilization).

Antimicrobial susceptibility test

This test was carried out on a VITEK-2 compact system using an AST N281 card according to the directions provided by the manufacturer. The VITEK-2 AST N281 susceptibility card is intended for use in clinical laboratories as an in-vitro test for assessing the sensitivity of clinically significant aerobic gram-negative bacteria to antibiotics (12). Antibiotics assessed in AST N281 card comprised. Ceftriaxone, ephotaxime, Piperacillin/Tazobactam, Tobramycin , Colistin , Amikacin , Doripenem , Levofloxacin , Gentamicin ,Meropenem , icarcillin/Clavulnic acid , Cefepime , Imipenem , Tigecycline , Ceftazidime , Cefoperazone/Sulbactum , Ciprofloxacin , Minocycline, Trimethoprim/Sulfomethoxazole (Cotrimoxazole) and



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Cefuroxime, The identification cards were then flooded with inoculations (ready by transferring 200 μ L of cultures solution from the 0.5 McFarland culture solution used for fulfilling the identity cards to an uncontaminated 3mL sterile saline solution with an ending turbid of 8x10⁶ cfu/mL).

3. Results

Throughout the study, 80 stool samples were obtained from children with diarrhea. Growth of bacteria was seen in 60 samples, whereas 20 samples did not exhibit growth of bacteria, as indicated in Table (1).

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

All sample	Positive samples %	Negative samples %
80	60 (75%)	20 (25%)

Table (2) indicates the distribution of the number of children in diarrhea as a consequence of bacteria between the hospitals in Basra Governorate, in which the greatest number of infections was recorded in Al-Fayhaa (83%), followed by a Teaching hospital (80%), and a low percentage in the Basra General Hospital (66.6%). The findings indicate highly significant differences ($p < 0.005$).

Table (2): Distribution of Diarrhea from children in various hospitals in Basra Governorate

collected place	N0. Sample	Positive samples %	Negative samples %
Al-Fayhaa	30	24 (83%)	6 (20%)
Basra General Hospital	30	20 (66.6%)	10 (33.3%)
Teaching hospital	20	16 (80%)	4 (20%)



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Total	80	60 (75%)	20 (25%)
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The study's findings revealed an increased rate of bacterial diarrhea amongst children aged (1-2), (2-3), (3-4), and (4-5) years, at a ratio of (36.66%, 26.66%, 20%, and 16.66%), respectively, shown in Table 3. In this graph, the findings showed that positive samples increased significantly ($p < 0.005$) in all ages more than negative samples. In addition, positive specimens raised substantially ($p < 0.005$) in ages (1-2 years and 4-5 years) than in other times and age groups, whereas negative samples raised substantially in ages (1-2 years) greater compared to additional times of age.

Table (3): Distribution of children diarrhea in the study according to age

Age	Positive sample %	Negative sample%	Total
1-2 years	22(36.66%)	7 (35%)	29 (36.25%)
2-3 years	16(26.66%)	6 (30%)	22(27.5%)
3-4 years	12 (20%)	4 (20%)	16(20%)
4-5 Years	10(16.66%)	3 (15%)	13(16.25%)
Total	60(75%)	20(25%)	80(100%)

In the present investigation, 60 bacterial specimens were collected and examined for positive growth. They were assigned to 7 different kinds of pathogenic microbes that result in diarrhea among kids under 5 years, including 14 cases of isolated (23.33%) from *E. coli*, then 12 isolates (20%). *Salmonella typhimurium*: 10 isolates (16.66%) *Klebsiella pneumonia*: 9 isolates (15%) *Pseudomonas aeruginosa*: 10 isolates (16.66%) *Shigella* spp, three separate isolates (5%). *Enterobacter areogenosa*, two isolates (3.33%). *Proteus mirabilis* shows up in table (4).



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Table (4) Percentage isolation of bacteria Species

Bacterial species	No.Bacteria isolates	Ratio
<i>Escherichia coli</i>	14	23.33%
<i>SalmonellaTyphimurium</i>	12	20%
<i>Klebsiella pneumonia</i>	10	16.66%
<i>Pseudomonas aeruginosa</i>	9	15%
<i>Shigella spp</i>	10	16.66%
<i>Enterobacter areogenosa</i>	3	5%
<i>Proteus mirabilis</i>	2	3.33%
Total	60	(75%)

As shown in Figure (1), A and B isolates of bacteria reacted differently to the antibiotics used in the research, showing all of the samples are susceptible to Norfloxacin, followed by Ciprofloxacin, Trimethoprim, and Amoxicillin. A majority of samples were 100% susceptible to Ciprofloxacin and 100% resistant to Amoxicillin, although their susceptibility to the other drugs differed. In conclusion, our research showed that multiple-drug resistant, gram-negative enterobacteria was the cause of diarrhea in children below the tender age of five.



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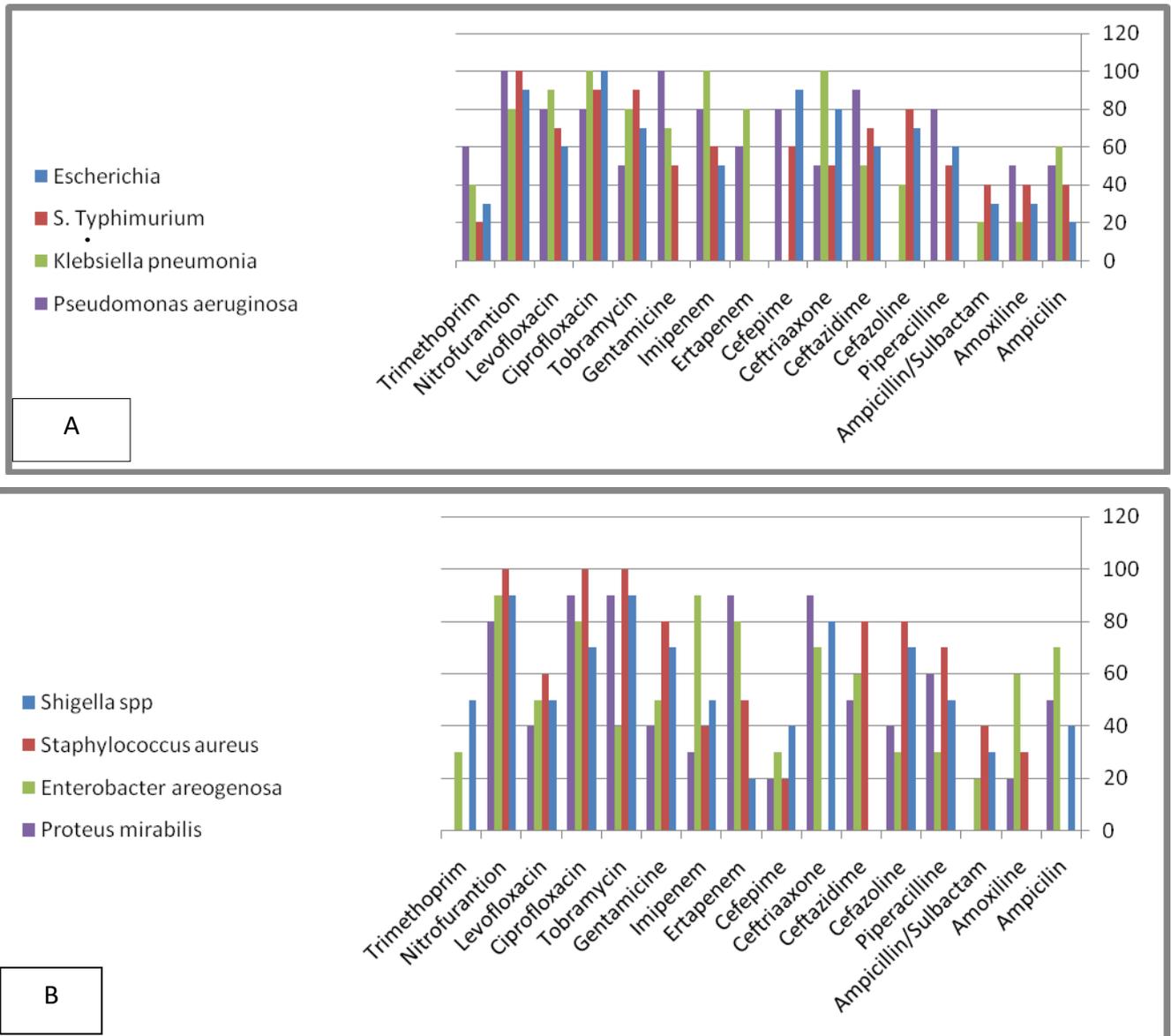


Figure (1): A and B Diagram showing the proportion sensitivities of the bacterial isolated from diarrhea towards a variety of antibiotics



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4. Discussion

Diarrhea was the most common disease in children, indicating an important health worry globally, especially as the second leading cause of death for children following respiratory illnesses (13). Children diarrhea below the threshold of five makes up roughly 63 percent of all global illnesses. Diarrheal pathogens must be accurately and timely identified. Conventional methods for identifying bacterial causes of diarrhea are arduous and time-consuming; however, these fail to identify diseases that are hard to grow in the laboratory. Previous research found that identifying the causal agent proved difficult in around forty percent of cases of diarrhea (14). The results of the study revealed that just sixty of the specimens (75%) were positive for growth of bacteria, whereas 20 samples (25%) displayed negative growth. Diarrhea is a serious illness for children below the age of five because of a significant amount of fluid in their bodies. Diarrhea reduces the body's electrolytes and fluids, producing dehydration, and is regarded as a serious condition needing medical attention in newborns, particularly when it is accompanied by temperature and endures for more than 24 hours (15).

The present research found that the average rate of diarrhea amongst hospitalized children in Women and Children Hospital / Al-Fayhaa / Basrah province was 24 (83%), significantly greater than previous findings released by other investigators (16). Yet, the current prevalence rate for diarrhea is reaching global records. It also discovered that diarrhea is the leading cause of child death across multiple countries, due to dangerous variables like infant feeding with bottles, polluted food and water, and insufficient hygiene and sanitation procedures. The present research also found that the largest prevalence of diarrhea was at one year 22 (36.66%). At the same time, the amount and severity of diarrhea differed among age groups. The results correspond with previous investigations (17). Diarrhea is more prevalent in children compared to adults globally, particularly among children under the age of five (18). These studies approved that diarrhea among children is highest in the age group less than 24 months and decreases with increasing age (19). Childhood diarrhea is mostly caused by a lack of antibodies to enterotoxin-producing bacteria such as *Escherichia coli*. Yet, the level of such antibodies rises increasing time as the exposure of kids to these microorganisms rises (20). The present study also revealed that *E. coli* had the highest proportion of isolated bacteria in feces, with 14 (23.33%), whereas the proteus



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mirabilis had the lowest rate: (3.33%). This finding is consistent with those of prior investigations (18). A recent study concluded that the high rate of bacterial isolation could be caused by milk and food pollution, climate variables, socioeconomic status, and environmental circumstances (21).

The present examination additionally found that the bacterial isolates are multi-drug resistant to antimicrobial disc in an artificially produced antibiotic sensitivity test. These results are consistent with prior research demonstrating the identification of antibiotic-resistant bacteria in stool biopsies of children with diarrhea. Recent research demonstrated that resistant *Escherichia coli* was isolated at an elevated rate (97.5%) (22). A further investigation confirmed the removal of multidrug-resistant intestinal coliform organisms from children's chronic diarrhea. Antibiotics ought to only be employed to treat *E. coli* and infections caused by *Salmonella* after a test for sensitivity to figure out the best course of action, as misuse of antibiotics may result in major antibiotic resistance problems (23). The research discovered that bacterial isolates were adaptable to different amounts of antibiotics. Antibiotic-resistant strains have grown more prevalent because of neglect (24).

The occurrence of antibiotic resistance events in separated pathogenic microbes is an excellent sign of the development of antibiotic resistance among people as a result of the overuse and misuse of antibiotics for the treatment of numerous illnesses with a prescription from a doctor. Resistance to antibiotics in microorganisms is intricate, extremely successful, and promotes the development of numerous medical difficulties, especially when it comes to the treatment of infections caused by bacteria (25). Antibiotic resistance can result from a variety of sources. They involve the unsuitable and at-random consumption of antimicrobial agents, the inability to follow the therapy stipulated by the trained physician, and the presence of several processes such as antibiotic impermeability, mutations, and changes in the structure of the infectious bacteria's outer protective membrane. Furthermore, the prevalence of antibiotic-resistant *Escherichia coli* allows for the successful or effective replacement of genetic material with other bacteria such as *Salmonella*, *Shigella*, *Yersinia*, and *Vibrio*. Furthermore, a chromosomal change affects the permeability of the cell membrane, reducing the accumulation of drugs or altering the drug's absorption characteristics (26). The foregoing demonstrates that the choice of antibiotic suitable for treatment is not at will, but rather relies on sensitivity testing of isolated microbes



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to choose the proper antibiotic to remove bacteria. To prevent tolerance and achieve optimal treatment effectiveness, an effective antibiotic against the infectious germ must be selected and its worth and dosage determined(27).

5. Conclusions

This study approved the high incidence of diarrhea in children under the age of five. On the other hand, a higher proportion of diarrhea was observed in one year than in other ages. *E. coli* was shown to have the greatest number of bacteria samples in comparison to *Salmonella typhimurium*, *Klebsiella sp.*, and *Shigella sp.* The in vitro antibiotic sensitivity test revealed that every single bacterial isolate was multidrug resistant.

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