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ARTICLE

Molecular Study of *Escherichia coli* Bacteria Isolated From Stool Samples of Children Less Than 5 Years Old in Anbar Governorate

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Abstract

Objectives: **Diarrhea is the leading infectious cause of childhood morbidity and mortality. Among bacterial agents, diarrheagenic *Escherichia coli* (DEC) is the major causal agent of childhood diarrhea in developing countries, particularly in children under the age of 5 years. Here, we performed a hospital-based prospective study to explore the pathotype distribution, epidemiological characteristics and antibiotic resistance patterns of DEC from < 5-year-old diarrheal children.**

Methods: **Stool samples were collected for 100 children suffering from gastroenteritis, and the bacterial species infecting the children were diagnosed using the Vitek2 compact system, sensitivity test, DNA extracted and Polymerase chain reaction (PCR) was performed, in addition to a and Serological test of isolated *E.coli*.**

Results: **In total, 100 samples were collected from children suffering from bacterial enteritis, and It was diagnosed by biochemical test and the Vitek2 compact and *E. coli* was the most common of the diagnosed bacteria With a percentage of 62%, as it showed resistance to Ceftazidime (50%), nalidixic acid (25%), and amikacin (30%), Azithromycin(20%),vancomycin(16%). Imipenem was found (8%). PCR was performed and results were obtained no results to (LT eltB1, STp estA1, STh estA2−4, ST1, LT1). The results of Serological test of isolated *E. coli* were of the isolates for children younger than two years, 8 out of 20 isolates were *Enteropathogenic E. coli*.**

Conclusions: **The presented research displayed the bacterium under consideration (*E. coli*) has a higher resistance rate to the commonly antibiotics used for bacterial gastroenteritis.**

Keywords: *Escherichia coli*, Diarrhea, Bacterial agents

1. Introduction

Bacterial gastroenteritis, sometimes referred to as bacterial food poisoning, is an infection of the intestines and stomach brought on by bacteria. It is a widespread ailment that can impact individuals of any age. Bacterial gastroenteritis usually manifests as symptoms 12−72 h after ingestion of tainted food or water. Bacterial gastroenteritis can cause the following symptoms: fever, loss of appetite, vomiting, diarrhea, cramping and pain in the abdomen, and bloody stools. Bacterial gastroenteritis can cause electrolyte imbalance, dehydration, and even mortality in extreme situations [1].

Complications of gastroenteritis: **Dehydration**, which can occur when you lose too much fluid through diarrhea and vomiting. Dehydration can be especially serious in infants and young children, as well as in older adults. **Malnutrition**, which can occur if you are unable to eat or drink enough due to gastroenteritis. **Intussusception**, a serious condition that can occur in infants and young children. It is caused by the telescoping of one part of the intestine into another part [2].

Causes of bacterial gastroenteritis: **Contaminated food or water:** The most common cause of bacterial gastroenteritis is consuming food or water that has been contaminated with bacteria. This can happen

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when food is not cooked properly, stored at the wrong temperature, or handled by someone who is sick., **Contact with infected person**: Bacterial gastroenteritis can also be spread through contact with an infected person. This can happen through direct contact, such as sharing food or drinks with someone who is sick, or through indirect contact, such as touching a surface that has been contaminated with bacteria [1]. Many bacterial, viral, and parasitic species can produce diarrhea, which is typically a sign of digestive tract illnesses. Worldwide, diarrhea has a wide range of characteristics and a seasonal distribution. Each year, there are around 1.7 billion episodes of diarrheal illness worldwide. This illness is a major source of morbidity and mortality among children and represents a substantial global public health issue [3].

Nevertheless, other research indicated that the predominant pathogenic agents linked to diarrhea [4,5] were enteric bacteria, such as DEC, *Salmonella* spp., *Escherichia coli* and *Shigella spp*., and *Klebsiella pneumonia*. Coastal zones were the sites of more frequent observations of *Vibrio parahaemolyticus* [6,7]

2. Materials and methods

2.1. Collection of stool samples

All specimens were taken from September 2022 to December 2022. Patients' information and history were recorded using a formal sheet questionnaire. Samples were collected from patients who attended al-Ramadi Maternity and Children Teaching Hospital. Samples were isolated from the stool of children under five years of age. and then cultured on media blood agar, macconkey agar, SSagar.

2.2. Isolation and identification of bacterial isolate

100 samples were collected from the excreta of infected children, and 62 isolates were identified as *E. coli*. Using morphological and cultural criteria on the MacConkey agar. The Vitek 2 compact automated system (Biomérieux, France) was used to confirm the identification.

2.3. Antimicrobial susceptibility test (AST)

The disk diffusion method, also known as the Kirby–Bauer method is a standardized system for testing the efficacy of antimicrobials against micro-organisms. Isolated pure colonies [2–4] were inoculated in brain-heart broth (2 ml) to obtain a bacterial suspension, which was compared with a McFarland turbidity tube 1 × 108 CFU/ml, Muller Hinton agar plates were inoculated using cotton swabs from that prepared suspension and incubated under standard condition. Anti-microbial discs were positioned upon the medium's surface.then incubated at 37 °C for 24 h. For interpreting the results, the Clinical and Laboratory Institute's standards were followed (Clinical and Laboratory Institute Standards 2022).

2.4. Molecular identification

To find some virulence genes, such as (LT eltB1, STp estA1, STh estA2–4, ST1, LT1) we use the PCR approach.

2.5. Extraction of bacterial DNA

With the aid of the Genomic DNA Mini Bacteria Kit supplied by the business Favrgen, Taiwan, the entire bacterial DNA was recovered from bacterial isolates. The DNA solution was kept cold until it was put to use in a PCR.

2.6. PCR amplifications

PCR amplification was used to identify the virulence genes present in *E. coli* isolates. The ALPHA & LIGO firm provided the PCR primers. Table 1 displays the PCR primer sequences and product size, TM(°C),.

Table 1. The size and sequences of primers used in current study.

Primers	Sequence (5'→3')	Tm(oC)	Product size (bp)
LT eltB1	LThF1 CATAATGAGTACTTCGATAGAGGAAC LTh R1 GAAACCTGCTAATCTGTAACCATCC	58	402
STp estA1	STpF1 ATGAAAAAGCTAATGTTGGCA STpR1 TTAATAACATCCAGCACAGGCA	58	239
STh estA2–4	estA2–4F AATTGCTACTATTCATGCTTTCAGGAC estA2–4R TCTTTTTCACCTTTCGCTCAGG	58	133
ST1	F- 5′-CTT TCC CCT CTT TTA GTC AG-3′ R- 5′-TAA CAT GGA GCA CAG GCA GG 3′	56	175
LT1	F - 5′-TTA CGG CGT TAC TAT CCT CTC TA-3′ R - 5′-GGT CTC GGT CAG ATA TGT GAT TC-3′	56	275

*F: Forward sequences, R: Reverse sequences.

2.7. Serological test of isolated E. Coli

Serological detection of isolated *E. coli* strains from human test material or other origin by slide agglutination.

Art. No	Product	Contains antibodies against	Liquid | Lyo.	Packing
TS2111 TS2111-01	**Anti-Coli I**	O 26:K 60, O 44:K 74, O 114:K90, O 125:K 70, O 142:K 86, O 158:K -	lyophilised lyophilised	1 ml 5 ml
TR2121 TR2121-01	**Anti-Coli II**	O 55:K 59, O 86:K 61, O 91:K -, O 111:K 58, O 119:K 69, O 126:K 71, O 127:K 63, O 128:K 67	lyophilised lyophilised	1 ml 5 ml
TR2131 TR2131-01	**Anti-Coli III**	O 25:K 11, O 78:K 80, O 103:K -, O 118:K -, O 124:K 72, O 145:K -, O 157:K -, O 164:K -	lyophilised lyophilised	1 ml 5 ml

Slide agglutination is a rapid and simple test that can be used to identify and characterize *E. coli* strains. The test is based on the principle of antigen–antibody binding. In this case, the antigen is a surface component of *E. coli*, and the antibody is a specific antiserum that has been raised against that component.

The test is performed by mixing a small amount of bacterial culture with a drop of antiserum on a glass slide. If the bacteria are of the same type as the antiserum, they will agglutinate, or clump together. This is because the antibodies will bind to the surface components of the bacteria, causing them to stick together. A positive result is indicated by the presence of visible agglutination within 1–2 min. The agglutination should be firm and should not break up when the slide is tilted. A negative result is indicated by the absence of agglutination [5].

3. Result and discussion

3.1. Isolation and identification of bacteria

100 samples were collected from the discharge of children with gastroenteritis from al-Ramadi Maternity and Children Teaching Hospital. The samples infected with bacteria were identified and diagnosed using morphological and cultural criteria and the Vitek2 compact automated system, 62 isolates of the *E. coli* bacteria were obtained, with a percentage of (62%).

3.2. Antimicrobial susceptibility testing

The result showed a highest resistance of *E. coli* to Ceftazidime(31) (50%), then amikacin(19) (30%), nalidixic acid [16] (25%), Azithromycin [13] (20%), vancomycin [10] (16%), Imipenem was found [5] (8%) as shown in Fig. 1.

3.3. Detection of ST1 and LT1 genes in E. coli

For every isolate under investigation, every test for these genes was negative. Some strains of *enterotoxigenic E. coli (ETEC)* carry the virulence factor ST1 gene. Diarrhea can be caused by ETEC (*E. coli* type E) bacteria, especially in underdeveloped nations and among travelers and children. A protein that aids ETEC in adhering to small intestinal cells is encoded by the ST1 gene. Depending on the strain of *E. coli*, different ST1 gene prevalences exist [8,9].

Another virulence factor present in certain ETEC strains is the LT1 gene. The heat-labile enterotoxin (LT) toxin is encoded by this gene. A protein called LT makes the intestines secrete more fluid, which results in diarrhea. Depending on the strain of *E. coli*, different LT1 gene prevalences exist. In contrast [10], estimate that it is approximately 30% in ETEC strains.

3.4. Detection of STp estA1 and STh estA2–4 genes in E.coli

The two genes of the sixty-two isolates that were the subject of our investigation yielded negative results. Certain strains of *enterohemorrhagic E. coli (EHEC)* carry the virulence factor STp estA1 gene. One kind of E. Coli called EHEC can result in severe diarrhea, bloody stools, and even fatality. A protein that aids in EHEC attachment to intestinal cells is encoded by the STp estA1 gene. Depending on the strain of *E. coli*, different STp estA1 genes are more or less common. But for EHEC strains, it's expected to be about 10% [11].

Other virulence factors present in certain strains of EHEC are the STh estA2-4 genes. These genes produce proteins that aid in the survival and pathogenicity of EHEC in the gut. Depending on the strain of *E. coli*, different STh estA2-4 genes have different frequencies. With a prevalence of about 20% in EHEC strains, they are thought to be more prevalent than the STp estA1 gene [11,12].

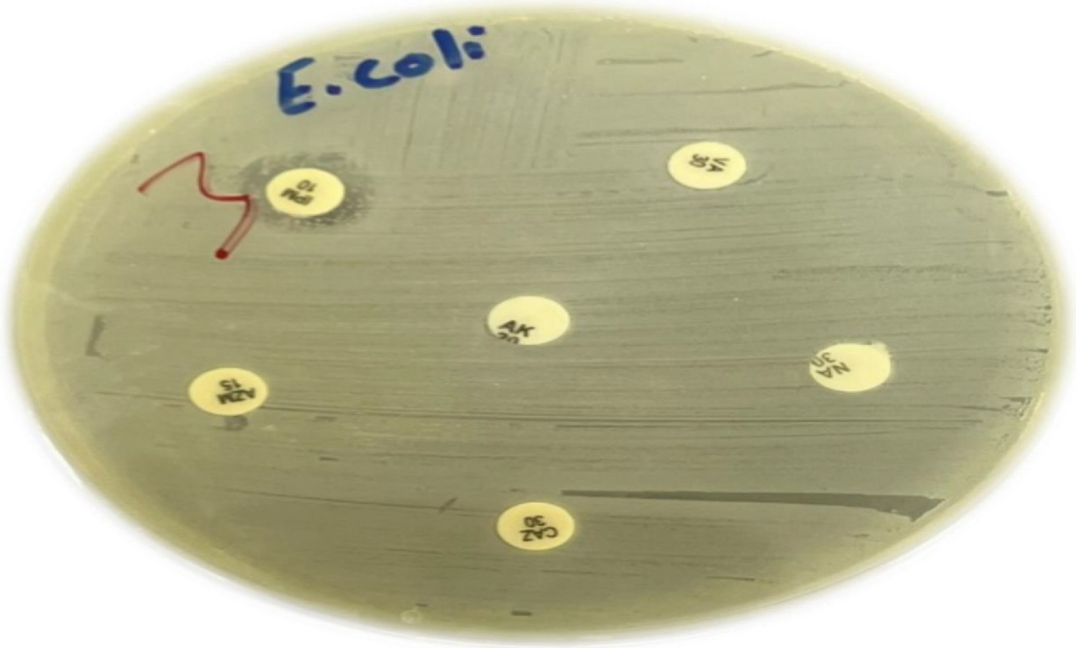

Fig. 1. Antibiotic resistance of Escherichia coli *on Molar Hinton Agar.*

3.5. Detection of LTeltB1 genes in E.coli

The results of our molecular investigation, which involved the use of uniplex PCR to detect LTeltB1 genes, revealed that 62 clinical isolates of *E. coli* were negative. Depending on the study and demographic, different *E.Coli* strains isolated from children under five years old have different prevalence levels of the LT eltB1 gene. On the other hand, the expected prevalence across the board is 20%. The low-temperature elongation factor B1 (LT-EF-B1) protein, an essential part of the bacterial protein translation machinery, is encoded by the LTeltB1 gene in *E.coli*. LT-EF-B1 is essential for ribosome translocation during peptide elongation because it makes the ribosome travel more easily along the mRNA molecule [13].

3.6. Serological test for E. coli

The lack of positive results for the LT1 and ST1 genes in *E. coli* strains led to the introduction of an alternate technique for strain identification, which is based on surface antigens, namely lipopolysaccharide (LPS) and flagella. We refer to this categorization scheme as serotyping. Of the twenty strains of *E.Coli*, only fifteen percent (3/20) belonged to the EPEC type 0.55 serogroups; ten percent (2/20) to the EPEC type for each of the 0111 and 0.25 serogroups, respectively; and five percent (1/20) to the EPEC strains 0.044 serotype. Furthermore, 60%, 12/20, were isolates of *E.coli*.

Only forty percent (8 out of 20) of the strains of atypical *enteropathogenic E. coli* (aEPEC) were found to be linked to the traditional EPEC serogroups in this study. This implies that many aEPEC strains would be difficult to identify using serotyping alone. However, serotyping is still a standard procedure in many clinical labs, including those in China. This may need immediate attention and raises questions about the accuracy of diagnoses. Interestingly, strains of aEPEC that were found in Mexico showed high levels of antibiotic resistance [14].

Similarly, considerable multidrug resistance was shown by aEPEC isolates recovered from a food-poisoning outbreak in China, exhibiting enhanced resistance to both quinolones and extended-spectrum cephalosporins [15]. The aEPEC strains in our investigation showed resistance to a wide range of widely prescribed therapeutic medications. Persistent diarrhea is defined as diarrhea that lasts for 14 days or longer, especially in children from developing nations who are younger than 3 years old. Roughly 10% of children in underdeveloped nations suffer from this kind of diarrhea, which can lead to severe weight loss. In fact, up to one-third to half of all diarrhea-related deaths may be caused by chronic diarrhea [16]. *Entropathogenic E.Coli* (EPEC),

EnteroAggregassive E.Coli (EAggC), and Cryptosporidium are the pathogens that cause persistent diarrhea [17].

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