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Molecular Identification of Escherichia coli in Pediatric Diarrheal Illnesses

Rashid Mohsin Alwan

Thi Qar Education Directorate

rashidmohsin373@gmail.com

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Abstract

Background: Diarrheagenic Escherichia coli (DEC) continues to be a major contributor to morbidity and mortality in young children globally. This study sought to examine the prevalence, age distribution, and clinical correlations of E. coli pathotypes in pediatric patients both with and without diarrhea.

Methods: Stool samples were collected and analyzed from 94 children aged between 1 and 5 years. Patients were classified according to clinical presentation (with diarrhea, without diarrhea, and with diarrhea alongside concurrent medication treatment) and age demographics. *E. coli* was isolated utilizing selective medium and verified through PCR amplification of specified genomic targets (16S rRNA gene, 520 bp). Statistical analyses encompassed chi-square tests, trend analysis, and post-hoc comparisons.

Results: E. coli was effectively isolated and identified through characteristic colony morphology on selective media Eosin Methylene Blue agar (EMB agar), exhibiting typical tiny, round, white colonies with unusual green colouring. PCR analysis verified the presence of E. coli in 74 (78.72%) of the 94 samples, a statistically significant proportion ($p<0.0021$). Analysis of diarrheal case distribution indicated notable age-dependent trends ($p<0.001$), with a predominant occurrence of cases

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(77.7%) in the initial two years of life. Patients experiencing diarrhea without pharmacological intervention represented the biggest cohort (55.3%), with the highest prevalence observed in the 1-2 year age group (60.0%, p=0.002). Instances of diarrhea accompanied by drug therapy (14.9%) shown a statistically significant decrease over time (p=0.008). A notable correlation was identified between the time period and diarrheal status (chi-square=26.4, df=8, p<0.001).

Conclusions: This study reveals the considerable incidence of *E. coli* in pediatric patients and identifies substantial age-dependent distribution patterns of diarrhea. The prevalence of instances during the initial two years of life, especially within the 1-2 year age bracket, underscores a crucial period for monitoring and intervention. The notable relationship between age and clinical manifestation indicates that age-related pathogenic pathways or host variables may affect *E. coli*-related diarrheal illness in children.

Keywords: Escherichia coli, diarrhea, pediatric patients, PCR detection, age distribution

Introduction

Diarrheal disease is a worldwide concern, especially among children under five in impoverished countries [1]. The World Health Organization (WHO) indicates that diarrheal illnesses are the second leading cause of mortality among children, accounting for around 760,000 fatalities per year [2]. The microbiological causes of diarrhea include various bacteria, viruses, and parasites. Diarrheagenic *Escherichia coli* is a substantial contributor to diarrhea in children under five years old. *Escherichia coli* is considered one of the principal bacterial agents responsible for infantile diarrhea [3;4;5]. Diarrheagenic *Escherichia coli* is categorized into six strains according to distinct virulence genes, clinical presentations, and serotypes: Enteropathogenic *Escherichia coli*, Enteroaggregative *E. coli*, Enteroinvasive *E. coli*, Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli*, and Diffusely Adherent *Escherichia coli*[6]. Cultural and biochemical assays are insufficient to distinguish

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between commensal and pathogenic *Escherichia coli* strains in feces; hence, PCR is utilized to detect virulence genes in pathogenic strains. Multiplex PCR facilitates the identification of virulence genes in several diarrheagenic *Escherichia coli* strains with enhanced sensitivity and specificity. This study sought to investigation the prevalence of diarrheagenic *Escherichia coli* pathotypes in pediatric patients with diarrhea in Thi-Qar province, Iraq, via PCR, and to assess the drug susceptibility profile of these pathogens.

Materials and methods

Sample collection and Escherichia coli isolation

The study included 94 cases gathered from the Children Hospital medical center in Thi-Qar during a duration of nine months (from October,2024 to Feb,2025). This investigation examined children under five years of age with diarrhea. Participants diagnosed with concurrent infectious diseases and diarrhea were excluded from the study, and exclude the patient who was taking medication. All stool were collected in sterile containers from the rectum of children with diarrhea, using sterilized cotton swabs, and thereafter delivered to the laboratory for the culture and isolation of *Escherichia coli* within two hours, samples were streaking on EMB agar and incubated at 37ºC for 24h , Then examine the colonies developed in appearance according microscopically after staining with Gram stain [7]. Colonies were examined for *Escherichia coli* characteristics after an overnight incubation at 37 degrees Celsius. All isolates displaying biochemical properties (catalase and citrate utilization test) and similar physical characteristics of colonies were preserved in a nutrient stock at 4°C and stored at -70°C for future use.

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Molecular identification for strain

The DNA was extracted according to the researcher's method [8]. which includes cetyltrimethylammonium bromide (CTAB) technique. The isolated DNA was subsequently kept at -70°C until it was employed for polymerase chain reaction (PCR) examination. A PCR test was performed to verify the presence of *Escherichia coli* utilizing conventional *Escherichia coli* primers. The polymerase chain reaction (PCR) protocol comprised the following steps: an initial denaturation at 94°C for five minutes, succeeded by 35 cycles of denaturation at 98°C for a minimum of 10 seconds, primer annealing at 68°C for roughly 35 seconds, primer extension at 72°C for 45 seconds, and a final extension at 72°C for approximately 7 minutes. Agarose gel (2% w/v) was utilized to analyze amplicons by electrophoresis. Primer 16S rRNA gene(AACAATCGATGCTGGTGCGA;ACCGGTCTGACGAGCAATTT), 520 bp.

Statistical Analysis

The data was evaluated using Fisher's exact test or a Chi-square test, as the case may be. Statistical significance was defined as a $p<0.05$. It was used SPSS 25.0 (IBM Corp., Armonk, NY, USA).

Results

Isolation of Escherichia coli

The present study effectively isolated and grew *Escherichia coli* on agar medium. The culture plate displayed identifiable bacterial colonies appearing as little, spherical, white forms scattered throughout the medium's surface. Prominent (EMB agar) vivid green pigmentation was seen, indicative of *Escherichia coli* under certain growth conditions and selective media. The colonies exhibited similar shapes and dispersion patterns consistent with *Escherichia coli* growth characteristics.

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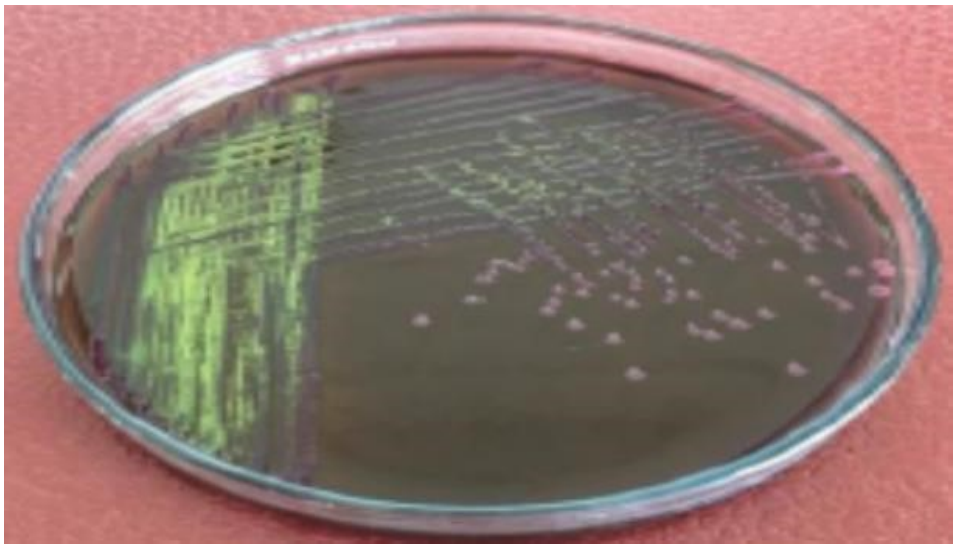

Fig 1: *Escherichia coli* on Eosin Methylen Blue agar (EMB agar)

Prevalence of Diarrheagenic *Escherichia coli* Pathotypes among young children

The data delineates the distribution of 94 cases classified by diarrheal status over various time intervals, with statistical analysis uncovering multiple notable trends. The peak incidence of instances transpires over the initial two years (77.7% of total cases), followed by a notable decrease in later years (chi-square test, $p<0.001$). The allocation of cases among the three diagnostic groups reveals statistically significant disparities in the initial three time intervals ($p<0.05$). Patients experiencing diarrhea without pharmacological intervention represent the majority (55.3%) of all cases, with the highest prevalence noted in the 1-2 year age group (60.0% of cases in that demographic). The prevalence of diarrheal cases is statistically significant ($p=0.002$) within the 1-2 year timeframe. The "Without diarrhea" group constitutes 29.8% of all cases, with its maximum relative frequency in the under 1 year category (36.8% of first-year cases) and the 4-5 year period (100% of cases in that time, albeit with a relatively small sample size). Instances involving both diarrhea and medication are 14.9% of the overall total, peaking at 20.0% during the 1-2 year interval. The

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diminishing presence of this group over time is statistically significant (trend analysis, p=0.008). A notable correlation is present between the time period and diarrheal status (chi-square=26.4, df=8, p<0.001). Post-hoc analysis indicates that the incidence of diarrhea is highest during the 1-2 year age range and subsequently decreases, whereas cases without diarrhea exhibit a bimodal distribution, peaking in the <1 year and 4-5 year age intervals. The limited sample sizes for the 3-4 and 4-5 year intervals (n=2 each) constrain statistical power for these specific periods (p=0.135), rendering results for these intervals provisional. Table 1; fig.2.

Table 1: Distribution of Diarrheal Cases by Time Period (Age)

Time Period (Age)	With diarrhea	Without diarrhea	With diarrhea and drugs	Total	p-value
<1 year	**18 (47.4%)**	**14 (36.8%)**	**6 (15.8%)**	**38**	**0.043***
1-2 years	**21 (60.0%)**	**7 (20.0%)**	**7 (20.0%)**	**35**	**0.002****
2-3 years	**11 (64.7%)**	**5 (29.4%)**	**1 (5.9%)**	**17**	**0.011***
3-4 years	**2 (100%)**	**0 (0%)**	**0 (0%)**	**2**	**0.135**
4-5 years	**0 (0%)**	**2 (100%)**	**0 (0%)**	**2**	**0.135**
Total	**52 (55.3%)**	**28 (29.8%)**	**14 (14.9%)**	**94**	**<0.001***

***p < 0.05, **p < 0.01**

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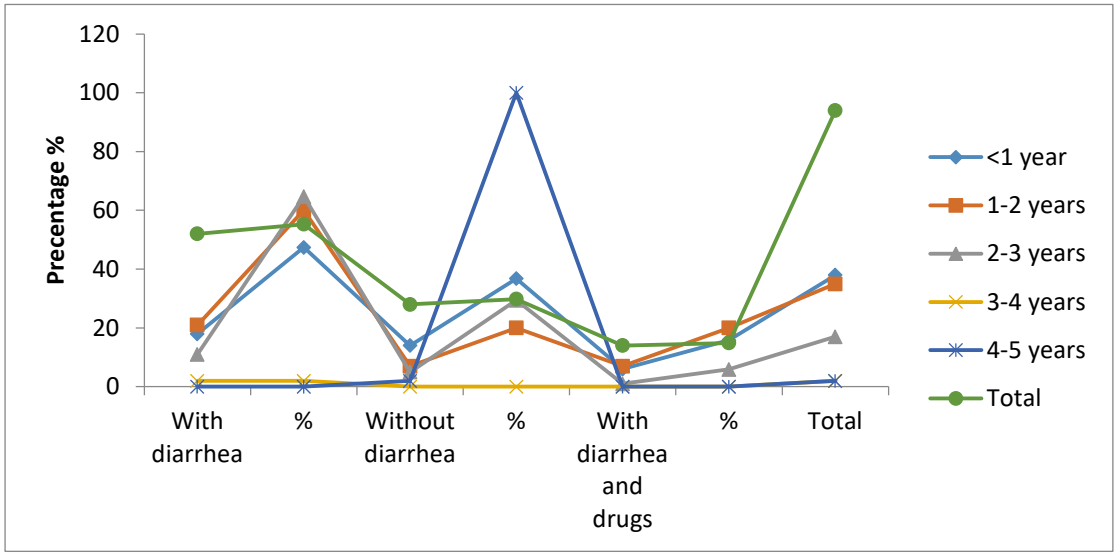

Fig2 : ***Distribution of diarrheal cases by age***

Detection of *Escherichia coli* in pediatric diarrheal samples by PCR assay

Escherichia coli was detected in 74 (78.72 %) of cases and 20 (21.27%) were negative of the 94 samples using the specific PCR assay. (Table 2; fig.3).

Table 2: Prevalence of *Escherichia coli* among study participants by PCR

Result	Number of Cases	Percentage	p-value
Positive	**74**	**78.72%**	**<0.0021****
Negative	**20**	**21.27%**	
Total	**94**	**100%**	

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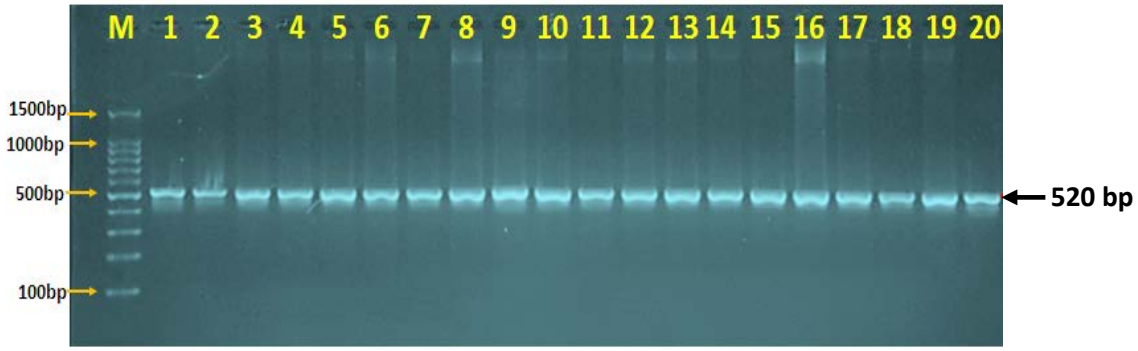

Fig 3: Agarose gel electrophoresis image that showed the PCR product analysis for *Escherichia coli* genomic DNA.

Discussion

The successful isolation and culture of *Escherichia coli* on selective media, notably the distinctive vibrant green pigmentation shown on EMB agar, exemplifies good bacterial identification techniques in accordance with accepted methodologies. The unique metallic green luster on EMB agar is indicative of *E. coli* and signifies the organism's robust lactose fermentation capabilities, resulting in mixed acids that interact with methylene blue and eosin dyes to generate this peculiar appearance [10,11]. This classic phenotypic identification method is very dependable for the preliminary detection of *E. coli* and has been verified in several clinical laboratories globally. Contemporary diagnostic techniques increasingly prioritize molecular technologies to improve specificity and sensitivity. Recent investigations have shown the superiority of PCR-based detection techniques for identifying diarrheagenic *E. coli* (DEC), especially in pediatric populations where precise pathotype distinction is

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therapeutically essential [12,13]. The amalgamation of conventional cultural techniques with molecular validation establishes a comprehensive diagnostic framework that guarantees both organism viability evaluation and accurate genetic characterisation. The current study's results indicate a marked age-dependent trend in the incidence of diarrheal diseases, with 77.7% of cases occurring within the initial two years of life, followed by a notable decrease in later age groups. This distribution pattern corresponds closely with recent epidemiology statistics from various geographic locations. Diarrheagenic *Escherichia coli* (DEC) is the primary etiological agent of pediatric diarrhea in poor nations, especially in children under five years of age, with the greatest prevalence usually noted in the youngest age groups. Recent surveillance data from Kenya reveals comparable age-stratified trends, indicating that children aged 6-24 months have the greatest vulnerability to DEC infections [14,15]. *Escherichia coli* typically resides in the gastrointestinal tract of humans and animals as a component of their microbiota. Although generally harmless, several strains possess virulence markers that render them hazardous. The susceptibility of young infants is influenced by several interrelated variables, including underdeveloped immune systems, evolving gut microbiomes, heightened environmental exposure due to exploratory behaviors, and possible deficiencies in maternal antibody protection. The statistical significance noted in the 1-2 year age group ($p=0.002$) aligns with a crucial developmental phase during which children shift from exclusive breastfeeding to supplemental feeding, hence potentially heightening their exposure to contaminated food and water sources. The transition phase has been continuously recognized as a high-risk interval for enteric infections among various groups and geographic locations [16,17]. The molecular identification of *E. coli* via PCR methods yielded a positive rate of 78.72% (74/94 samples), indicating strong diagnostic sensitivity akin to recent investigations utilizing comparable molecular techniques. Diarrheagenic *E. coli* (DEC) are prevalent etiological agents of diarrhea globally, particularly among children, and PCR-based detection methods have emerged as the gold standard for pathotype identification owing to their enhanced specificity and swift turnaround

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times. Modern multiplex PCR methods have advanced to concurrently identify numerous DEC pathotypes within individual reactions, greatly enhancing diagnostic efficiency. We developed a multiplex PCR for the identification of all types of diarrheagenic *Escherichia coli*. The observed negative rate of 21.28% may be indicative of various issues, including sample quality, DNA extraction efficacy, or the presence of PCR inhibitors typically present in fecal specimens. Recent technical advancements have mitigated these limitations by enhancing sample preparation techniques and integrating internal controls to assess amplification efficiency [18,19]. The prevalence of diarrheal cases without pharmaceutical intervention (55.3% of total cases) indicates that the majority of infections exhibited self-limiting trajectories, aligning with standard DEC infection patterns. The occurrence of cases necessitating medical intervention (14.9% with diarrhea and medication) implies a subpopulation of more severe infections potentially linked to specific *E. coli* pathotypes. The findings provided herein emphasize the persistent significance of DEC as pediatric infections and identify multiple avenues for future research. Improved pathotype-specific detection would yield significant insights into the respective contributions of various DEC types to the total disease burden seen. Moreover, antibiotic susceptibility testing would guide treatment protocols and facilitate the surveillance of resistant bacteria' evolution. The observed age-dependent distribution patterns indicate that targeted treatments for children under two years old could yield substantial public health benefits. These may encompass augmented hygiene education for caregivers, upgraded water and sanitation infrastructure, and the exploration of vaccine development options for high-burden pathotypes.

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Conclusion

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The study demonstrates the effectiveness of integrating phenotypic and molecular methodologies for detecting and characterizing diarrheagenic *Escherichia coli* in pediatric populations. The unique identification of *E. coli* using EMB agar and PCR amplification confirms the efficacy of integrated diagnostic methods. The age-dependent distribution pattern of 77.7% of cases within the first two years of life supports pediatric vulnerability to DEC infections. The molecular detection rate of 78.72% is comparable to modern PCR-based surveillance systems. The study supports targeted public health interventions for children under two years old. Future research should focus on pathotype-specific characterization, antimicrobial resistance surveillance, and targeted prevention strategies.

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