**Original Research Article**

**Prevalence, Molecular Characterization And Antibiotic Susceptibility Pattern of Enteropathogenic Escherichia coli (EPEC) Strains Associated with Pediatric Diarrhoea in Abuja, Nigeria**

**ABSTRACT**

**Aim:**

To determine the prevalence, molecular characteristics, and antibiotic susceptibility pattern of Enteropathogenic Escherichia coli (EPEC) strains isolated from children under five years of age presenting with acute diarrhoea in Abuja, Nigeria, and to evaluate the utility of combining serotyping with molecular diagnostics in EPEC identification.

**Study Design:**

Cross-sectional descriptive study.

**Place and Duration of Study:**

Abuja Municipal Area Council (AMAC), Federal Capital Territory, Abuja, Nigeria. Garki Hospital, Federal Medical Center, Abuja and National Hospital, Abuja between March 2022 and February 2023.

**Methodology:**

A total of 200 faecal samples were collected from pediatric patients (aged <5 years) with acute diarrhoea. E. coli was isolated using standard biochemical techniques. Confirmed isolates were serotyped with polyvalent and monovalent EPEC antisera. Molecular identification of the eaeA (intimin) and bfpA (bundle-forming pilus) virulence genes was carried out using polymerase chain reaction (PCR). Isolates were classified as typical EPEC (positive for both genes) or atypical EPEC (positive for eaeA only). Antimicrobial susceptibility testing was performed using the disc diffusion method for commonly used antibiotics.

**Results**:

Of the 200 stool samples analyzed, 19 (9.5%) were confirmed as EPEC. The most frequently detected serotype was O55:K59 (B5). Molecular analysis revealed that 5 isolates (26.3%) were positive for bfpA and 1 (5.2%) for both eaeA and bfpA genes, indicating a predominance of atypical EPEC strains. The remaining isolates lacked both virulence genes, suggesting the limitations of serotyping alone. Antimicrobial susceptibility testing showed 100% resistance to ampicillin, with high resistance to trimethoprim (89.5%) and tetracycline (84.2%). Ciprofloxacin, ceftazidime, and ceftriaxone exhibited the highest effectiveness. Multidrug resistance was observed in 26.3% of isolates.

**Conclusion**:

Atypical EPEC strains are more prevalent than typical EPEC in children with diarrhoea in Abuja. Combining molecular diagnostics with serotyping enhances detection accuracy. The high rate of antibiotic resistance, including multidrug resistance, underscores the need for routine surveillance to guide effective treatment and public health interventions.

**Keywords**: Enteropathogenic E. coli (EPEC), Virulence genes, eaeA, bfpA, Diarrhoea, Molecular diagnostics, Antibiotic resistance, Nigeria, Children under five

**1. Introduction**

Diarrhoeal disease remains a leading cause of morbidity and mortality among children under five years of age, particularly in low- and middle-income countries (LMICs) such as Nigeria. The United Nations Children’s Fund (UNICEF, 2024) reports that diarrhoea is responsible for approximately 9% of global childhood deaths, resulting in an estimated 444,000 fatalities annually, despite the availability of effective treatment interventions. Among the various etiological agents, Enteropathogenic *Escherichia coli* (EPEC) has been consistently implicated in paediatric diarrhoea, particularly in settings marked by inadequate sanitation, limited access to clean water, and suboptimal hygiene practices (Akinlabi *et al.,* 2023; Thakur et al., 2018).

EPEC lacks the shiga toxin unlike EHEC, which makes it a non-toxigenic pathotype of *E. coli* characterized by its ability to induce attaching and effacing (A/E) lesions on the intestinal epithelium. These lesions result from the intimate adherence of the bacterium to the mucosal surface, leading to the effacement of microvilli and subsequent disruption of nutrient absorption, which manifests clinically as watery diarrhea (Park et al., 2022). The pathogenicity of EPEC is closely associated with several virulence determinants, most notably the *eae* gene encoding intimin—a key adherence protein—and the locus of enterocyte effacement (LEE), a pathogenicity island that encodes a type III secretion system (Platenkamp & Mellies*,* 2018). This secretion apparatus enables the direct translocation of bacterial effector proteins into host enterocytes, triggering cytoskeletal rearrangements that culminate in lesion formation (Sirous et al., 2020).

Two subtypes of EPEC have been identified based on their genetic profiles: typical EPEC (tEPEC), which harbours both the *eae* and *bfpA* genes (the latter encoding the bundle-forming pilus), and atypical EPEC (aEPEC), which lacks *bfpA* but retains *eae*. Atypical strains have increasingly been recognized as emerging diarrhoeal pathogens, especially in developing countries, and their epidemiological and clinical significance warrants further investigation.

Accurate detection of EPEC is essential for both clinical diagnosis and epidemiological monitoring. While traditional culture-based methods such as growth on MacConkey agar and biochemical identification remain useful for isolating *E. coli*, they are insufficient for differentiating EPEC from other diarrhoeagenic *E. coli* pathotypes. Molecular approaches, particularly PCR targeting *eae* and *bfpA*, have emerged as the diagnostic gold standard due to their sensitivity and specificity. Complementary techniques, including enzyme-linked immunosorbent assays (ELISA) and immunofluorescence microscopy, are also employed for the identification of EPEC-specific antigens in clinical specimens (Hassan *et al.,* 2024).

Information on the aetiology of diarrheas is needed for epidemiological surveillance, devising preventive measures such as vaccinations, and empiric treatments, yet national surveillance programmes are either lacking or non-functional. Thus, the current understanding is based on limited research data merely covering a handful of countries (Mero et al., 2021). In light of the ongoing burden of diarrhoeal disease and the need for precise pathogen identification, this study aimed to determine the prevalence of EPEC among children with diarrhoea in Abuja, Nigeria, and to characterize their virulence gene profiles using molecular techniques.

**2. Materials and Methods**

**2.1 Study Design and Setting**

A cross-sectional descriptive study was conducted among children under five years of age presenting with diarrhoea at selected health facilities within the Abuja Municipal Area Council (AMAC), Federal Capital Territory, Nigeria. The primary objective was to determine the prevalence, molecular characteristics and antibiotic susceptibility pattern of typical and atypical *Enteropathogenic Escherichia coli* (EPEC) strains in this population.

**2.2 Sample Size Determination**

The sample size was calculated using the prevalence rate of diarrhoeagenic *Escherichia coli* (15%) previously reported among children under five in Gwagwalada, Abuja (Adebola *et al.,* 2014). Employing the standard formula for sample size estimation in cross-sectional studies described by Daniel (1999), a minimum of 200 samples was determined sufficient at a 95% confidence level and a 5% margin of error.

**2.3 Sample Collection and Transport**

Stool specimens were collected aseptically from children aged 0–59 months presenting with acute diarrhoea at participating hospitals. Following ethical clearance and parental consent, each sample was obtained in sterile, leak-proof stool containers, appropriately labeled with the patient's identification code, age, sex, and collection date. The samples were immediately transported on ice to the microbiology laboratory and stored at −20°C until processing.

**2.4 Bacterial Isolation and Identification**

Initial isolation of *E. coli* was carried out by culturing each stool sample on MacConkey agar (Oxoid, UK), followed by incubation at 37°C for 24 hours. Pink colonies indicative of lactose fermentation were subcultured onto Eosin Methylene Blue (EMB) agar and re-incubated under identical conditions to confirm the presence of *E. coli* through the observation of characteristic green metallic sheen colonies. Presumptive *E. coli* isolates were subjected to a series of standard biochemical tests, including indole, methyl red, Voges–Proskauer, citrate utilization, and triple sugar iron (TSI) reactions, to confirm their identity.

**2.5 Serological Identification of EPEC**

Biochemically confirmed *E. coli* isolates were serotyped using slide agglutination with commercial polyvalent and monovalent O:K antisera (Guangdong Huankai Microbial Sci. & Tech. Co. Ltd., China), according to the manufacturer's instructions. Initial screening was conducted with polyvalent O antisera specific for EPEC strains. Isolates testing positive were subsequently subjected to serotyping using monovalent antisera targeting the following serotypes: O55:K59(B5), O26:K60(B6), O127:K63(B8), O111:K58(B4), O126:K71(B16), O119:K69(B14), O86:K61(B7), O114:K90(B), O125:K70(B15), O128:K67(B12), O18c:K77(B21), O112:K66(B11), O142:K86(B), O124:K72(B17), and O44:K74(L).

**2.6 Molecular Detection of EPEC Virulence Genes**

Confirmed EPEC isolates were subjected to molecular analysis using polymerase chain reaction (PCR) to detect two key virulence-associated genes: *eaeA* (encoding intimin) and *bfpA* (encoding bundle-forming pilus). Genomic DNA was extracted using the Zymo Research Quick-DNA™ Miniprep Kit, following the manufacturer’s protocol. Primer sequences specific for each target gene and corresponding cycling conditions are detailed in **Table 1**.

**Table 1: Primer Sequences and PCR Conditions for Detection of EPEC Virulence Genes**

| **Target Gene** | **Primer Sequence (5’–3’)** | **Annealing Temp (°C)** | **No. of Cycles** | **Amplicon Size (bp)** | **Reference** |
| --- | --- | --- | --- | --- | --- |
| *eaeA* | F: CATTATGGAACGGCAGAGGT R: ATCTTCTGCGTACTGCGTTCA | 55 (1 min) | 35 | 790 | Beaudry *et al.,* 1996 |
| *bfpA* | F: AATGGTGCTTGCGCTTGCTCG R: GCCGCTTTATCCAACCTGGTA | 60 (1 min) | 30 | 326 | Gunzburg *et al.,* 1995 |

### Polymerase Chain Reaction (PCR) assays were conducted in a total reaction volume of 50 µL. Each reaction mixture contained 25 µL of 2X PCR Master Mix (Inqaba Biotec West Africa Ltd., 2024), 2 µL each of the forward and reverse primers, 2 µL of extracted DNA template, and 21 µL of nuclease-free water. The thermal cycling conditions began with an initial denaturation step at 94°C for 5 minutes. This was followed by repeated cycles of denaturation, primer annealing, and strand extension as outlined in Table 1. A final extension was carried out at 57°C for 7 minutes, after which the samples were held at 4°C.

### The PCR products were separated by electrophoresis using a 1.5% agarose gel prepared with ethidium bromide. The gel was run at 100 mV for 60 minutes, and a 100 base pair (bp) DNA ladder was used as a molecular size standard. DNA bands were visualized under ultraviolet (UV) light using a transilluminator.

### **2.8 Antibiotic Susceptibility Test**

Susceptibility of isolated EPEC strains to different antibiotics was determined by Kirby- Bauer disc diffusion technique as specified by the Clinical and Laboratory Standard Institute (CLSI, 2017). The antibiotic discs used in this study were Amoxicillin- clavulanate (20/10µg), ceftriazone (30µg), ceftazidime (30µg), gentamycin (10µg), tetracycline (30µg), ciprofloxacin (5µg), ampicillin (10µg), trimethoprim (5µg).

**3. RESULTS AND DISCUSSION**

**3.1 Prevalence of *Escherichia coli* Among Study Participants**

Out of the 200 stool samples collected from children under five years presenting with diarrhoea, 64 isolates (32%) were confirmed as pure cultures of *Escherichia coli* based on colony morphology and biochemical characteristics.

**3.2 Identification of Enteropathogenic *Escherichia coli* (EPEC) via Serotyping**

Among the 64 *E. coli* isolates, 19 strains (29.7%) were identified as belonging to classical EPEC serogroups using serological agglutination assays (Table 2). The most frequently detected serogroup was O55:K59 (31.5%), followed by O44:K74 (21.0%), O114:K90 (15.8%), and O128:K67 (10.5%). Less frequently observed serotypes included O86:K61, O111:K58, O125:K70, and O126:K71, each contributing 5.3% to the total. No isolates belonging to O26:K60, O119:K69, O127a:K63, or O142:K86 were identified.

**Table 2: Distribution of EPEC O-Serotypes Among Positive Isolates**

| **Serotype** | **Number of Isolates** | **Percentage (%)** |
| --- | --- | --- |
| O55:K59 | 6 | 31.5 |
| O26:K60 | 0 | 0.0 |
| O44:K74 | 4 | 21.0 |
| O86:K61 | 1 | 5.3 |
| O125:K70 | 1 | 5.3 |
| O114:K90 | 3 | 15.8 |
| O111:K58 | 1 | 5.3 |
| O126:K71 | 1 | 5.3 |
| O119:K69 | 0 | 0.0 |
| O127a:K63 | 0 | 0.0 |
| O128:K67 | 2 | 10.5 |
| O142:K86 | 0 | 0.0 |
| **Total** | **19** | **100.0** |

**3.3 Molecular Detection of EPEC Virulence Genes**

Of the 19 EPEC isolates, 10 were randomly selected for molecular analysis using PCR to determine the presence of two key virulence genes: *eaeA* (intimin) and *bfpA* (bundle-forming pilus). Among these, five isolates were positive for *bfpA* alone, and only one isolate harbored both *eaeA* and *bfpA*, identifying it as a typical EPEC strain. Four isolates tested negative for both genes (Table 3).

**Table 3: Distribution of EPEC Virulence Genes and Classification**

| **Isolate No.** | ***eaeA*** | ***bfpA*** | **Virulence Category** |
| --- | --- | --- | --- |
| 1 | + | + | Typical EPEC |
| 2 | – | + | Atypical EPEC |
| 3 | – | – | Negative |
| 4 | – | – | Negative |
| 5 | – | + | Atypical EPEC |
| 6 | – | + | Atypical EPEC |
| 7 | – | – | Negative |
| 8 | – | – | Negative |
| 9 | – | + | Atypical EPEC |
| 10 | – | + | Atypical EPEC |

*Key: + = gene detected; – = gene not detected*

**3.4 Antimicrobial Susceptibility Pattern of EPEC Strains**

A total of nineteen (19) *Enteropathogenic Escherichia coli* (EPEC) strains isolated from children with diarrhoea were subjected to antimicrobial susceptibility testing to determine their resistance profiles. The results are presented in Table 4. The isolates exhibited variable susceptibility to the panel of antibiotics tested. Ciprofloxacin showed the highest activity against the EPEC isolates, with 17 (89.5%) strains being sensitive. This was closely followed by ceftazidime and ceftriaxone, with susceptibility rates of 79.0% and 73.7%, respectively. Moderate sensitivity was also observed with gentamicin and amoxicillin-clavulanate (68.4%).

Conversely, the highest resistance rates were observed with ampicillin, to which all 19 isolates (100%) were resistant. High resistance was also recorded against trimethoprim (89.5%) and tetracycline (84.2%). These findings are consistent with earlier studies in Nigeria and other parts of Africa, which have reported increasing resistance to older, commonly used antibiotics such as ampicillin and tetracycline among diarrhoeagenic *E. coli* strains (Odetoyin et al., 2016).

Multidrug resistance (MDR), defined as resistance to at least three classes of antibiotics, was observed in 5 (26.3%) of the isolates. This poses a significant therapeutic challenge and underscores the importance of continuous surveillance and prudent use of antibiotics in clinical settings. The observed resistance pattern may be attributed to the widespread and often unregulated use of antibiotics in both hospital and community settings in Nigeria (Abatur et al., 2023).

These results highlight the need for region-specific antibiotic stewardship and routine monitoring of antimicrobial resistance in enteric pathogens, especially among vulnerable populations such as children.

**Table 4: Antibiotic Susceptibility Pattern of EPEC Strains**

| **Antimicrobial Agent** | **Sensitive n (%)** | **Resistant n (%)** |
| --- | --- | --- |
| Ampicillin | 0 (0.0%) | 19 (100.0%) |
| Amoxicillin-Clavulanate | 13 (68.4%) | 6 (31.6%) |
| Ceftriaxone | 14 (73.7%) | 5 (26.3%) |
| Gentamycin | 13 (68.4%) | 6 (31.6%) |
| Tetracycline | 3 (15.8%) | 16 (84.2%) |
| Ciprofloxacin | 17 (89.5%) | 2 (10.5%) |
| Ceftazidime | 15 (79.0%) | 4 (21.0%) |
| Trimethoprim | 2 (10.5%) | 17 (89.5%) |

**Key:** S – Sensitive, R – Resistant

**3.5. Discussion**

Understanding the etiological agents of childhood diarrhoea is critical for designing targeted public health interventions and advancing vaccine development. This study identified *Enteropathogenic Escherichia coli* (EPEC) as a contributing agent to paediatric diarrhoea in Abuja, Nigeria, with a prevalence rate of 9.5%. These findings align with earlier studies in the region that have also documented EPEC as a significant diarrhoeagenic pathotype in children (Akinlabi *et al.,* 2023; Abba & Dutsinma, 2021).

Serological analysis in this study confirmed the presence of eight serogroups among the 12 O-serotypes recognized by the World Health Organization (WHO) as classical EPEC strains (Trabulsi *et al.,* 2002). The dominant serogroup identified was O55:K59, which accounted for 31.5% of the EPEC isolates. This observation corroborates earlier findings that have reported a high frequency of this serotype in sub-Saharan Africa (Gomes *et al.,* 2016).

Molecular characterization of the virulence genes revealed a predominance of atypical EPEC (aEPEC) strains, defined by the presence of *bfpA* in the absence of *eaeA*. Five out of the ten tested isolates exhibited this gene pattern, while only one isolate possessed both *bfpA* and *eaeA*, qualifying it as a typical EPEC (tEPEC). These results suggest that atypical EPEC strains may now be more prevalent than their typical counterparts in this region—an emerging trend that has been observed both in Nigeria and internationally (Odetoyin *et al.,* 2016; Mwanga *et al.,* 2019; Snehaa *et al.,* 2021).

The increasing detection of aEPEC has significant epidemiological implications. Though the pathogenic role of aEPEC remains less clearly defined, multiple studies have implicated them in acute diarrhoeal episodes, especially among young children (Mandomando *et al.,* 2019; Olarinmoye *et al.,* 2018). Their apparent rise may reflect evolutionary adaptations allowing these strains to persist in new ecological niches and host environments, potentially leading to altered virulence gene profiles, such as the absence of *eaeA*.

Geographical variation in EPEC prevalence and genetic diversity underscores the need for continued regional surveillance. The findings in this study support the growing body of evidence highlighting the dominance of aEPEC in diarrhoeal infections, which may be due to either underreporting of *eaeA*-negative strains or genuine shifts in circulating genotypes.

**4. Conclusion**

This study demonstrates that *Enteropathogenic Escherichia coli* (EPEC) continues to play a significant role in paediatric diarrhoeal disease in Abuja, Nigeria. The overall prevalence of EPEC among diarrhoeic children under five years was 9.5%, with serogroup O55:K59 (B5) being the most prevalent. Molecular analysis revealed a higher occurrence of atypical EPEC strains (26.3%) compared to typical EPEC (5.2%), highlighting the emerging role of atypical variants in childhood diarrhoea. These findings underscore the need for diagnostic approaches that combine classical serotyping with molecular methods for accurate identification and characterization of EPEC strains.

In addition, antimicrobial susceptibility testing revealed a concerning resistance profile. All EPEC isolates showed 100% resistance to ampicillin, with high resistance also observed against trimethoprim (89.5%) and tetracycline (84.2%). Although ciprofloxacin (89.5%), ceftazidime (79.0%), and ceftriaxone (73.7%) demonstrated relatively high effectiveness, the detection of multidrug resistance (MDR) in 26.3% of isolates raises significant public health concerns. This level of resistance suggests the potential for treatment failure with commonly prescribed antibiotics and highlights the urgent need for rational antibiotic use, particularly in paediatric populations.

Overall, the study emphasizes the critical importance of continued epidemiological surveillance, molecular profiling, and antimicrobial resistance monitoring of EPEC strains. These measures are essential to inform public health strategies, guide empirical treatment protocols, and improve clinical outcomes in children suffering from diarrhoeal diseases in Nigeria and other resource-limited settings.

**ETHICAL APPROVALAND CONSENT**

**Ethical approval for the study was obtained from the Health Research Ethical Committee (HREC) at the Federal Ministry of Health, Abuja with approval number – FHREC/2023/01/221/01/221/01-11-23. Additionally, administrative permission was secured from the management of each participating health facility. Written informed consent was obtained from caregivers before the commencement of data and sample collection. Participants' confidentiality and the anonymity of their data were ensured throughout the research process.**

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

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