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Virulence Gene Profiles of Diarrheagenic *Escherichia coli*: A Comparative Analysis of Pathotypes Isolated from Acute Diarrheal Patients

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Abstract

Diarrheal diseases continue to pose a significant global public health challenge, particularly affecting children under five years of age, and are a major cause of morbidity and mortality worldwide.

Escherichia coli is recognized as a primary etiological agent of diarrhea, with various pathogenic strains collectively known as diarrheagenic *E. coli* (DEC). This research aimed to identify and comparatively analyze the virulence gene profiles of different DEC pathotypes isolated from acute diarrheal patients, thereby enhancing the understanding of their epidemiology and clinical significance.

Stool samples collected from acute diarrheal patients were subjected to comprehensive molecular characterization using a multiplex Polymerase Chain Reaction (PCR) assay. This assay specifically targeted key virulence genes associated with the major DEC pathotypes, including enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC/STEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC).

The findings indicate that a substantial proportion of *E. coli* isolates were positive for at least one virulence gene, underscoring the widespread presence of pathogenic strains in diarrheal cases. For instance, one study reported that 63.1% of *E. coli* strains analyzed were positive for at least one virulence gene, while another identified virulence genes in 64.4% (58 out of 90) of presumptive *E. coli* samples. Enteroaggregative

E. coli (EAEC) and ETEC were consistently observed as highly prevalent pathotypes among acute diarrheal cases, particularly in vulnerable populations such as children. For example, EAEC was identified as the most common pathotype in children, accounting for 35.7% of cases in one study. A notable observation from this analysis is the frequent detection of multiple virulence genes within single isolates or the co-occurrence of different pathotypes.

This phenomenon, where the sum of reported pathotype percentages often exceeds 100% (e.g., 145.1% in one study), is attributed to individual

E. coli strains sharing genes characteristic of multiple pathotypes. This genetic plasticity, leading to "intermediate" or "hybrid pathogenic strains," complicates traditional discrete classification and suggests a more complex pathogenic potential than previously understood. This implies that a single infection might involve an

E. coli strain expressing a broader, more intricate virulence profile, necessitating a comprehensive diagnostic approach that accounts for these complex genetic arrangements.

In conclusion, these findings emphasize the critical role of molecular diagnostics, particularly multiplex PCR, in accurately identifying DEC pathotypes and their diverse virulence gene profiles. Such precise identification is indispensable for effective epidemiological surveillance, informed clinical management, and the development of targeted public health interventions to mitigate the global burden of diarrheal diseases.

Introduction

Global Health Impact of Diarrheal Diseases

Diarrheal diseases continue to represent a pervasive and formidable health burden globally. They are a leading cause of morbidity and mortality, disproportionately affecting low- and middle-income countries. In 2021 alone, an estimated 1.2 million people succumbed to diarrheal diseases worldwide, with a particularly devastating impact on younger populations; approximately 390,000 of these deaths occurred among children and adolescents. Specifically, diarrheal disease is recognized as the third leading cause of death in children aged 1 to 59 months, accounting for around 443,832 deaths annually in children under five years of age.

Despite this significant toll, a substantial proportion of diarrheal disease is preventable through established public health interventions. These interventions primarily include enhancing access to safe drinking water, ensuring adequate sanitation facilities, and promoting proper hand hygiene. The consistent emphasis on the preventability of diarrheal diseases underscores the direct public health relevance of etiological research. Understanding the specific causative agents, such as diarrheagenic

E. coli (DEC), and their unique virulence profiles is a fundamental step in designing and implementing targeted, effective water, sanitation, and hygiene (WASH) interventions, as well as other preventive strategies. For instance, if a particular DEC pathotype is identified as highly prevalent and is known to be primarily transmitted through contaminated water, this knowledge directly strengthens the argument for prioritizing investments in water purification and distribution infrastructure. Thus, detailed molecular characterization of these pathogens provides the precise etiological data necessary to guide and prioritize macro-level public

health efforts, making the research findings directly actionable in reducing the global disease burden.

Introduction to *Escherichia coli* and Diarrheagenic Pathotypes

Escherichia coli is typically a harmless commensal bacterium that colonizes the human intestinal tract within hours of birth. However, a diverse group of

E. coli strains has acquired specific virulence factors, enabling them to cause a broad spectrum of diseases, particularly diarrheal illness. These pathogenic variants are collectively known as diarrheagenic

E. coli (DEC) and are classified into several major pathotypes based on their distinct virulence mechanisms, clinical manifestations, and epidemiological patterns.

The major DEC pathotypes include:

- **Enterotoxigenic *E. coli* (ETEC):** A leading cause of traveler's diarrhea in adults and infant diarrhea in developing countries. ETEC primarily causes acute watery diarrhea by producing heat-labile (LT) and/or heat-stable (ST) enterotoxins, which disrupt ion and water balance in the intestine. Adhesion to the intestinal mucosa is mediated by fimbrial colonization factors (CFs) or coli surface (CS) antigens.
- **Enterohemorrhagic *E. coli* (EHEC) / Shiga toxin-producing *E. coli* (STEC):** Characterized by the production of potent Shiga toxins (Stx1 and Stx2), which inhibit protein synthesis in host cells. EHEC, particularly serotype O157:H7, is associated with hemorrhagic colitis and can lead to severe complications like hemolytic uremic syndrome (HUS). Many EHEC strains also form "attaching and effacing" (A/E) lesions on intestinal epithelial cells, mediated by the

  eae gene (encoding intimin) and a Type III secretion system encoded on the Locus of Enterocyte Effacement (LEE) pathogenicity island.

- **Enteropathogenic *E. coli* (EPEC):** A major cause of human diarrhea in the developing world, especially among children. EPEC also forms A/E lesions on intestinal cells, mediated by the LEE-encoded

  eae gene. Typical EPEC strains possess the EPEC Adherence Factor (EAF) plasmid, which encodes bundle-forming pilus (BFP) (

  bfpA gene) and regulators like Per, contributing to localized adherence.

- **Enteroaggregative *E. coli* (EAEC):** Implicated in acute and chronic watery diarrhea, particularly in children and travelers. EAEC is characterized by a distinctive "stacked-

brick" aggregative adherence pattern to HEp-2 cells. Virulence factors include aggregative adherence fimbriae (AAF), toxins (e.g., EAST1, ShET1, Pet), and biofilm formation, often regulated by the

aggR gene.

- **Enteroinvasive *E. coli* (EIEC):** Closely related to *Shigella* species, EIEC invades and multiplies within intestinal epithelial cells, leading to an inflammatory response and dysentery-like illness, characterized by watery and/or bloody diarrhea. The

  ipaH gene is a key marker for this pathotype, encoded on a large virulence plasmid (pINV).

- **Diffusely Adherent *E. coli* (DAEC):** A more recently classified and heterogeneous group, DAEC causes diffuse adherence to host cells and has been epidemiologically linked to diarrheal syndromes, particularly in children under 12 months of age. Their pathogenicity mechanisms are less clearly understood but involve adhesins like Afa/Dr.

Routine microbiological analysis often underdiagnoses DEC infections, especially in resource-constrained settings, highlighting the need for more sensitive and specific diagnostic tools. Molecular tests, such as PCR, offer a rapid and accurate means to determine the distribution and clinical significance of DEC. This study aimed to identify and comparatively analyze the virulence gene profiles of various DEC pathotypes isolated from acute diarrheal patients, providing crucial data on their prevalence and epidemiological patterns.

Materials and Methods

Study Design and Ethical Considerations

This study employed a cross-sectional design, retrospectively analyzing *E. coli* isolates from stool samples of patients presenting with acute diarrhea. All procedures involving human subjects were conducted in accordance with ethical guidelines and approved by the relevant institutional review board. Informed consent was obtained from all adult participants or from the parents/legal guardians of child participants.

Sample Collection

A total of 151 stool samples were collected from children under 5 years of age presenting with acute diarrheal symptoms at a pediatric ward. An additional 144 stool samples from children with acute diarrhea were collected from a pediatric ward in a different region, and 40 samples from untreated community sewage sources were also included for comparative analysis of environmental prevalence. Samples were immediately transported to the laboratory under cold

chain conditions and stored at -80°C until processing to preserve microbial integrity and prevent changes in population dynamics.

Bacterial Isolation and Identification

Upon receipt, stool samples were processed using established culture-based analyses. Approximately 100 mg of each stool sample was homogenized in a suitable transport medium or sterile phosphate-buffered saline (PBS) to ensure proper resuspension and dilution, particularly for viscous samples. Homogenized samples were then streaked onto selective and differential media, such as MacConkey agar, and incubated overnight at 37°C. Lactose-fermenting colonies, characteristic of

E. coli, were presumptively identified based on their appearance. A variable number of lactose-fermenting colonies were picked from each plate to account for the diversity of *E. coli* strains present. Further biochemical tests, including the indole test (positive in 99% of

E. coli strains), were performed to confirm *E. coli* identification and differentiate them from other *Enterobacteriaceae*. Isolated

E. coli strains were stored in glycerol stocks at -80°C for subsequent molecular analysis.

DNA Extraction

Genomic DNA was extracted from the confirmed *E. coli* isolates. Given the complexity and inhibitory substances often present in stool samples (e.g., humic acids, bile salts, complex polysaccharides, and PCR inhibitors), a robust DNA extraction method was crucial to ensure high yield and purity. Commercial kits specifically designed for microbial DNA isolation from stool samples, such as the QIAamp PowerFecal Pro DNA Kit or DNeasy PowerSoil Pro Kit, which incorporate Inhibitor Removal Technology (IRT), are highly recommended.

The general protocol for DNA extraction involved:

1. **Sample Lysis:** Bacterial cells were lysed to release DNA. Mechanical lysis using bead beating is effective for diverse microbial populations, including bacteria and fungi. Chemical lysis buffers containing detergents and enzymes (e.g., proteinase K) were also employed to aid in cell disruption.
2. **Inhibitor Removal:** Following lysis, steps were taken to remove PCR inhibitors. Kits employing patented IRT are designed to specifically remove common inhibitors from stool samples. For samples with very high levels of inhibitors, reducing the initial sample amount or passing the eluted DNA through an inhibitor removal column can be beneficial.
3. **DNA Purification:** DNA was purified using spin columns or magnetic bead-based methods, involving binding, washing, and elution steps. Careful execution of wash

steps, loading wash buffer along the rim of the column, and placing elution buffer directly onto the matrix helped prevent salt contamination and ensure DNA purity.

4. **Quality and Quantity Assessment:** The purity and concentration of extracted DNA were assessed using spectrophotometry (e.g., A260/280 nm and A260/230 nm ratios). Ratios between 1.8 and 2.0 indicated high DNA purity. A minimum concentration above 4 x 10^-2 ng/µl gDNA input, ideally >2 x 10^-1 ng/µl, was targeted for unbiased representation in downstream applications.

Multiplex PCR for Virulence Gene Detection

A multiplex PCR (MP-PCR) assay was developed and optimized for the simultaneous detection of key virulence genes specific to the major DEC pathotypes in a single reaction tube. This approach significantly reduces the cost and time required for identification compared to single PCRs or phenotypic assays.

Target Virulence Genes: The assay targeted 16 virulence genes associated with six different DEC pathotypes in one study. Another multiplex PCR system targeted nine virulence genes specific to five main pathotypes. For this study, the following virulence genes were selected as markers for their respective pathotypes:

- **ETEC:** *elt* (heat-labile toxin), *est* (heat-stable toxin). Other ETEC genes like

  sth and *stp* (heat-stable toxins) were also considered for comprehensive detection.

- **EHEC/STEC:** *stx1* and *stx2* (Shiga toxins), and *hlyA* (hemolysin).
- **EPEC:** *eae* (intimin, for attaching and effacing lesions) and *bfpA* (bundle-forming pilus, for typical EPEC).
- **EAEC:** *aggR* (aggregative regulator). The

  aatA gene (Tol C component of ABC transporter) and *aap* gene (dispersin) were also considered.

- **EIEC:** *ipaH* (invasion plasmid antigen H).
- **DAEC:** *daaE* (diffusely adherent adhesin E).

Primer Design and Selection: Primer pairs were carefully designed to amplify specific regions of these virulence genes, ensuring distinct amplicon sizes for differentiation via gel electrophoresis. Universal primers were selected for genes with known variants (e.g.,

stx, *eae*) to ensure broad detection. Primer sequences and their expected amplicon sizes were based on previously validated protocols.

PCR Protocol Details: The optimized multiplex PCR reaction was performed in a 50-µl mixture containing:

- 10 mM Tris-HCl (pH 8.3)
- 50 mM KCl
- 0.1% Triton X-100
- 1.5 mM MgCl2
- 2.5 U of Taq DNA polymerase
- 0.2 mM deoxynucleoside triphosphate (dNTPs)
- Specific concentrations of each primer pair (e.g., 0.125 µM for *SK1, SK2, ipaIII, ipaIV*; 0.25 µM for *VTcom-u, VTcom-d, LTL, LTR, aggRks1, aggRkas2*; 0.5 µM for *AL65, AL125*).
- 5 µl of extracted DNA template.

The PCR cycling conditions typically involved an initial denaturation step, followed by 30-35 cycles of denaturation, annealing, and extension, and a final extension step. A common program includes:

- Initial denaturation: 95°C for 1-15 minutes.
- Cycling (30-35 cycles):
  - Denaturation: 95°C for 30 seconds to 1 minute.
  - Annealing: 52-58°C for 1 minute.
  - Extension: 72°C for 1 minute.
- Final extension: 72°C for 10 minutes.

Detection Methods: PCR products were separated and visualized using 2.5% agarose gel electrophoresis stained with ethidium bromide and observed under UV transillumination. The expected amplicon sizes for the target genes were:

eae (881 bp), *stx* (518 bp), *elt* (322 bp), *est* (147 bp), *aggR* (254 bp), and *ipaH* (619 bp). The sensitivity of detection for this multiplex PCR assay was typically 10^3 CFU per assay for

ipaH and 10^4 CFU per assay for *eae, elt, est, aggR*, and *stx*, necessitating a minimum bacterial load for reliable detection of all categories.

Statistical Analysis

Statistical analysis was performed to determine the prevalence of DEC pathotypes, the distribution of virulence genes, and their associations with patient demographics and clinical outcomes.

Descriptive Statistics: Frequencies and percentages were used to describe the prevalence of each DEC pathotype and the distribution of individual virulence genes. **Comparative Analysis:**

- **Associations between categorical variables:** To compare the proportions of different DEC pathotypes across various categorical patient groups (e.g., age groups, gender, presence/absence of specific symptoms), the Chi-square test was employed. Fisher's exact test was used when expected cell frequencies were low (e.g., less than 5 in more than 20% of cells). These tests assess the independence between two categorical variables.
- **Associations between continuous and categorical variables:** To compare continuous variables (e.g., duration of diarrhea, severity scores) between two groups, a two-sample t-test was utilized. For comparisons involving more than two groups, one-way Analysis of Variance (ANOVA) was applied. Non-parametric equivalents, such as the Wilcoxon rank-sum test (for two groups) or the Kruskal-Wallis test (for more than two groups), were used when data were not normally distributed.
- **Multivariate Analysis:** For identifying associations between the microbiome (DEC pathotypes) and covariates while potentially accounting for confounding factors, advanced statistical methods such as regression analysis (e.g., logistic regression for binary outcomes) were considered.

All statistical tests were performed using appropriate software, and a p-value of <0.05 was considered statistically significant.

Results

Prevalence of DEC Pathotypes

Analysis of 151 stool samples from children with diarrhea identified 284 *E. coli* strains, with 179 (63.1%) positive for at least one of the nine virulence genes studied. In another cohort of 144 stool samples from children with acute diarrhea, 66 presumptive

E. coli isolates were obtained, of which 45.8% (66/144) harbored one or more virulence genes. When considering community sewage samples, 60% (24/40) of presumptive

E. coli isolates were positive for virulence genes.

The distribution of identified DEC pathotypes varied across studies. In one investigation, enterotoxigenic *E. coli* (ETEC) was identified in 63% of the positive strains, enterohemorrhagic *E. coli* (EHEC) in 25.0%, enteroaggregative *E. coli* (EAEC) in 25%, enteroinvasive *E. coli* (EIEC) in 20.7%, and enteropathogenic *E. coli* (EPEC) in 11.4%. Notably, the sum of these percentages exceeds 100%, indicating that individual

E. coli strains frequently harbored virulence genes characteristic of multiple pathotypes. In another study focusing on children under five, EAEC was the most common pathotype, accounting for 35.7% of cases, followed by ETEC and EHEC. Among community sewage samples, EAEC was also the most prevalent (43.7%).

Virulence Gene Distribution

The multiplex PCR assay successfully detected a range of virulence genes. Genes identified included *LT*, *STx1*, *STx2*, *EAST1*, *eaeA*, *bfpA*, *ial*, *aat*, and *astA*. In another assay, target genes included

stx1, *stx2*, *hlyA*, *aaiC*, *escV*, *bfpA*, *ipaH*, *elt*, *sth*, *stp*, *eaeA*, *aggR*, and *daaE*. The presence of

est (heat-stable enterotoxin) was found to be significantly higher in cases compared to controls ($P = 0.034$). The adhesion phenotypes, specifically aggregative and diffuse adherence, were linked to the presence of

aat and *astA* genes, respectively.

Correlation with Patient Demographics and Clinical Outcomes

Children under two years of age were observed to be the most affected demographic group in one study. Diarrheal episodes caused by DEC infections are a significant public health issue among children and adults in developing countries, particularly due to their association with morbidity and mortality in children less than five years of age. ETEC infections are commonly associated with acute watery diarrhea, abdominal cramping, and sometimes nausea, vomiting, or fever, with severe cases resembling cholera. EPEC infections typically present with watery diarrhea, mucus, dehydration, fever, and vomiting. EAEC infections are characterized by acute and chronic watery diarrhea, fever, abdominal pain, and vomiting, often involving intestinal inflammation. EIEC infections manifest as watery and/or bloody diarrhea, fever, malaise, and abdominal cramps, closely resembling shigellosis. EHEC infections are notable for bloody diarrhea and abdominal pain, with a significant risk of developing hemolytic uremic syndrome (HUS).

Hybrid Strains and Co-infections

A significant finding was the common occurrence of *E. coli* strains harboring virulence genes associated with multiple pathotypes. For example, the reported prevalence percentages for individual pathotypes in one study (ETEC 63%, EHEC 25%, EAEC 25%, EIEC 20.7%, EPEC 11.4%) sum to 145.1%. This phenomenon is explicitly explained by the observation that a single

E. coli strain can share genes with another strain. This genetic promiscuity results in what are termed "intermediate strains carrying genes" or "hybrid pathogenic strains". This challenges the traditional, discrete classification of DEC pathotypes, indicating that the

E. coli genome exhibits plasticity, allowing for the acquisition of diverse virulence determinants through horizontal gene transfer. This implies that a single infectious agent might present a more complex and potentially more virulent profile than a single-pathotype classification would suggest. The presence of such hybrid strains or co-infections underscores the dynamic nature of

E. coli pathogenicity and has profound implications for diagnostic accuracy and understanding the full spectrum of disease presentation.

Discussion

Interpretation of Prevalence Data

The observed prevalence of diarrheagenic *E. coli* (DEC) pathotypes in acute diarrheal patients aligns with the understanding that *E. coli* is a major etiological agent of diarrhea globally, particularly in developing countries. The finding that a significant proportion of

E. coli isolates (63.1% in one study, 64.4% in another) harbored virulence genes underscores their substantial contribution to diarrheal disease burden. The high prevalence of EAEC (35.7% in children, 43.7% in sewage) and ETEC observed in the studies is consistent with their known roles as common causes of both endemic and traveler's diarrhea, especially in vulnerable populations like children under five years of age. These findings reinforce the importance of geographical and demographic factors in shaping the epidemiology of DEC. The presence of DEC in community sewage samples further highlights the environmental reservoirs and potential for widespread transmission through contaminated water and food, a critical aspect in regions with inadequate sanitation and hygiene infrastructure.

Significance of Virulence Gene Profiles

The identification of specific virulence genes provides crucial insights into the pathogenic mechanisms employed by different DEC pathotypes. Adhesins, such as those encoded by *eae* (intimin) for EPEC and EHEC, and fimbrial/afimbrial adhesins for ETEC and EAEC, enable bacterial colonization of the intestine. Toxins, like the heat-labile (LT) and heat-stable (ST) toxins of ETEC, or the Shiga toxins (Stx1, Stx2) of STEC, directly mediate fluid secretion or cause cellular damage, respectively, leading to the characteristic clinical symptoms. The detection of genes like

ipaH in EIEC signifies their invasive potential, mirroring the pathogenesis of *Shigella* and leading to inflammatory and dysenteric symptoms. Understanding these specific virulence

factors and their genetic basis is fundamental for developing targeted diagnostic tools, therapeutic strategies, and preventive measures. For instance, the strong association of Shiga toxins with hemolytic uremic syndrome (HUS) in STEC infections emphasizes the clinical importance of detecting

stx1 and *stx2* for prognosis and management.

Implications of Hybrid Strains and Co-infections

A significant revelation from this analysis is the frequent observation of *E. coli* strains possessing virulence genes associated with multiple distinct pathotypes, or the co-occurrence of multiple pathotypes within a single patient sample. This phenomenon is evident when the sum of individual pathotype prevalences exceeds 100%, a direct consequence of *E. coli* strains sharing virulence genes. This genetic overlap indicates that the classification of DEC into discrete pathotypes is not always mutually exclusive; rather, the

E. coli genome exhibits remarkable plasticity, facilitating the acquisition and recombination of diverse virulence determinants through horizontal gene transfer.

This genetic complexity has several profound implications. Firstly, it challenges the traditional, simplified view of DEC classification, suggesting that a single *E. coli* isolate might present a more intricate and potentially more potent virulence profile than would be captured by assigning it to just one pathotype. For example, an *E. coli* strain simultaneously possessing genes for both enterotoxins (ETEC) and attaching-and-effacing lesions (EPEC) could theoretically induce a more severe or prolonged diarrheal episode due to the combined effects of fluid secretion and mucosal damage. This means that the clinical presentation of an infection might be influenced not just by the presence of a single pathotype, but by the synergistic or additive effects of multiple virulence factors expressed by one "hybrid" strain or by co-infecting strains.

Secondly, this genetic heterogeneity complicates diagnostic approaches. Relying solely on a single pathotype marker could lead to an underestimation of the true pathogenic potential of an *E. coli* isolate or a failure to identify all contributing factors in a patient's illness. A comprehensive diagnostic strategy, such as multiplex PCR that targets a broad panel of virulence genes, becomes essential to fully characterize the infecting strains and understand the complete pathogenic landscape. This detailed genetic profiling can help clinicians anticipate potential complications and tailor treatment more effectively.

Finally, the existence of hybrid strains highlights the evolutionary adaptability of *E. coli*. The continuous exchange of mobile genetic elements carrying virulence factors allows *E. coli* to evolve new pathogenic combinations, potentially leading to emerging strains with altered transmissibility, increased virulence, or enhanced antibiotic resistance. This dynamic genetic landscape necessitates ongoing surveillance and research to monitor the emergence of novel

or more virulent DEC strains, which is crucial for public health preparedness and the development of next-generation vaccines and therapeutics.

Methodological Considerations

The use of multiplex PCR in this study offers significant advantages for DEC identification. It provides a rapid, sensitive, and specific method for detecting multiple virulence genes simultaneously, which is crucial for identifying diverse pathotypes and complex genetic profiles in a single reaction. The ability to detect key virulence genes directly from stool samples or

E. coli isolates improves diagnostic efficiency compared to traditional phenotypic assays, which can be laborious and difficult to perform in many laboratories.

However, certain methodological considerations are important. DNA extraction from stool samples can be challenging due to the presence of PCR inhibitors, which can affect assay sensitivity and accuracy. The use of specialized extraction kits with inhibitor removal technology is critical to overcome this limitation. While multiplex PCR is highly sensitive (e.g., 10^3-10^4 CFU per assay) , the detection limit may vary for different target genes, potentially leading to an underestimation of less abundant pathotypes or genes in a mixed infection. Furthermore, while molecular methods detect the

presence of virulence genes, they do not directly confirm gene *expression* or the functional activity of the encoded virulence factors. Therefore, correlating genetic profiles with clinical outcomes remains essential.

Public Health Implications

The findings of this comparative analysis have substantial public health implications. Accurate identification of circulating DEC pathotypes and their virulence gene profiles is fundamental for effective epidemiological surveillance, allowing public health agencies to monitor trends, identify outbreaks, and implement targeted control measures. Knowing the predominant pathotypes in a region can inform public health campaigns focusing on specific transmission routes (e.g., contaminated food for EHEC, water for ETEC).

From a clinical management perspective, rapid and precise diagnosis of DEC infections can guide appropriate treatment strategies. For instance, distinguishing between ETEC (often managed with rehydration and sometimes antibiotics) and EHEC (where antibiotics may be contraindicated due to HUS risk) is critical. The identification of hybrid strains or co-infections suggests that clinical presentations might be more severe or complex, necessitating a more comprehensive approach to patient care. Furthermore, understanding the virulence gene landscape is vital for vaccine development efforts, particularly for ETEC and EPEC, which remain major causes of childhood morbidity and mortality. Identifying key adhesins

and toxins as vaccine targets could lead to broadly protective vaccines, especially for children in high-burden regions. The data also highlight the ongoing need for improved water, sanitation, and hygiene infrastructure, as these remain primary drivers of diarrheal disease transmission.

Conclusion

This comparative analysis of virulence gene profiles of diarrheagenic *Escherichia coli* pathotypes isolated from acute diarrheal patients underscores the complex and multifaceted nature of these enteric pathogens. The study confirms the significant prevalence of DEC in diarrheal cases, with EAEC and ETEC emerging as particularly dominant pathotypes, especially in pediatric populations. A critical observation is the frequent occurrence of *E. coli* strains harboring virulence genes from multiple pathotypes, challenging traditional discrete classifications and highlighting the genetic plasticity of DEC. This genetic overlap suggests the presence of "hybrid" strains that may possess enhanced pathogenic capabilities or contribute to more complex clinical presentations.

The findings emphasize the indispensable role of molecular diagnostic tools, such as multiplex PCR, for accurate and comprehensive identification of DEC pathotypes and their diverse virulence gene repertoires. Such detailed genetic characterization is not only crucial for precise epidemiological surveillance and outbreak investigations but also for guiding clinical management and informing the development of targeted public health interventions. Future research should focus on longitudinal studies to better understand the long-term clinical implications of hybrid DEC strains and co-infections, as well as continued efforts to identify novel virulence factors and develop broadly protective vaccines and therapeutics to reduce the global burden of diarrheal diseases.

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