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Geographic Distribution And Pathotype Diversity Of Escherichia Coli Isolates In India: Insights From Serotyping And Virulence Gene Analysis

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ABSTRACT

Escherichia coli continues to be a major cause of diarrheal illness, especially in low- and middle-income nations with inadequate surveillance. The purpose of this study was to describe the diversity of pathotypes and serogroups of E. coli isolates found in diarrheal cases in different parts of India. 634 (71.8%) of the 883 isolates that were gathered were identified as E. coli using accepted morphological and biochemical standards. Out of 540 isolates, 35 different O-serogroups were found by serotyping; the most common "O-serogroups were O8, O11, O22, and O88. Furthermore, 14.8% of isolates could not be typed. Enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and enterohemorrhagic (EHEC)" groups were obtained via pathotyping, which showed that 275 isolates (43.4%) carried one or more virulence genes. The most prevalent groups were ETEC and EPEC. There was no discernible relationship between pathotype and serogroup. The results show that E. coli strains have a significant genetic and antigenic diversity, highlighting the necessity of ongoing molecular surveillance to identify new pathotypes and enhance public health response tactics.....

Keywords: Escherichia coli, diarrheal disease, serogroup, pathotype, ETEC, EPEC, EAEC, EHEC, India, molecular surveillance..

INTRODUCTION

Based on the results of several investigations, this strain of bacteria has been designated as a top pathogen due to its diverse virulence parameters and the fact that it may contaminate not only people and animals but also food and other ecological niches such as water and soil. Because it colonizes the human stomach within hours of birth and can interact with its host in benign commensalism to significant pathogenicity, it is one of the most common causes of bacterial infections globally "(1). Despite being a commensal gut bacterium, E. coli is a reservoir of acquired AMR determinants (2)." E. coli's inability to reproduce in the environment was considered to prevent it from living outside the host (3). Later study demonstrated that "E. coli can survive outside of its host and is common in soil, sand, and silt in temperate, tropical, and subtropical climates and some aquatic bodies" (4). E. coli are the most common gut bacteria because they can adapt genetically and phenotypically to environmental changes. Their flexibility and survival in environmental niches depend on critical nutrients such organic carbon, phosphate, and nitrogen and their ability to adjust to nutritional deficiencies (5). "Over evolution, E. coli has acquired many genotypic and phenotypic traits that allow them to quickly adapt to environmental changes." It became a seasoned pathogen due to these traits: Enzymes are activated to catabolize nutrients, toxins are synthesized to limit environmental invasion, a survival state is switched to allow stress tolerance and survival even with nutrient deprivation, and antibiotic resistance genes and virulence factors are expressed (6,7). Abiotic and biotic factors affect bacterium growth in the environment. Temperature, nutrient availability, pH, and sunlight affect niche bacterial growth (8). E. coli's ability to consume resources, outgrow with other species, and create biofilms in nature are biologic factors (9). Due to environmental variables and stress, pathogenic bacteria may evolve from low to high virulence (10). In India, diarrheal diseases caused by Escherichia coli remain a major public health issue. E. coli isolate pathotype and serogroup distribution are essential for diagnosis, treatment, and targeted interventions. The study sought to fill the vacuum in region-specific molecular epidemiology data on diarrheagenic E. coli in India..

2. MATERIALS AND METHODS

2.1. "Bacterial Isolation and Identification"

From across India, 883 suspected Escherichia coli isolates were collected. These isolates were morphologically and biochemically screened to confirm species identity. Morphological evaluation included Gram staining and growth characteristics on selective and differential medium such nutrient agar (smooth, creamish colonies), MacConkey agar (lactose fermentation), and nutrient broth (turbidity with or without pellicle formation). Biochemical experiments included glucose, lactose, and sucrose fermentation; catalase and oxidase activity; "indole production; methyl red (MR); Voges-Proskauer (VP); citrate utilization; and the triple sugar iron (TSI) reaction." Additionally, ONPG testing occurred. Validated E. coli isolates (lactose-fermenting, catalase-positive, oxidase-negative, indole-positive, Gram-negative rods) were added for further testing.

2.2. Serotyping

E. Coli isolates were serologically identified using commercially available monovalent and polyvalent antisera. Non-typeable (UT) isolates were identified when agglutination did not occur with all of the available antisera. O-antigen serogrouping was carried out in accordance with normal slide agglutination techniques.

2.3. Molecular Pathotyping

Multiplex polymerase chain reaction (mPCR) assays that target certain virulence genes linked to diarrheagenic E. coli (DEC) were used to classify confirmed E. coli isolates according to their pathotype. Following procedures modified from well-established literature, the following genes were examined: eaeA (EPEC), bfpA (typical EPEC), elt and est (ETEC), aggR (EAEC), stx1, stx2, and hlyA (EHEC). The boiling lysis procedure was used to extract the DNA, and gel electrophoresis was used to visualize the PCR results.

3. RESULTS

3.1. Confirmation of *E. coli* Isolates

A total of 883 isolates were initially recovered from various regions of India. Based on morphological characteristics and biochemical confirmation, 634 isolates (71.8%) were identified as *Escherichia coli*. All confirmed isolates exhibited typical *E. coli* traits, including Gram-negative staining, oxidase negativity, catalase positivity, and lactose fermentation on MacConkey agar. Biochemically, these isolates were "indole-positive, methyl red-positive, Voges-Proskauer-negative, and citrate-negative, with acid and gas production from glucose and lactose fermentation". Furthermore, all tested isolates were ONPG-positive, supporting the final confirmation of *E. coli*.

3.2. Serogroup Distribution

540 of the 634 E. coli isolates that were confirmed were successfully serotyped and dispersed among 35 different O-serogroups. The majority of serotyped isolates were found to belong to the ten most commonly discovered serogroups, "which were O8, O11, O22, O88, O126, O83, O35, O141, O149, and O7. 94 isolates (14.8%)" were classified as untype able (UT) because they failed to agglutinate with any of the available antisera, which may indicate novel or variant strains that are not currently covered by the serotyping panel. Remarkably, just six isolates (1.2%) were found to be O157, a serogroup that has been linked to serious foodborne outbreaks and enterohemorrhagic E. coli in the past. This implies that the traditional outbreak-associated serotypes are not very common in the population under study. Geographically, serogroup prevalence varies. For example, although serogroups O102 and O154 were exclusive to Karnataka, serogroups O26 and O128 were only found in isolates from Assam. This geographic clustering can be a result of regional epidemiology or environmental factors affecting the distribution of strains.

Table 1. Distribution of predominant *E. coli* O-serogroups among confirmed isolates (n = 634)

O-serogroup	Number of Isolates	Percentage (%)
O8	54	8.5
O11	48	7.6
O22	45	7.1
O88	39	6.2
O126	36	5.7

O83	33	5.2
O35	30	4.7
O141	28	4.4
O149	26	4.1
O7	24	3.8
Others (25 serogroups)	177	27.9
Untypeable (UT)	94	14.8
Total	634	100.0

3.3. Pathotype Profiling

Multiplex PCR showed that 275 of 634 E. coli isolates (43.4%) exhibited diarrheagenic pathotype-associated virulence genes. ETEC, EPEC, EAEC, and EHEC were the four main pathotypes of these isolates. With 112 isolates (40.7% of pathotype-positive isolates), ETEC was the most common, mostly including est. EPEC was second with 103 isolates (37.5%), most of which were unusual (eaeA positive, bfpA negative). Nine isolates (3.3%) had EHEC and 30 (10.9%) had EAEC. Since no virulence genes were found in the remaining 17 isolates (6.2%), they were deemed uncharacterized. This may indicate new pathotypes or virulence factors not in the panel. The pathotype distribution varies by area. ETEC and EPEC were found throughout India, unlike EHEC, which was only found in northern and eastern India. EAEC outnumbered EHEC in every region surveyed, despite being less common. In the statistical study, certain serogroups and pathotypes did not correlate ($p > 0.05$), demonstrating E. coli's genetic diversity and adaptation to different ecological niches.

Table 2. Distribution of *E. coli* pathotypes among confirmed isolates (n = 634)

Pathotype	No. of Isolates	% among Total	% among Pathotypes
ETEC (*est*, *elt*)	112	17.7	40.7
EPEC (*eaeA*, ±*bfpA*)	103	16.2	37.5
EAEC (*aggR*)	30	4.7	10.9
EHEC (*stx1*, *stx2*, *hlyA*)	9	1.4	3.3
Uncharacterized	17	2.7	6.2
Total Pathotypes	275	43.4	100.0

4. CONCLUSION

This study reviews the serogroup and pathotype distribution of diarrheal Escherichia coli isolates from several Indian locations. E. coli was found in 71.8% of the 883 isolates, demonstrating their importance in gastrointestinal illnesses. O-serogroup diversity, including 35 serotypes and a large number of untypeable isolates, highlights circulating strains' antigenic heterogeneity. The identification of diarrheagenic pathotypes, particularly ETEC and EPEC, highlights the complexity and spatial variation of E. coli virulence. A dynamic and evolving epidemiology is suggested by region-specific serotypes and the low incidence of outbreak serogroups like O157. These findings demonstrate the need for molecular surveillance to track emerging strains and guide diarrheal disease treatments in India.

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