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Epidemiology of Diarrheagenic Enteroaggregative and Enterotoxigenic *Escherichia coli* and their Prevalence in Children Under Five In Western Rajasthan

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To better understand circulating diarrheagenic pathotypes, researchers often focus on investigating the fimbrial profiles, toxin subtypes, and dominant colonization factors of Escherichia coli (E. coli) in specific geographic areas. Few studies have evaluated the health outcomes of children in western Rajasthan. Bikaner has a high prevalence of diarrhea, according to surveys on waterborne illnesses. It is crucial to understand the distribution and variability of these components, as their frequency can vary temporally and across regions, with direct implications for epidemiological surveillance and tailored intervention strategies. However, many studies focusing on ETEC (Enterotoxigenic E. coli) and EAEC (Enteroaggregative E. coli) do not conduct in-depth genotyping or phenotyping. For EAEC, there is a need to determine which virulence factors, such as plasmid-encoded genes, toxins, and aggregative adherence fimbriae, are linked to more severe disease, as well as to understand the differences between persistent and self-limiting diarrhea. Due to the variability of EAEC strains, it remains unclear which subsets pose a "high risk." Furthermore, data on antibiotic susceptibility for EAEC and ETEC in many regions are limited. The focus is particularly on diarrheagenic E. coli strains, such as EAEC and ETEC, as opposed to extraintestinal strains. The resistance mechanisms of these pathogens, such as extended-spectrum beta-lactamases (ESBLs) and plasmids, have not been extensively studied among EAEC and ETEC. There are still interrogations regarding how often EAEC and ETEC lead to severe disease outcomes, such as dehydration and hospitalization, compared to mild illness. It is also imperative to investigate how EAEC and ETEC co-infection with other enteric pathogens and to consider the impact of host factors such as nutrition, microbiome composition, immunity, previous exposures, and age. The present review highlights and analyses how monitoring changes over time in prevalence, virulence factors, and resistance is crucial for understanding the dynamics of EAEC and ETEC.

Keywords: Diarrheagenic Escherichia coli; EAEC; Epidemiology; ETEC; Prevalence.

E. coli is a rod-shaped, Gram-negative, motile by peritrichous flagella, regularly found within the gastrointestinal tract of humans and animals, and spread by fecal contamination. *E. coli* can be found and spread through soil, water, and food.[1] *Escherichia coli* can be broadly classified into two types: commensal and pathogenic. Although most *E. coli* strains live harmlessly in the colon and typically do not pose a threat to healthy individuals, some pathotypes can lead

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to serious extraintestinal and intestinal illnesses in healthy and immunocompromised hosts. *Escherichia coli* strains involved in diarrheal diseases are among the most significant etiological agents of diarrhoea, having evolved through the successive acquisition of specific virulence traits via horizontal gene transfer (HGT) that enable successful persistence in the host.[2] Under specific conditions, such as environmental stress or other opportunistic factors, pathogenic strains of *E. coli* can lead to severe infections, potentially making it a fatal pathogen. These strains often colonise the intestines, leading to diarrheal symptoms. Diarrheal disease is also a major cause of malnutrition and represents the third leading cause of mortality in children under five years of age, predominantly in developing countries. Diarrheagenic *E. coli* (DEC) stands out as a prominent and diverse group of enteric pathogens. DEC are classified into six distinct pathotypes based on their unique virulence mechanisms and clinical expression: EAEC (Enteroaggregative *E. coli*), ETEC (Enterotoxigenic *E. coli*), EHEC (Enterohemorrhagic *E. coli*), EPEC (Enteropathogenic *E. coli*), EIEC (Enteroinvasive *E. coli*) and DAEC (Diffusely Adherent *E. coli*). Among these, EAEC and ETEC are recognised as major agents, causing persistent and acute watery diarrhea, respectively. EAEC is an emerging gut pathogen most commonly associated to both acute and persistent diarrhea, malnutrition, and growth retardation in children, particularly in developing countries.[3,4] EAEC, however, it is a major public health issue because of its ability to induce infections even in the absence of typical diarrheal symptoms, as well as its association with symptomatic cases. In such instances, the bacterium may still cause complex gastrointestinal disturbances that lead to significant discomfort and health complications.[5] Emphasising the urgent need to prioritise the diagnosis, prevention, and intervention of ETEC and EAEC is vital for substantially mitigating the incidence of childhood diarrheal morbidity and mortality in India and similar regions. By employing specific strategies that address these pathogenic risks and associated antimicrobial and multidrug resistance, we can significantly improve the health outcomes of vulnerable children. This proactive strategy seeks to save lives but build a healthier and more resilient future for communities facing these widespread health challenges.

A modified systematic method was used in this review to compile data on diarrheagenic *E. coli* and childhood diarrhea in western Rajasthan. Important questions about prevalence, risk factors, and regional health outcomes were determined and matched with gaps in local research. Comprehensive electronic searches of PubMed and other databases were used to find pertinent papers using MeSH terms such as "diarrhea under-five Rajasthan," "EAEC and ETEC pathotypes," and "childhood health western Rajasthan." Due to diverse data, identified papers (published between 1998 and 2025) were individually evaluated against inclusion criteria (such as pediatric focus and geographical relevance), quality-rated, and abstracted for synthesis without meta-analysis. Contextual generalizations were informed by district reports from Bikaner and other regional surveys.

Diarrheagenic *Escherichia coli* (DEC)

Diarrheagenic *Escherichia coli* (DEC) is a bacterial pathogen that is the main cause of endemic and epidemic diarrhea in the world.[6] In underdeveloped nations, diarrhea and related gastrointestinal problems are a major source of sickness and mortality, especially for newborns and young children.[7] An estimated 1.17 million casualties worldwide were attributed to diarrheal illnesses in 2021. This indicated almost 60% decrease since 1990, when there were 2.93 million deaths. Although there has been a decline in deaths caused by diarrheal diseases, these conditions still represent approximately 59 million Disability-Adjusted Life Years (DALYs) worldwide. Out of this total, approximately 30.9 million DALYs are attributed to children under the age of 5, influenced by various assessed risk factors associated with diarrheal diseases.[8] Similarly, India is projected to experience significant gains due to its large population, with a reduction in disability-adjusted life years (DALYs) from 16.8 million to 2.11 million. The prevalence and occurrence of diarrheal cases among children under 5 years of age in India during 2015–16 were reported at 9.2%.[9] According to the National Health Survey-5 (2019-21), 7.3 per cent of children in India suffer from diarrheal diseases. The country's prevalence has significantly decreased from 2016 to 2019 in

the country.[10] However, in the western region of Rajasthan, very few studies have been conducted to assess childhood health outcomes. A survey on waterborne diseases reported that the city of Bikaner had the highest incidence, with 5.4% of the population experiencing diarrhea.[11] According to a study conducted between 2020 and 2022, diarrhea, pneumonia, low birth weight, and asthma were the leading causes of mortality across the blocks of Sirohi district. The study further revealed that 67.9% of children aged 0–5 years experienced diarrhea.[12] There is still much to explore in understanding and addressing diarrheal disease caused by EAEC and ETEC pathotypes.

Burden Associated Diarrhoea in Under-Five Children

Gastrointestinal diarrhea is a prominent cause of illness and death in children under the age of five, especially in low-and middle-income countries. *Escherichia coli* is a major bacterial etiological agent in infantile diarrhea. Including food and water, as well as inadequate sanitation standards, are, the primary modes of transmission for *E. coli*-induced diarrhea. Infected children usually exhibit symptoms such as watery or persistent diarrhea, stomach discomfort, vomiting, fever, and, in extreme cases, dehydration and malnutrition. Persistent infections, particularly those caused by EAEC and ETEC, are closely linked to poor growth, poorer cognitive development, and an increased vulnerability to subsequent infections. The pathogenesis of *E. coli* associated diarrhoea involves bacterial adhesion to the intestinal epithelium, toxin production, disruption of epithelial barrier integrity, and induction of host inflammatory responses. Virulence factors such as enterotoxins, adhesins, flagellin, and cytoskeleton-altering toxins contribute to intestinal inflammation, fluid secretion, and epithelial damage. Host immune responses, including Toll-like receptor–mediated signaling, further amplify inflammation and disease severity. Despite a decline in diarrhoeal prevalence at the national level in recent years, regional disparities persist, with under-five children in arid and resource-limited regions remaining highly vulnerable. Limited access to clean drinking water, inadequate sanitation, malnutrition, and poor healthcare infrastructure continue to exacerbate disease burden.[12,13]

Epidemiological Studies and the Clinical Relevance of EAEC and ETEC

Extensive research has consistently demonstrated a strong association between diarrhea and the presence of specific virulence genes in EAEC. These VRGs (Virulence-related genes) play a vital role in the bacterium's pathogenicity by enabling it to adhere to intestinal epithelial cells and evade host immune responses, thereby exacerbating the severity of gastrointestinal infections. Furthermore, the remarkable genetic diversity among EAEC strains complicates our understanding of their pathogenic mechanisms and presents considerable challenges in developing effective treatment and prevention strategies. Differences in virulence factors driven by this genetic variability amplify public health concerns surrounding EAEC infections.[14] Epidemiological studies reinforce the clinical relevance of EAEC, particularly in India. In Chandigarh, a study investigating children under the age of five identified EAEC in 16% of diarrheal cases, compared to only 6% among well-nourished controls. The disparity was statistically significant, especially among children under two years of age, indicating heightened vulnerability within this age group.[3] Similarly, in Kolkata, a comprehensive analysis of 3,826 stool specimens collected between 2008 and 2011 found EAEC to be the most prevalent pathotype of diarrheagenic *E. coli* (DEC), detected in 5.7% of samples. Followed by Enterotoxigenic *E. coli* (ETEC) at 4.2% and Enteropathogenic *E. coli* (EPEC) at 1.8%, underscoring the need for targeted interventions and further research into EAEC's public health impact.[15] A case-control study involving 355 children under age five with diarrhea and 150 control subjects revealed that EAEC was present in 16.05% of cases, compared to just 5.33% of controls. Additionally, ETEC was detected in 8.73% of cases and only 1.33% of controls. These findings illustrate a statistically significant link between EAEC and ETEC with pediatric diarrhea, further emphasising the critical role these pathogens play in gastrointestinal illnesses among children.[16]

Supporting this evidence, earlier observations by Anvikar et al.[17] and Hegde et al.[18] also recognized EAEC as the predominant strain of diarrheagenic *E. coli*, reinforcing its significant role in childhood gastrointestinal infections. ETEC is a

common source of traveller's diarrhea, particularly among visitors to places with a very poor sanitation and limited access to clean drinking water, such as many developing countries.[19] It is also recognised as the most recurrent reason of infectious diarrhea in children under five in these regions.[20] According to a study among 79 countries, they estimated that 200 million episodes of childhood diarrhoea caused by ETEC and *Shigella* occurred in one year, including 75 million cases in children under five years of age.[21] ETEC spread primarily occurs through the ingestion of contaminated food and water infected with pathogenic bacteria, posing a significant health risk. This transmission is particularly exacerbated by inadequate sanitation facilities, where human waste is often improperly disposed of and can seep into water sources. The situation is further aggravated by the consumption of unsafe drinking water, which may be tainted with fecal matter, and by the widespread practice of open defecation in many communities. Such conditions are especially prevalent in numerous low-income countries.[22] ETEC diarrheal infection stems from the production of two potent enterotoxins: heat labile (LT) enterotoxins and heat-stable (ST) enterotoxins encoded by the *elt* and *est* genes, respectively. And intriguingly, some ETEC strains can produce both toxins at once, intensifying their deleterious effects on the host. ETEC also produces colonisation factors (CFs), protein-rich surface polymers that assist the bacteria adhere tightly to the intestinal mucosa. Among the many colonisation factors identified, seven CFA/I and CS1 through CS6 are notably more prevalent than the others.[23,24]

Epidemiological relevance of ETEC pathotype in Odisha, with ETEC has been found as the leading cause of diarrhea. A study examining tourists with diarrhea who visited Goa, India, found that enterotoxigenic *Escherichia coli* (ETEC) was the most prominent pathogen which was identified in more than 25% of cases.[25] Another study in Puri, India, revealed that ETEC was the most prevalent diarrheal pathogen among hospitalised patients, accounting for 20.3% of cases.[26] These findings align with broader meta-analyses that have identified ETEC as the major bacterial cause of traveller's diarrhea in South Asia and other regions.[25] According to a 2023 study conducted in Wenzhou, EAEC and ETEC are the most common pathotypes responsible for causing diarrhea.[27] Similarly, a study conducted in Puri reported that ETEC was the most prevalent pathotype, followed by EAEC and EPEC pathotypes.[26]

Multiple DEC Pathotypes and the Emergence of Hybrid Strains

Previous studies conducted in the western Rajasthan have established a significant prevalence of EAEC and ETEC in clinical samples from diarrheal patients.[28] However, these investigations are crucial and are specifically focused on identifying the presence of DEC pathotypes and their prevalence. The true landscape of *E. coli*-associated diarrhea is likely more complex. Reconfirmation of the prevalence of EAEC and ETEC in clinical isolates derived from patients with diarrheal illness, alongside environmental samples such as sewage water, is imperative for comprehensive characterisation of these pathogens and for substantiating their regional circulation. Moreover, there is a critical knowledge gap regarding the co-infection with multiple DEC pathotypes, the emergence of hybrid strains carrying virulence genes from more than one pathotype, and the detailed molecular and serological characterisation of these isolates. Furthermore, the rising threat of AMR (antimicrobial resistance) among enteric pathogens necessitates a thorough investigation into the resistance profiles of prevalent strains and the genetic basis of this resistance, which has not been comprehensively addressed in the region.

EAEC and ETEC Pathotypes and their Pathogenesis

As discussed above, there are six distinct pathotypes of diarrheagenic *Escherichia coli* (DEC) but the present review focuses on EAEC and ETEC due to their significant roles in causing acute and persistent diarrhea, particularly in children under five.

Enteroaggregative *E. coli* (EAEC)

EAEC was first identified in 1987 as a disease linked to acute infantile diarrhea. In subsequent years, EAEC has been recognized as a significant contributor to both persistent and acute diarrhea in infants and people worldwide.[29,30] Several major mechanisms are involved in the enteroaggregative (EAEC) pathogenesis, including attachment to the intestinal mucosa, biofilm formation, mucus secretion, cytotoxic

damage, and mucosal inflammation triggered by cytokine release.[31] EAEC is a pathogen that produces a typical aggregative adherence pattern on cultured epithelial cells. In the Aggregative Adherence (AA) pattern, bacteria adhere to one another, the epithelial surface, and even to abiotic surfaces.[32,33] The pattern of adherence is determined by organelles known as aggregative adherence fimbriae (AAF), which can be classified into at least five categories.[34,35] Other adhesive structures including *E. coli* common pilus (ECP) and non-canonical adherence factors like Hra1 type I and fimbriae (T1F) are expressed by EAEC strains. Other potential virulence factors of EAEC include the cytotoxins Pet (plasmid-encoded toxin), EAST-1 (EAEC heat-stable enterotoxin 1), and both encoded by the pAA plasmid, in addition to the protein crucial for colonization (Pic) encoded by the bacterial chromosome, which possesses both mucus secretagogue and mucinase functions.[36] Components such as dispersin and flagellar protein encoded by *fliC* gene have been shown to stimulate the host immune response by increasing the production of interleukin-8 (IL-8), thereby inducing inflammation.[5]

The primary virulence factors in the case of EAEC that contribute to its pathogenic potential are located on a mega plasmid known as pAA. A type VI secretion system (T6SS) and Pic virulence gene are also encoded on the bacterial chromosome. The *aagR* a process regulated transcriptionally by *aagR* regulator (AraC/XylS family),[3,37] is expressed

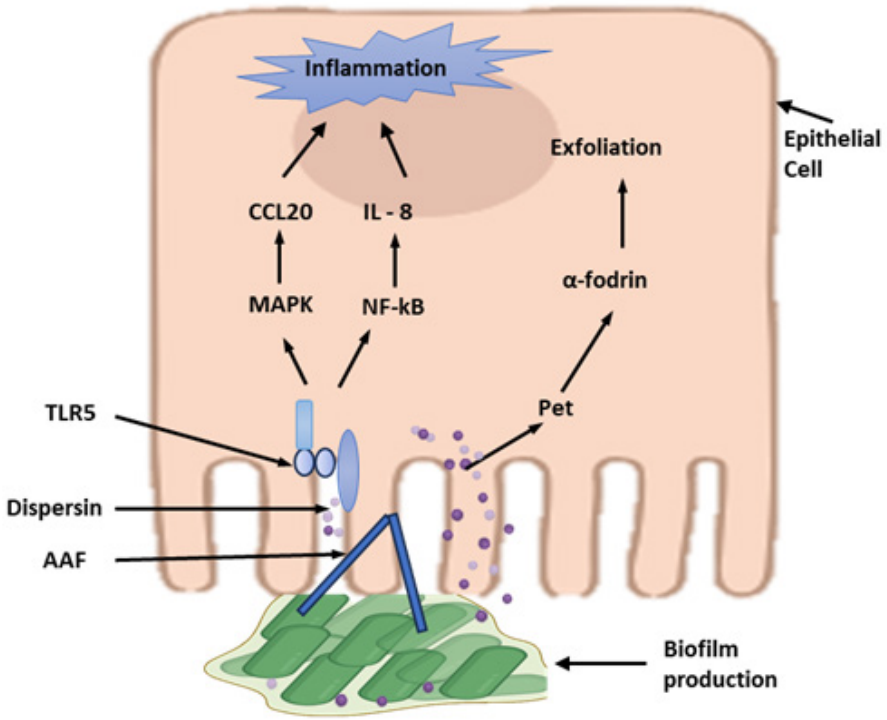

Fig. 1. EAEC Pathogenesis: EAEC attach and aggregate on colonic epithelial cells in a stacked brick pattern by AAF fimbria and the secreted protein encoded by *aap* known as dispersin. EAEC form a thick biofilm enabling protection against host or interventional antimicrobial responses. FliC surface flagella are then recognized by TLR5 receptors expressed on the apical surface of enterocytes. Bacterial-epithelial cell contacts trigger a cascade of events activating NF-*ê*B and MAPK pathways that result in the upregulation of proinflammatory cytokines IL-8, TNFá and CCL20 responsible for recruiting dendritic cells and neutrophils to the site of infection. Pet enters the cell via clathrin-mediated endocytosis and is translocated into the cytosol after being transferred from the Golgi complex to the endoplasmic reticulum through retrograde trafficking. In the cytosol, Pet cleaves the actin-binding protein á-Fodrin inducing cytotoxic disruption of the cytoskeleton.

by the pAA plasmid, regulating the expression of both plasmid and chromosomal genes. However, the pathogenesis of EAEC is complicated and involves genes that are not regulated by AggR, which describes the virulence factors that this master regulator controls.[38] The AA phenotype in EAEC strains encoded by *pAA* gene; is a magaplasmid encode the several virulence gene like aggregative adherence fimbriae (AAF/I–V) to effectively colonize the intestinal mucosa,[34,35] plasmid-encoded toxin (Pet), enteroaggregative heat-table toxin (EAST, *astA*), which corresponds to CVD432 fragment, antiaggregation protein (Dispersin, *aap*), include anti-aggregation protein transporter (*aatA*) and the gene regulating the AggR transcriptional activator (*aggR*) needed for the expression of the huge number of virulence genes.[39] AAF, which is encoded on the pAA plasmid and stimulates biofilm formation for intestinal colonization in the host, promotes EAEC adherence to the epithelial cell.[40] Strains that carry the *aggR* gene are classified as typical EAEC, while those lacking this gene are considered atypical EAEC.[3] Typical EAEC strains often harbour toxin genes such as *pet* and *astA*, which are located on the pAA plasmid, as well as *pic*, which is chromosomally encoded. Additionally, chromosomal regions of EAEC strains may contain genes implicated in iron acquisition, such as *fyuA* and *irp2*.[40]

Pathogenesis of EAEC

Bacterial adherence to the gastrointestinal mucosa, mainly in the colonic region, is critical in gastrointestinal infections.[29] Bacterial adherence to the gastrointestinal mucosa, especially in the

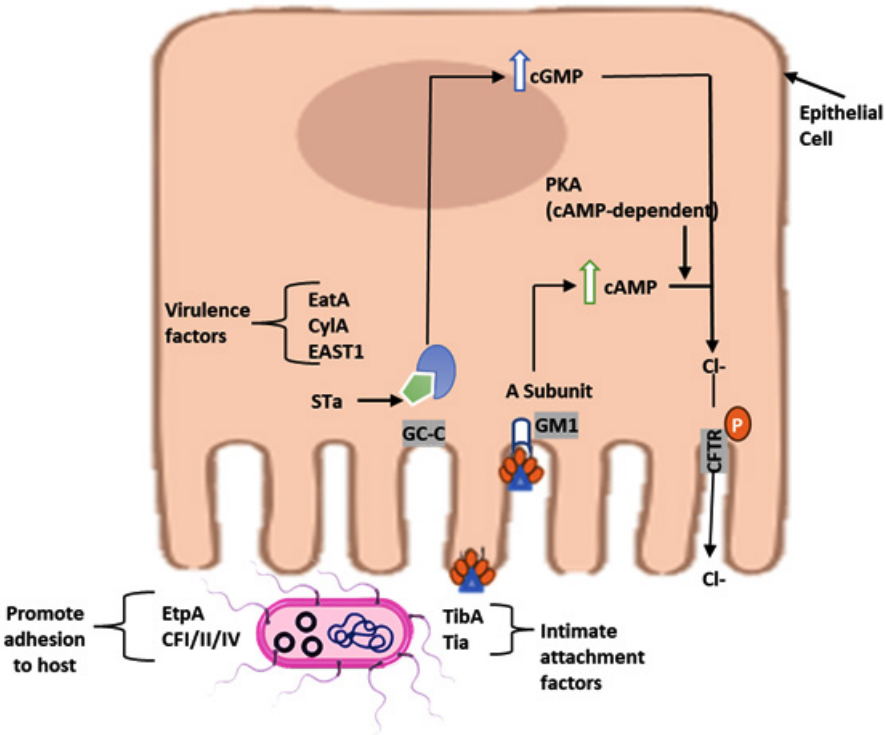

Fig. 2. ETEC Pathogenesis: ETEC Pathogenesis: Tia/TibA are the surface proteins that mediate the surface adhesion between ETEC and host epithelial cells. STa toxin binds to the GC-C receptor, activating its catalytic domain and converting GTP into cGMP. elevated cGMP levels activate kinases that phosphorylate the regulatory domain of CFTR, enhancing Cl- secretion, thereby disrupting electrolyte balance LT binds to the GM1 receptor on epithelial cells, facilitating its entry. Once internalized, the A1 subunit of LT converts ATP into cAMP. Increased cAMP activates kinases that phosphorylate CFTR, exacerbating electrolyte imbalance. The cumulative effects of these toxins disrupt ion transport, promoting water secretion into the small intestine and resulting in diarrhea.

colonic region, occurs via the regulatory gene *aggR* with aggregative adherence fimbriae (AAF) (AAF/I-AAF/II) and (*aatA*) dispersin protein. This is followed by biofilm production, which is facilitated by genes such as *AAF*, *shf*, *aatA*, *yafK*, *fis*, and *set1A*.[41] The bacterial surface protein Dispersin (*aap*) whose expression is positioned upstream of the *aggR* regulator. It forms a surface layer that facilitates bacterial dispersal and is highly immunogenic because of its availability on the bacterial surface. Dispersin binds noncovalently to the outer membrane via electrostatic interactions. Dispersin is capable of binding to extracellular matrix proteins such laminin and plasminogen. With the aid of a plasminogen activator, it plays an essential role that produces plasmin. This specifies that dispersin may be involved in the pathogenic mechanisms pertaining to systemic infections, such as bacteremia.[42]

Aat, an ABC transporter complex comprised of five genes (*aatPABCD*) located on the pAA plasmid, is necessary for dispersin to be transported to the bacterial surface. The expression of these genes is also positively controlled by the product of *aagR*. The *aatPABCD* locus encodes a permease protein, a N-acyltransferase, an ABC transporter, a putative protein, and a TolC-like outer membrane protein, respectively.[43] Binding of EAEC flagellin to Toll-like receptor 5 (TLR5) activates MyD88-dependent signalling pathways, leading to the activation of downstream kinases and transcription factors, including NF-êB and mitogen-activated protein kinases (MAPKs). Activation of NF-êB induces the production of pro-inflammatory cytokines and chemokines such as IL-8, IL-6, TNF-á, CCL20, and CXCL1. Among these, IL-8 plays a critical role in recruiting neutrophils to the site of infection, thereby contributing to intestinal inflammation. Although TLR5-mediated immune responses are essential for controlling EAEC infection, excessive or prolonged activation promotes intestinal inflammation, epithelial damage, and diarrhoea, which are hallmark features of EAEC pathogenesis. Furthermore, EAEC biofilm formation and persistent colonization can amplify TLR5 signalling, leading to sustained and chronic inflammatory responses. [42,43]

In addition to inflammatory signaling, EAEC produces a cytoskeleton-altering toxin known as plasmid-encoded toxin (Pet). Pet induces cytoskeletal contraction, loss of actin stress fibers, and disruption of focal adhesions in epithelial monolayers, followed by complete cell rounding and detachment. Notably, both the cytotoxic and enterotoxic effects of Pet depend on its serine protease activity. Pet has been shown to enter eukaryotic cells, and its trafficking through the vesicular system is essential for the induction of cytopathic effects (Fig. 1). Following clathrin-mediated endocytosis, Pet undergoes retrograde transport to the endoplasmic reticulum and is subsequently translocated into the cytosol. An intracellular target of Pet, á-fodrin, has been identified. Pet binds to and cleaves epithelial fodrin, resulting in its redistribution throughout the cell and the formation of intracellular aggregates known as membrane blebs.[29,31,32]

Enterotoxigenic *E. coli* (ETEC)

ETEC is internationally acknowledged as the predominant cause of traveller's diarrhea and a prevalent contributor to mild to severe diarrhea in children.[24,44] The clinical signs of ETEC infections resemble those of cholera, produced by *Vibrio cholerae*, characterized by watery diarrhea and dehydration.[6] ETEC strains produce enterotoxins that induce diarrhea. Enterotoxins are categorized into heat-labile enterotoxins (LT), heat-stable enterotoxins (ST), and CFs (colonization factors). The strains of ETEC may express either *st*, *lt*, or both genes.[45,46] CFA/I (Colonization factor antigen I) is a major colonization antigen identified in many ETEC isolates. These factors facilitate the close contact with the host that is essential for colonization and the release of toxins. Enterotoxigenic *Escherichia coli* (ETEC) can bind to the specific receptors on enterocytes by CFs, which are usually non-fimbrial, fimbrial, and fimbrial structures encoded by plasmid genes. More than 29 human ETEC colonization factors (CFs) have been identified and classifies into 4 main groups: class 1b, CFA/A-like, CS5-like, and a diverse group with less structural similarity. CS1, CS3, CS6, and CFA/I are studied the most. It is well known that CFA/I-specific IgY antibodies significantly reduce ETEC adhesion in the HT-29 cell line.[47] Tia and Tib are outer membrane proteins that bind to the intestinal mucosa and can cause the infection. These proteins are crucial for the bacteria to attachment to the epithelial cells in the host's intestine.[48]

Pathogenesis of ETEC

The non-invasive pathogen ETEC utilises colonization factors (CFs) to adhere to and colonize the small intestinal mucosa to cause secretory diarrhea. It then secretes two enterotoxins LT and ST. LT is 84 kDa, a high-molecular-weight enterotoxin featuring an active alpha subunit surrounded by five identical binding B subunits, whearas ST is a low-molecular-weight peptide encoded by the *eltAB* and *estA* genes, consisted of 18 to 19 amino acid (aa) residues.[24] These attach to the epithelial cells and interfere with normal secretion and absorption processes which subsequently triggers the cyclic nucleotide cascades in enterocytes wherein LT and AB5 toxin enter host cells via ganglioside receptor 1 (GM1), activating adenylyl cyclase.

Following activation, adenylyl cyclase raises cAMP (cyclic adenosine monophosphate) in cells and causes the intestinal lumen to secrete water and electrolytes. The heat-stable toxin (ST) binds to guanylyl cyclase (GC) on enterocytes, stimulating the secretion of sodium ions and activating CFTR channels. In contrast, Lt, the heat-labile toxin binds to ganglioside-GM1. It activates adenylate cyclase, leading to the activation of protein kinase A (PKA), which further interrupts ion transport and contributes to diarrhea (Fig.2).[49]

EAEC and ETEC Susceptibility of the Under-Five Gut and Antimicrobial Resistance (AMR)

EAEC and ETEC pathotypes can more easily adhere, colonize, and upset the gut's fluid balance in children under five because of their immature intestinal barrier, which includes developing tight junctions, a mucus layer, and epithelial receptor profiles. Because crucial mucosal immunity components like secretory IgA, antigen-specific memory T cells, and balanced cytokine responses are still developing in this age group, pathogen-specific neutralization of adhesins and enterotoxins remains less effective, allowing these pathotypes to cause diarrhea at lower infectious doses than in older age groups.[24,50] Moreover, EAEC biofilms and ETEC fimbrial colonization factors have a competitive advantage on the mucosal surface because the gut microbiota of children under five years of age is less diverse, less stable, and more easily disturbed. This biological vulnerability, along with high-force transmission settings, contaminated food and water, and intense fecal–oral exposure from hand-to-mouth behaviour, explains why EAEC and ETEC are major causes of acute and persistent diarrhea, growth faltering, and morbidity in children under five, particularly in low- and middle-income countries.[51,52,53]

Antibiotic resistance in DEC is a significant health threat to humans, especially when treating gut infections. The rising resistance of DEC strains, including enteroaggregative *E. coli* and enteropathogenic *E. coli*, to widely used antibiotics makes treating infections more challenging. The overuse of antibiotics, which aid in the selection of resistant bacteria, are mostly to blame for the setting up of multidrug-resistant (MDR) bacteria in humans.[54] A number of antibiotics, such as beta-lactams, fluoroquinolones, and aminoglycosides, can cause MDR *E. coli* to develop resistance, which severely restricts the available treatment options and raises the risk of more extended hospital stays, more expensive medical care, and higher mortality. The capacity of *E. coli* to transfer genes horizontally, enabling resistant characteristics to proliferate among bacterial strains, is what propels the development of MDR in these pathogens. Resistance genes on plasmids, which can impart resistance to several antibiotics, including those essential for treating severe infections, are frequently involved in this gene transfer.[55] The emergence of MDR *E. coli* emphasizes the critical need for improved antibiotic stewardship, infection prevention strategies, and the creation of novel antimicrobial treatments to fight these resistant organisms.

CONCLUSION

In regions like Western Rajasthan, EAEC and ETEC represent significant microbial causes of diarrheal illness in children under five, significantly increasing childhood morbidity. Research from similar contexts in India and other poor nations consistently identified EAEC and ETEC as the most common diarrheagenic *E. coli* strains affecting this vulnerable age group, despite the absence of many prevalence studies for these particular pathotypes in Western Rajasthan. To characterize antimicrobial resistance patterns, multidrug resistance prevalence, and potential transmission dynamics, a comprehensive

investigation is required to ascertain the regional prevalence of enteroaggregative EAEC and ETEC, along with thorough molecular epidemiological profiling and antibiogram analysis. By employing molecular approaches, the presence of these pathotypes, prevalence of hybrid strains and the genetic determinants of their antimicrobial resistance can be confirmed. These kinds of studies may provide critical insights into the circulating pathogenic strains, aid in the development of more effective treatment strategies, and guide public health interventions for the control and prevention of diarrheal diseases. A semi-arid and hot region of Western Rajasthan, faces significant challenges due to persistent water scarcity. This ongoing issue severely impacts sanitation and hygiene standards, thereby increasing the population's vulnerability to waterborne infections. Many rural and some urban areas of Western Rajasthan rely on groundwater sources or stored water, which may be contaminated due to poor sewage systems and open defecation in surrounding regions. Contaminated water is a known vector for the transmission of ETEC and EAEC. Western Rajasthan is a popular destination for domestic and foreign tourists, and it is known for its historical sites and cultural events such as the Camel Festival. This popularity increases the risk of traveller's diarrhea, often caused by ETEC, highlighting the need for effective surveillance and research. Research on the prevalence, strain diversity, and antibiotic resistance patterns of EAEC and ETEC in Western Rajasthan is limited. Conducting local studies would provide valuable epidemiological data for targeted interventions and effective public health planning. The misuse or overuse of antibiotics in both human and veterinary practices in the region raises concerns about multidrug-resistant pathotypes of *E. coli*. Investigating EAEC and ETEC pathotypes in this area is crucial for monitoring trends in antibiotic resistance. Children in Western Rajasthan often experience malnutrition, increasing their vulnerability to severe impacts from diarrheal pathogens such as EAEC and ETEC. Therefore, understanding the local strains is essential for improving clinical management and developing prevention strategies, such as vaccines or probiotics.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent is not required.

Clinical Trial Registration

The research does not involve any clinical trials.

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Not Applicable.

Author contributions

Parvinder Kumar: Prepared the final draft of the manuscript; Sweta Barupal: Assisted in the data collection and editing; Chetna Aggarwal: Data analysis and completed all references; Jyoti Lakhani: Visualization and formatting; Sanjay Kumar Kochar: Conceptualization, analysis and editing; Dharmesh Harwani: Funding Acquisition, writing and supervision

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