2. REVIEW OF LITERATURE

2.1 PROBLEM STATEMENT

Diarrheal diseases are the major cause of death in children under 5 years of age in resource-poor countries, resulting in approximately 2.5 million deaths each year worldwide.\(^{20}\) Along with other related gastrointestinal disorders, they are the most important causes of illness and death, particularly among infants and young children from developing countries.\(^{21}\)

However, since most of the burdens of diarrheal diseases are incurred among infants and children in developing countries, the majority of diarrhea etiology studies are performed on children in such regions.\(^{4,22-27}\) Of the 6.3 million global deaths of children below 5 years of age due to infectious diseases, diarrhea alone has caused 0.58 million deaths (about 9\%) in 2013.\(^{21}\)

Abdullah Al-Sorchee et al in 2013 have shown Enteropathogenic \textit{E.coli} (EPEC) to be one of the major causative agents of children’s diarrhea followed by ETEC, STEC and EAggEC in diarrheal patients (infants and children).\(^{28}\)

Ballal et al conducted an etiological study in 2014 in rural coastal India and discovered DEC, mainly Entero-aggregative \textit{Escherichia coli} (EAEC), as the predominant isolate, followed by Salmonella, Shigella, Vibrio cholera and parasites from patients under 5 years of age.\(^{29}\)

2.2 THE ORGANISM

In 1885, Escherich first isolated \textit{Escherichia coli} (\textit{E. coli}) from stools of children with enteritis. The bacterium \textit{E.coli} belongs to the family \textit{Enterobacteriaceae} and tribe \textit{Escherichiae}. It is a predominant normal flora of the intestinal tracts of humans and animals. Mostly, the \textit{E. coli} strains are harmless commensals except few pathogenic ones. \textit{E.coli} has been identified as a diverse species both in terms of phenotypic and genotypic appearances. It
is identified on the basis of ‘O’, ‘H’ and ‘K’ antigens, the three of which together constitute the serotype. The serological typing is determined by somatic antigens (O: O1-O173) and flagella antigen (H: H1-H56). The categorization is based on virulence, pathogenicity and serogroups.

Along with other enteric gram-negative bacteria, such as Salmonella and Shigella, E. coli is known for its ability to adapt and colonize a diverse array of reservoirs, including open-air environments and the gastrointestinal (GI) tracts of mammals and birds. E. coli bacteria are mesophilic organisms that replicate at temperatures of 7 to 45°C. Under optimal temperature conditions (35 to 42°C and 95 to 104°F) the pathogen can replicate at pH values of 4 to 10 and in the presence of up to 8% sodium chloride.

There are wide differences in the prevalence of different categories of diarrheagenic E. coli (DEC). These groups include Enteropathogenic E. coli (EPEC), Enteroinvasive E. coli (EIEC), Enterotoxigenic E. coli (ETEC), Enteroaggregative E. coli (EAggEC), Diffuse-adhering E. coli (DAEC) and Verotoxin-producing E. coli (VTEC) or Shiga-toxin producing E. coli (STEC) [includes Enterohaemorrhagic E. coli (EHEC)]. The incidence of DEC is largely unknown in India as very few laboratories can identify these organisms. ETEC and EPEC are the major bacterial enteric pathogens amongst DEC but the newer increasingly reported DEC in the recent years are the Shiga-like toxin producing E. coli (STEC) and Enteroaggregative E. coli (EAggEC).

While most strains of E. coli are not human pathogens, certain serotypes are responsible for three types of human illness: (i) neonatal meningitis, (ii) chronic urinary tract infections, and (iii) gastroenteritis. Each type of E. coli diarrhea is associated with a different pathotype of E. coli, and each pathotype has characteristic virulence determinants that contribute to its pathogenic mechanisms.

ETEC has been reported to be the most commonly isolated bacterial enteropathogen in children under 5 years of age in developing countries, and accounts for almost 20% of cases, which translates to several hundred million cases of diarrhea and several tens of thousands of deaths every year. ETEC is also the most common cause of travelers’ diarrhea responsible for 10–60% of infections depending on geographical location.
EAEC is the second most common cause of travelers’ diarrhea after ETEC and its role in endemic and epidemic disease is gradually getting more & more recognition.\(^4\) It causes persistent diarrhea in children in developing countries.\(^41,42\)

EPEC was reported to be the first strain of \textit{E. coli} generally accepted to cause diarrheagenic outbreaks in the developed world and its role in causing diarrhea has been well established.\(^43,44\)

Cajetan Ifeanyi et al in 2015 detected DEC in 12.5\% of all children reported with diarrhea in Abuja, Nigeria and emphasized the need for routine evaluation of diarrheic children for virulence properties of infectious DEC.\(^45\)

2.3 DIARRHEAGENIC ILLNESS

Diarrhea is a disease of poor hygiene, lack of clean and safe drinking water, over-crowding and insanitary practices including the widespread use of bottle-feeding instead of breastfeeding. Consumption of contaminated food, water or direct fecal contamination through fingers is the major cause of transmission of entero-pathogens prevailing especially in the rural settings of developing countries. The under five children are most prone to acquire diarrheal infections as such young children tend to pick everything they see and eat or swallow, leading to acquisition of a variety of infections.\(^46\)

\textit{E. coli} infections generally include loose, watery stools (diarrhea) and mild to severe abdominal pain and/or cramping. Other symptoms may include mild fever, headache, nausea, or vomiting. Persons with immature or impaired immune systems are more prone to both mild and severe illnesses and are also more likely to sustain secondary infections and/or other health complications.\(^47\) Symptoms associated with severe infections of certain serotypes include bloody diarrhea (hemorrhagic colitis), as well as renal (kidney) malfunction and failure, thrombocytopenia (inadequate platelet count), microangiopathic hemolytic anemia (lysis of red blood cells), or hemolytic uremic syndrome (HUS), in which patients exhibit all of these symptoms.\(^10,48\)
Traditional HUS is most commonly observed in children less than 5 years of age, while adults are more likely to develop an atypical form of HUS characterized by a lack of prodromal diarrhea (D-HUS). D-HUS is commonly misdiagnosed as thrombotic thrombocytopenic purpura, a strikingly similar type of thrombotic microangiopathy, also characterized by extensive and irreversible neurological damage. Seizure, stroke, herniated bowels, and/or chronic renal malfunction may also accompany severe infections.

2.4 MECHANISMS OF VIRULENCE

*E. coli* bacteria are particularly promiscuous microorganisms and continually exchange genetic material with other related and unrelated species of bacteria. A large number of virulence factors have been identified in the diarrheagenic *E. coli* strains. Correspondingly, the virulence genes associated with each class of diarrheagenic *E. coli* (Table 1) were acquired through extensive lateral transfers of genetic material. Colonization of host GI tissue is rather methodical, and evasion of host defenses, colonization, replication, and damage to the host are observed with all diarrheagenic *E. coli* infections.

<table>
<thead>
<tr>
<th>Class</th>
<th>Adhesion site</th>
<th>Adhesion mediator</th>
<th>Invasion potential</th>
<th>Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>Small Intestine</td>
<td>Intimin</td>
<td>Moderate</td>
<td>Possible enterotoxin (EAST1)</td>
</tr>
<tr>
<td>EAEC</td>
<td>Small and large intestine</td>
<td>AAF</td>
<td>None</td>
<td>EAST1, Pet, Pic</td>
</tr>
<tr>
<td>EIEC</td>
<td>Large Intestine (colon)</td>
<td>Unclear</td>
<td>High</td>
<td>Enterotoxin</td>
</tr>
<tr>
<td>ETEC</td>
<td>Small Intestine</td>
<td>Fimbrial CFs</td>
<td>None</td>
<td>LT, ST</td>
</tr>
<tr>
<td>EHEC</td>
<td>Large Intestine (colon)</td>
<td>Intimin</td>
<td>Moderate</td>
<td>Stx, enterohemolysin α- hemolysin, EAST1</td>
</tr>
</tbody>
</table>
2.5 SOURCES OF DIARRHEAGENIC E. COLI

Diarrheagenic E. coli bacteria have been recovered from a variety of environmental locations that include, but are not limited to, ranches and livestock feeding operations, livestock harvesting facilities, natural and human-made bodies of water, municipal water sources, urban and rural soils and landscapes, sewage treatment effluent, and agricultural fairs, as well as from hospitals and day care centers. Animals shown to shed the organism include beef and dairy cattle, sheep, swine, horses, rodents, dogs, deer, and wild and domesticated birds. Animal feces are the source of contamination for animal hides, water, soil, and inanimate objects, leading to transfer of contamination to raw food ingredients, processing water, equipment, and workers. Microorganisms from the gastrointestinal tract and feces of animals may contaminate meat and poultry carcasses, as well as the processing environment, which may then become a secondary source of contamination or cross contamination.

Raw products may become contaminated through intimate contact with contaminated water and/or soil and animal manure during cultivation or harvest, leading to contamination of associated products, such as juices, salads etc. In reality, however, nearly all food products (raw and processed) are subject to cross-contamination, which may occur at nearly all processing or handling steps leading up to consumption.

2.6 METHODS FOR ISOLATION, DETECTION, IDENTIFICATION AND CONFIRMATION OF DIARRHEAGENIC E. COLI

Microbiological tests can be used to detect, identify, or quantify the presence of specific microorganisms. Selective E. coli enrichment broths include brain heart infusion and gram-negative or E. coli broth supplemented with novobiocin. Additional compounds added to selective media to suppress the growth of background flora include antimicrobials to which E. coli are particularly resistant, such as cefixime, cefsulodin, potassium tellurite, and vancomycin.
Conventional culture methods remain the gold standard in pathogen detection, and numerous selective and differential media, which require as little as 18 hours for the detection of Diarrheagenic E. coli, are available. Popular solid media used to isolate generic biotype I E. coli, EPEC, ETEC, and EAEC include violet red bile agar, Levine’s eosine methylene blue agar, MacConkey agar (MAC), and 3M Petri Film rehydratable films. MAC and Hektoen enteric agar are most useful when isolating EIEC.

Sorbitol is added to MAC (SMAC) to differentiate E. coli O157 from non-O157 strains, which readily ferment sorbitol, while E. coli O157 strains typically do not in addition to SMAC, other media used to differentiate E. coli O157: NM and O157:H7 from non-O157 E. coli include SMAC supplemented with cefixime and potassium tellurite, rainbow agar, hemorrhagic colitis agar, and other proprietary chromogenic media.

PCR is the most extensively used rapid technology for detection of STEC and involves the extraction of genetic material; DNA hybridization, or the coupling of specific probes with target cell DNA; and the amplification of the target sequence. In general, PCR products are visualized via electrophoresis on agarose gels and enzyme-linked hybridizations, or Southern blot assays using labeled probes can be used to confirm the identity of a product.

The development of multidrug resistance in all pathotypes of E. coli isolated from children with diarrhea. In the last decades, resistance against the commonly used drugs for the treatment of enteric infections including ampicillin, tetracycline, and co-trimoxasole has increased among DEC. Misuse and overuse of antibiotics in the treatment of diarrheal illness has played an important role in the development of drug resistance, which complicates treatment of those infections in which antibiotics are indicated. There is a paucity of data regarding the efficacy of antimicrobials in a number of the pathogens detected on the panel and in these cases antibiotics are generally only recommended in severe or non-resolving cases or those at risk for severe disease such as immune-compromised patients.

Enos Ngoso et al in 2016 did the molecular characterization of diarrhoeogenic bacteria isolated from stools of under five children in Dar-es-Salaam, Tanzania and showed that a rapid and affordable PCR-based method (Multiplex polymerase chain reaction (mPCR)) may routinely
be used in hospitals for the identification of the most common bacteria associated with diarrhea in developing countries, including the six pathotypes of *E. coli*.  

In a study conducted in Tamil Nadu, India, Suganya et al in 2016 have concluded that multiplex PCR assays can correctly determine the presence of corresponding DEC virulence genes in all the reference strains of DEC and have further recommended that a clinical microbiology laboratory setting in a developing country could use these Multiplex PCR assays as a practical and rapid diagnostic tool for identification of DEC.