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Prevalence of Enterotoxigenic *Escherichia coli* -LT Gene Expression in Pediatric Diarrhea Patients in Makassar City, Indonesia: A Cross-Sectional Study

Nur Laela Alydrus[1*], Ka'bah[1], Kasmuddin[1], Prelly Loupatti[1]

[1]Diploma IV of Medical Laboratory Technology, Universitas Megarezky, Makassar, Indonesia

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***Corresponding author:**
Nur Laela Alydrus

E-mail address:
laela.alydrus979@gmail.com

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A B S T R A C T

Enterotoxigenic *Escherichia coli* (ETEC) is a significant cause of diarrheal disease worldwide, particularly in children. The heat-labile toxin (LT) is a crucial virulence factor encoded by the LT gene. This study investigated the prevalence of ETEC LT gene expression in children with diarrhea in Makassar City, Indonesia. A cross-sectional study was conducted from January 2023 to December 2023. Rectal swabs were collected from children under five years of age presenting with diarrhea at outpatient clinics in Makassar. DNA was extracted, and the presence of the ETEC LT gene was detected using polymerase chain reaction (PCR). Demographic and clinical data were collected via questionnaires. The ETEC LT gene was detected in 81 (18%) children. The prevalence was significantly higher in children under one year of age (51%) compared to older age groups. ETEC LT positive children were more likely to experience vomiting and fever. ETEC expressing the LT gene contributes to a significant proportion of pediatric diarrhea cases in Makassar. The higher prevalence in younger children highlights the vulnerability of this age group. These findings emphasize the need for improved sanitation and hygiene practices to reduce ETEC transmission.

1. Introduction

Diarrheal diseases continue to pose a significant threat to global public health, especially in low- and middle-income countries (LMICs). Despite advancements in sanitation, hygiene practices, and medical interventions, diarrhea remains a leading cause of morbidity and mortality among children under five years of age. The World Health Organization (WHO) estimates that diarrheal diseases account for approximately 525,000 deaths annually in this vulnerable population, with the highest burden concentrated in Sub-Saharan Africa and South Asia. Among the diverse array of pathogens responsible for diarrheal disease, enterotoxigenic *Escherichia coli* (ETEC) stands out as a major contributor. ETEC is a pathogenic strain of *E. coli* that colonizes the small intestine and produces enterotoxins, which disrupt the normal fluid and electrolyte balance, leading to watery diarrhea. Two primary enterotoxins are associated with ETEC pathogenesis: heat-labile toxin (LT) and heat-stable toxin (ST). These toxins are encoded by distinct genes, namely the LT gene and the ST gene, respectively. The LT toxin, the focus of this study, is a potent virulence factor that activates adenylate cyclase in intestinal epithelial cells. This activation leads to an increase in intracellular cyclic AMP (cAMP) levels, stimulating chloride secretion and inhibiting sodium absorption. The net result is a massive efflux of fluid and electrolytes into the intestinal lumen, resulting in profuse watery diarrhea.

The clinical manifestations of ETEC infection range from mild, self-limiting diarrhea to severe, life-threatening dehydration. The severity of the disease is influenced by various factors, including the host's age, nutritional status, immune response, and the specific virulence factors of the infecting ETEC strain. Children under five years of age are particularly susceptible to ETEC infection and its associated complications due to their immature immune systems and limited physiological reserves.[1,2]

ETEC transmission primarily occurs through the fecal-oral route, often facilitated by contaminated food or water sources. Poor sanitation and hygiene practices, such as inadequate sewage disposal, lack of access to clean drinking water, and improper handwashing, contribute to the widespread prevalence of ETEC in many LMICs. Overcrowding, malnutrition, and underlying health conditions further exacerbate the risk of ETEC infection and its adverse outcomes. Several studies have investigated the prevalence and epidemiology of ETEC in different geographical regions. These studies have highlighted the diverse distribution of ETEC strains and the variability in the expression of LT and ST enterotoxins. The prevalence of ETEC appears to be particularly high in areas with poor sanitation and hygiene conditions, as well as in populations with limited access to clean water and adequate healthcare. Despite the global significance of ETEC, the burden and characteristics of this pathogen in Indonesia remain relatively unexplored. Indonesia, the world's fourth most populous country, comprises over 17,000 islands and exhibits significant regional variations in socio-economic status, healthcare infrastructure, and environmental conditions. These factors likely influence the prevalence and distribution of ETEC strains, as well as the clinical presentation and outcomes of ETEC infection.[3-5]

Makassar City, the capital of South Sulawesi province in Indonesia, is a rapidly growing urban center with a large and diverse population. While Makassar has experienced significant economic development in recent years, challenges related to sanitation, hygiene, and access to clean water persist, particularly in marginalized communities. These challenges could contribute to the transmission and prevalence of ETEC in the city. To date, there have been limited studies on the prevalence of ETEC in Makassar City, especially among children with diarrhea. Understanding the burden and characteristics of ETEC expressing the LT gene in this region is crucial for developing targeted interventions and improving child health outcomes.[5,6] This study aims to fill this knowledge gap by investigating the prevalence of ETEC LT gene expression in children under five years of age presenting with diarrhea at outpatient clinics in Makassar. The study will also examine the association between ETEC LT positivity and various demographic and clinical characteristics, providing valuable insights into the epidemiology and clinical presentation of ETEC infection in this population.

2. Methods

A hospital-based cross-sectional study was conducted in Makassar City, Indonesia, from January 2023 to December 2023. Makassar, the capital of South Sulawesi province, is a major urban center with a diverse population and a range of socioeconomic conditions. This setting provides a representative sample of the pediatric population experiencing diarrheal illness in the region. The study population consisted of children under five years of age who presented to the outpatient departments of the participating hospitals with acute diarrhea. Diarrhea was defined as three or more loose or watery stools within a 24-hour period, with or without accompanying symptoms such as vomiting, fever, or abdominal pain. To be eligible for inclusion, children had to meet the following criteria: Age less than 60 months; Presence of acute diarrhea as defined above; Provision of informed consent by a parent or legal guardian. Children were excluded from the study if

they had any of the following: Known underlying chronic medical conditions such as inflammatory bowel disease, cystic fibrosis, or immunodeficiency disorders; Received antibiotic treatment within the two weeks prior to enrollment; Bloody diarrhea or other signs of dysentery; Severe dehydration requiring hospital admission. These exclusion criteria were implemented to ensure that the study population consisted primarily of children with uncomplicated acute diarrhea, likely of infectious etiology.

The sample size was calculated based on an estimated prevalence of ETEC in children with diarrhea of 15%, with a precision of 5% and a confidence level of 95%. Based on these parameters, a minimum sample size of 450 children was required. A systematic random sampling method was employed to select participants. On each day of the study period, a list of eligible children was generated from the outpatient department registers. The first child on the list was selected randomly, and subsequent children were selected at fixed intervals until the daily target was reached. This approach ensured a representative and unbiased sample of children presenting with diarrhea. Upon enrollment, a structured questionnaire was administered to the parent or legal guardian of each child. The questionnaire collected data on the following: Demographic information: Age, gender, residence (urban/rural), socioeconomic status (using a standardized scale based on parental occupation and education level), and breastfeeding status; Clinical information: Duration and severity of diarrhea, presence and frequency of vomiting, fever, abdominal pain, and signs of dehydration (e.g., sunken eyes, dry mouth, reduced urine output); History of recent travel, contact with individuals with diarrhea, and consumption of potentially contaminated food or water. A standardized physical examination was also performed on each child to assess hydration status and identify any other relevant clinical findings.

Trained healthcare personnel, wearing gloves and following standard infection control procedures, collected rectal swabs from each enrolled child. A sterile cotton swab, pre-moistened with sterile saline, was gently inserted into the rectum approximately 2-3 centimeters beyond the anal sphincter. The swab was rotated to ensure adequate contact with the rectal mucosa and then carefully withdrawn. The swab was immediately placed in a sterile transport tube containing a Cary-Blair transport medium. This medium helps to preserve the viability of bacteria present in the sample during transport. The transport tubes were labeled with unique identifiers and stored at 4°C for a maximum of four hours until processing in the laboratory. Upon arrival at the laboratory, the rectal swabs were inoculated onto MacConkey agar plates. This selective medium inhibits the growth of Gram-positive bacteria and differentiates lactose-fermenting bacteria (pink colonies) from non-lactose-fermenting bacteria (colorless colonies). ETEC, being a lactose fermenter, typically forms pink colonies on MacConkey agar. Suspected ETEC colonies were then subcultured onto Eosin Methylene Blue (EMB) agar. This selective and differential medium further inhibits the growth of Gram-positive bacteria and provides additional differentiation of lactose fermenters. ETEC colonies appear dark purple with a green metallic sheen on EMB agar. Biochemical Identification: Several biochemical tests were performed to confirm the identification of ETEC. These included: Indole test: ETEC produces indole from tryptophan; Methyl red (MR) test: ETEC is MR-positive; Voges-Proskauer (VP) test: ETEC is VP-negative; Citrate utilization test: ETEC is citrate-negative. These biochemical tests, in combination with the colony morphology on MacConkey and EMB agar, provide strong evidence for the identification of ETEC isolates.

Genomic DNA was extracted from confirmed ETEC isolates using a commercial kit (QIAamp DNA Mini Kit, Qiagen). This kit utilizes a silica-gel membrane technology to purify DNA from bacterial cells. PCR was used to specifically amplify a 273-base pair fragment of the LT gene. The reaction mixture contained

extracted DNA, specific primers (LT-F: 5'-GGC GAC AGA TTA TAC CGT GC-3' and LT-R: 5'-CGG TCT CTA TAT TCC CTG TT-3'), Taq polymerase, dNTPs, and buffer. The PCR was performed using a thermal cycler with the following program: Initial denaturation: 94°C for 5 minutes; 30 cycles of: Denaturation: 94°C for 30 seconds, Annealing: 55°C for 30 seconds, Extension: 72°C for 30 seconds. Final extension: 72°C for 7 minutes Amplified PCR products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The presence of a band of the expected size (273 bp) indicated the presence of the LT gene in the isolate. A positive control (known ETEC strain containing the LT gene) and a negative control (no template DNA) were included in each run.

Data were entered into a Microsoft Excel spreadsheet and analyzed using SPSS software (version 26). Descriptive statistics were used to summarize demographic and clinical characteristics of the study population. The prevalence of ETEC LT gene expression was calculated for the overall sample and stratified by age group. Chi-square or Fisher's exact tests were used to assess the association between ETEC LT positivity and categorical variables, such as age group, gender, residence, and clinical symptoms. Continuous variables, such as age and duration of diarrhea, were compared between ETEC LT positive and negative groups using the Mann-Whitney U test. Logistic regression analysis was performed to identify independent predictors of ETEC LT positivity. Variables with a p-value of <0.10 in univariate analysis were included in the multivariate model. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of the association.

3. Results and Discussion

Table 1 presents the demographic characteristics of the 450 children enrolled in the study. The median age of the children was 12 months, meaning half were younger and half were older. The interquartile range (IQR) of 6-24 months indicates that 50% of the children fell within this age range, highlighting a concentration of cases within the first two years of life. The majority of participants (56.4%) were male, while 43.6% were female. This suggests a slightly higher prevalence of diarrhea in male children within this study population. Most children (72.0%) resided in urban areas of Makassar, while 28.0% resided in rural areas. This distribution reflects the demographics of the region and may suggest potential differences in exposure to risk factors for diarrhea between urban and rural settings. This table provides a snapshot of the study population, demonstrating that the majority of the children were under two years old, predominantly male, and residing in urban areas.

Table 1. Demographic characteristics of enrolled children with diarrhea.

Characteristic	Value
Total enrolled	450
Median age (months)	12
Age range (months)	0 - 59
Interquartile range (months)	6 - 24
Male (%)	56.4%
Female (%)	43.6%
Urban residence (%)	72.0%
Rural residence (%)	28.0%

Table 2 illustrates the prevalence of the ETEC LT gene across different age groups among children with diarrhea. The most striking finding is the significantly higher prevalence of the ETEC LT gene in children under 12 months of age (51.0%). This indicates that infants are particularly vulnerable to ETEC infections expressing the LT toxin, potentially due to factors such as immature immune systems and increased exposure to contaminated environments. The prevalence of the ETEC LT gene decreases notably with increasing age. Children aged 12-23 months had a prevalence of 24.5%, while those aged 24-35 months had a prevalence of 19.2%. This downward trend suggests that older children may develop some level of immunity or have decreased exposure to ETEC as they grow older. The differences in prevalence between the age groups are statistically significant ($p < 0.001$), reinforcing the observation that younger children, especially infants, are disproportionately affected by ETEC infections expressing the LT gene.

Table 2. Prevalence of etec lt gene by age group.

Age group (months)	Number tested	Number positive	Prevalence (%)
<12	196	100	51.0%
12-23	102	25	24.5%
24-35	73	14	19.2%
36-59	79	12	15.2%
Total	450	81	18.0%

Table 3 compares the clinical characteristics of children with and without the ETEC LT gene. A significantly higher proportion of children with the ETEC LT gene (65.4%) experienced vomiting compared to those without the gene (42.9%). This strong association suggests that vomiting may be a hallmark symptom of ETEC infection expressing the LT toxin, likely due to its effect on intestinal motility and fluid secretion. Children with the ETEC LT gene were also more likely to have a fever (58.0%) than those without the gene (39.3%). This indicates that ETEC LT-positive infections may be more likely to trigger a systemic inflammatory response, leading to elevated body temperature. There were no significant differences in the occurrence of abdominal pain or dehydration between the two groups. This suggests that these symptoms are not specific to ETEC LT infections and may be common to other causes of diarrhea as well. The differences in vomiting and fever between the two groups were statistically significant ($p<0.001$ and $p=0.003$, respectively), highlighting a strong association between these symptoms and the presence of the ETEC LT gene. Table 3 provides valuable insights into the clinical presentation of ETEC LT-positive infections in children. The presence of vomiting and fever, coupled with the high prevalence of the ETEC LT gene in this study, underscores the importance of considering ETEC as a potential cause of diarrhea in children presenting with these symptoms. This information can aid in the timely diagnosis and appropriate management of ETEC infections, ultimately improving outcomes for affected children.

Table 3. Clinical characteristics of children with and without ETEC LT gene.

Characteristic	ETEC LT positive (n=81)	ETEC LT negative (n=369)	p-value
Vomiting	53 (65.4%)	158 (42.9%)	<0.001
Fever	47 (58.0%)	145 (39.3%)	0.003
Abdominal pain	68 (84.0%)	312 (84.5%)	0.912
Dehydration	22 (27.2%)	98 (26.6%)	0.921

Table 4 presents the results of a multivariate logistic regression analysis, which examines the independent association of various factors with the presence of the ETEC LT gene in children with diarrhea. The presence of vomiting is the strongest predictor of ETEC LT gene positivity. Children who experience vomiting are 2.94 times more likely to have the ETEC LT gene compared to those who do not vomit, even after adjusting for age, gender, and residence. This finding suggests that vomiting is a specific symptom strongly associated with ETEC LT infection. Fever is also a significant independent predictor of ETEC LT gene positivity. Children with fever are 1.97 times more likely to have the ETEC LT gene than those without fever, after adjusting for other variables. This indicates that fever is another clinical manifestation associated with ETEC LT infection. While there was a trend toward younger age being associated with ETEC LT gene positivity, this association did not reach statistical significance in the adjusted model. This suggests that the effect of age might be explained by other factors included in the model. Both gender and residence (urban vs. rural) were not found to be significantly associated with ETEC LT gene positivity in the adjusted model. This implies that these factors do not independently influence the likelihood of having ETEC LT infection. The findings from this multivariate analysis have important clinical implications. They suggest that the presence of vomiting and fever in children with diarrhea should raise suspicion of ETEC LT infection. This information can guide clinicians in making timely diagnostic and treatment decisions, potentially leading to improved outcomes for affected children. Table 4 highlights the independent association between vomiting, fever, and ETEC LT gene positivity. The results emphasize the importance of considering ETEC LT infection in the differential diagnosis of children with diarrhea presenting with these symptoms. This information can be used to inform clinical practice and public health interventions aimed at reducing the burden of ETEC-related diarrhea in children.

Table 4. Multivariate logistic regression analysis of factors associated with ETEC LT gene positivity.

Variable	Adjusted odds ratio (aOR)	95% confidence interval (CI)	p-value
Vomiting	2.94	1.73 - 5.00	<0.001
Fever	1.97	1.16 - 3.35	0.012
Age (per year increase)	0.82	0.65 - 1.03	0.084
Gender (Male vs. Female)	1.15	0.78 - 1.68	0.485
Residence (Urban vs. Rural)	0.93	0.56 - 1.54	0.771

The human immune system is a complex network of cells, tissues, and organs that work in concert to defend the body against harmful pathogens. However, this intricate system undergoes significant maturation and development during the first years of life, leaving infants particularly susceptible to infections, including those caused by ETEC. The immune system comprises two main branches: innate and adaptive immunity. Innate Immunity: This first line of defense is present from birth and provides a rapid, non-specific response to a wide range of pathogens. It includes physical barriers like skin and mucous membranes, as well as cellular components such as neutrophils, macrophages, and natural killer (NK) cells. Adaptive Immunity: This branch develops over time through exposure to pathogens and is responsible for generating specific, targeted responses. It involves B cells, which produce antibodies, and T cells, which directly kill infected cells or help coordinate the immune response. In infants, the innate immune system is relatively functional at birth, but the adaptive immune system is still immature. This imbalance predisposes infants to infections, as their ability to mount targeted responses against specific pathogens like ETEC is limited.[7,8]

One crucial aspect of adaptive immunity is humoral immunity, which involves the production of antibodies by B cells. Infants receive some antibodies from their mothers through the placenta and breast milk, providing passive protection against certain infections. However, these maternal antibodies gradually decline in the first few months of life, leaving infants more vulnerable to pathogens like ETEC, which they have not yet encountered and developed their own antibodies against. Furthermore, the infant's own B cell response is less efficient compared to adults. Infants produce lower levels of antibodies with a narrower range of specificity, making them less effective at neutralizing and clearing pathogens. This is partly due to the immaturity of follicular dendritic cells in infant lymph nodes, which are essential for B cell activation and antibody production. Cellular immunity, mediated by T cells, is also immature in infants. T-cell responses are less robust and less diverse compared to adults, with a bias towards Th2 responses, which are associated with antibody production rather than direct cell killing. This may impair the infant's ability to clear intracellular pathogens like ETEC, which can invade intestinal epithelial cells. Additionally, regulatory T cells (Tregs), which play a role in suppressing excessive immune responses, are more abundant in infants. While Tregs are important for preventing autoimmune reactions, their heightened activity in infants may dampen the overall immune response to pathogens, including ETEC.[8,9]

The gut microbiota, a complex community of microorganisms residing in the gastrointestinal tract, plays a pivotal role in immune system development and function. It helps to educate the immune system, stimulate the production of antimicrobial peptides, and compete with pathogens for resources. In infants, the gut microbiota undergoes significant changes during the first year of life, influenced by factors such as delivery mode (vaginal vs. cesarean), feeding practices (breast milk vs. formula), and antibiotic exposure. This dynamic period of microbial colonization can impact the infant's susceptibility to infections, including ETEC. For example, breastfed infants tend to have a more diverse and beneficial gut microbiota compared to formula-fed infants. Breast milk contains prebiotics, which promote the growth of beneficial bacteria, and antibodies, which can neutralize pathogens. This may provide some protection against ETEC infection in breastfed infants. Conversely, disruptions to the gut microbiota, such as those caused by antibiotic use, can increase the risk of ETEC infection. Antibiotics can deplete beneficial bacteria, allowing opportunistic pathogens like ETEC to colonize the gut and cause disease. The immature immune system and the dynamic gut microbiota in infants create a window of vulnerability to ETEC

infection. The reduced capacity for adaptive immune responses, coupled with the ongoing establishment of a protective gut microbiota, leaves infants more susceptible to ETEC colonization and toxin-mediated diarrhea. Understanding these developmental aspects is crucial for designing effective strategies to prevent and manage ETEC infections in infants. This may include promoting exclusive breastfeeding, optimizing complementary feeding practices, minimizing antibiotic use, and exploring the potential of probiotics or prebiotics to enhance gut microbiota development and function.[10,11]

The first few months of life represent a critical period for infants, as they transition from the protective environment of the womb to the outside world, teeming with potential pathogens. While the maternal immune system equips newborns with a temporary shield of antibodies, this protection gradually wanes, leaving them increasingly susceptible to infections. This phenomenon, known as maternal antibody waning, plays a significant role in the epidemiology of enteric infections, including those caused by Enterotoxigenic *Escherichia coli* (ETEC). During pregnancy, maternal antibodies, primarily immunoglobulin G (IgG), are actively transported across the placenta to the developing fetus. This transplacental transfer provides passive immunity, protecting the newborn against various pathogens to which the mother has been exposed or vaccinated against. These maternal antibodies are particularly crucial for defending against enteric infections, as the infant's own immune system is still immature and developing. After birth, maternal antibodies continue to be transferred to the infant through breast milk, providing further protection against enteric pathogens. Breast milk contains various immune factors, including secretory IgA (sIgA), lactoferrin, lysozyme, and oligosaccharides, which contribute to gut health and immune function. However, the levels of these protective antibodies gradually decline over time. The initial concentration of maternal antibodies in the newborn is directly proportional to the mother's antibody levels at the time of delivery. Mothers with higher antibody titers will transfer more antibodies to their infants, providing longer-lasting protection. Each antibody has a specific half-life, which is the time it takes for the concentration to decrease by half. Maternal IgG antibodies typically have a half-life of about 21-28 days, meaning that their levels decrease by 50% every three to four weeks.[12-14]

Breastfeeding prolongs the duration of maternal antibody protection by continuously supplying antibodies and other immune factors to the infant. The longer a child is breastfed, the slower the rate of antibody decline. Maternal exposure to specific pathogens through natural infection or vaccination can boost antibody levels and extend the duration of protection in the infant. As maternal antibodies wane, infants enter a window of vulnerability, during which they become increasingly susceptible to enteric infections. This is particularly relevant for ETEC, as the peak incidence of ETEC diarrhea typically occurs between 6 and 18 months of age, coinciding with the period of declining maternal antibody levels. Several mechanisms contribute to the increased susceptibility of infants to ETEC infection during this period. Maternal antibodies play a crucial role in neutralizing ETEC toxins and preventing their binding to intestinal receptors. As antibody levels decline, the ability to neutralize these toxins diminishes, allowing ETEC to colonize the gut and cause disease. Maternal antibodies also facilitate the opsonization of ETEC, marking them for destruction by phagocytic cells. With waning antibody levels, this process becomes less efficient, enabling ETEC to evade the immune system. Maternal antibodies, particularly sIgA, contribute to mucosal immunity by preventing the adherence of ETEC to the intestinal epithelium. As these antibodies decline, ETEC can more readily attach to the gut lining, facilitating colonization and infection.[14-16]

Infants, particularly those in the first year of life, are inherently vulnerable to environmental pathogens

due to a confluence of biological, behavioral, and socioeconomic factors. Their immature immune systems, combined with increased exposure to contaminated food and water, create a perfect storm for enteric infections such as those caused by ETEC. The immune system of an infant is still developing. While they receive some passive immunity through maternal antibodies, this protection gradually wanes over the first few months of life. Key components of the adaptive immune system, such as the production of specific antibodies and T-cell responses, are not yet fully mature. This leaves infants susceptible to a wide range of pathogens, including ETEC, which they may encounter in their environment. The gut microbiota plays a crucial role in protecting against enteric infections. It acts as a barrier against pathogens, competes for nutrients, and produces antimicrobial substances. However, the infant gut microbiota is in a state of flux during the first year of life, as it is colonized by a diverse array of microorganisms from the environment. This dynamic process can leave windows of vulnerability, during which pathogens like ETEC can establish a foothold and cause infection. The mode of feeding (breastfeeding vs. formula feeding) and hygiene practices during feeding and weaning can significantly influence an infant's exposure to ETEC. Breast milk provides numerous protective factors, including antibodies, immune cells, and oligosaccharides that promote the growth of beneficial bacteria in the gut. Exclusive breastfeeding for the first six months of life is associated with a reduced risk of diarrheal disease, including ETEC infection. However, not all mothers are able to breastfeed exclusively, and mixed feeding (breast milk and formula) may still offer some protection compared to formula feeding alone.[15-17]

Formula feeding, especially in settings with limited access to clean water and poor hygiene practices, can increase the risk of ETEC exposure. Improper cleaning and sterilization of bottles and nipples, as well as the use of contaminated water to prepare formula, can introduce ETEC into the infant's diet. The introduction of complementary foods (solid or semi-solid foods) during weaning presents another opportunity for ETEC exposure. If these foods are prepared with contaminated water or handled with unhygienic practices, they can become a source of infection. Infants have a natural tendency to explore their environment through touch and taste. They frequently put their hands or objects in their mouths, which can transfer pathogens like ETEC from contaminated surfaces to their digestive system. This behavior is particularly common during crawling and early walking stages, when infants are in close contact with floors and other potentially contaminated surfaces. In many developing countries, including Indonesia, poor sanitation and limited access to clean water contribute to widespread environmental contamination with fecal matter. The practice of open defecation, still prevalent in some areas, leads to the direct contamination of soil and water sources with human feces, which may contain ETEC. In urban areas with inadequate sewage systems, fecal waste can overflow or leak into the environment, contaminating water sources and increasing the risk of ETEC transmission. Contact with animal feces, especially from livestock raised in close proximity to human settlements, can also expose infants to ETEC, as some strains of the bacteria can colonize both humans and animals.[16-18]

Poverty and low socioeconomic status exacerbate the risk of ETEC exposure in infants. Families living in poverty often have limited access to clean water, sanitation facilities, and healthcare. They may also lack the resources to purchase safe, nutritious food or to implement proper hygiene practices. This creates a cycle of vulnerability, where infants born into poverty are more likely to be exposed to ETEC and other pathogens, leading to diarrhea and malnutrition, which in turn can further compromise their immune systems and increase their susceptibility to future infections. Addressing the complex issue of infant exposure to ETEC requires a multi-faceted approach

that encompasses both individual and community-level interventions. Exclusive breastfeeding for the first six months of life should be encouraged and supported as a key strategy to reduce the risk of ETEC diarrhea. This includes educating mothers about the benefits of breastfeeding, providing lactation support, and creating supportive environments for breastfeeding in public spaces. Investments in infrastructure to improve access to clean water and sanitation facilities are essential for reducing the risk of environmental contamination with ETEC. This should be accompanied by community-based hygiene education programs that promote handwashing, safe food handling, and proper disposal of feces. Caregivers should be educated about safe practices for preparing and storing formula, as well as the importance of hygiene during weaning. This includes washing hands thoroughly before preparing food, using clean utensils and containers, and avoiding feeding infants food that has been left at room temperature for extended periods. The development and deployment of effective ETEC vaccines hold great promise for reducing the burden of ETEC-related diarrhea in infants. Ongoing research in this area should be prioritized, with a focus on developing vaccines that are affordable, accessible, and effective in the context of developing countries.[19,20]

4. Conclusion

ETEC expressing the LT gene is a prevalent cause of pediatric diarrhea in Makassar City, Indonesia. The higher prevalence in children under one year of age highlights the vulnerability of this group and the need for targeted interventions. These findings emphasize the importance of improving sanitation and hygiene practices to reduce ETEC transmission in the community.

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