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Non Lactose *Fermenting Escherichia Coli* and Shiga Toxin

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Abstract:

Non-lactose-fermenting Escherichia coli (NLFEC) has a few descriptive studies restricted to infections in humans. Isolates of NLFEC characterised by their virulence ability, biofilm formation capacity and antimicrobial susceptibility profile. E.coli lactose-fermenting strains with the same conditions were analysed to provide comparisons. The non-lactose-fermenting E. coli strains were classifiedes belonging to clade I E. coli, whereas the lactose-fermenting strains were classified in phylo group B2. The virulence markers were present in all strains. adhesion, iron accumulation, toxins, colicin and cytotoxin production biofilm regulation. In addition to the extracellular matrix components, the ability of the strains to form in vitro biofilms has been demonstrated. Multidrug resistance (MDR) profiles were observed by in vitro susceptibility tests to all NLFEC strains. Non-lactose-fermenting E. coli behaves similar to lactose-fermenting E. coli, exhibiting MDR. The lactose non-fermenters underwent PCR-based O typing, multilocus sequence.typing (MLST) analysis, phylogenetic grouping. Resistance mechanisms have been studied for ciprofloxacin resistant isolates. From (19.7%) isolates were lactose non-fermenters and the ciprofloxacin resistance rate was significantly higher than in lactose fermenters. We have investigated the prevalence and characteristics of lactose intolerance in isolates from E. coli in this study.

Keywords: Escherichia coli-Toxins –Lactose.

Litreture review

Pathogens found in food, like *E. coli* that produces Shiga toxin. Hemorrhagic colitis, bloody, watery diarrhea, and the potentially lethal hemolytic uremic syndrome (HUS) can all be brought on by *E. Coli* (STEC).Contaminated foods, tainted water, and person-to-person contact can all spread STEC (1) In addition to being vital for human health, fresh fruits and vegetables are also great sources of fiber, minerals, and vitamins (2) They are vulnerable to microbial contamination through a variety of channels, such as harvest and postharvest handling, as well as contact with soil, dust, and water (3) A major source of pollution is the fertilizer used in the production of fruits and

vegetables, which is untreated wastewater and manure (4) Contamination can occur from contact with sewage, irrigation water, animal dung, transport, and merchant handling (5) Human disease outbreaks associated with fresh or minimally processed fruit and vegetable consumption have increased in recent years. The main virulence factors of STEC are encoded by the two subfamilies of shiga toxins, Stx1 and Stx2, which are classified as (6) They become pathogenic when they adhere to the intestinal microvilli of the host cell and cause effacement lesions. One of the most common causes of foodborne illness globally is strains of STEC (7,8) Twelve percent of the *E.coli* isolates were STEC isolates, of which two had Stx1 and one had Stx2. *E.coli* is a common biphasic microorganism found in the digestive tracts of humans and animals. It can survive well in the environment or in the host, where it is released along with human waste and animal excrement. (9) Studying clarified that a growing number of isolates of *Escherichia coli* O157:H7 are coming from fresh produce, such as leaf lettuce, cantaloupes, apples, and bean sprouts. Although the exact mechanism by which the pathogen enters the plant is unknown, one theory suggests that it does so through cultivation in areas that have been fertilized with manure that has not been properly treated. Based on data from epidemiology, *E.coli*. Up to 83.3% of dairy and beef cattle carry *E. Coli O157:H7,* which is asymptomatic when excreted in the feces. Composting manure is advised by current manure-handling regulations prior to applying it as fertilizer to a field. (10)

Hemolytic anemia (characterized by broken red blood cells), thrombocytopenia (low platelet counts in the blood), and acute kidney injury (AKI) are the clinical manifestations of Shiga toxin-producing *Escherichia coli* (STEC)-associated hemolytic uremic syndrome (STEC-HUS). It is the main infectious factor that causes AKI in kids. Severe instances may result in neurological issues or possibly death (11). Treatment for STEC-HUS is difficult because patients frequently have organ damage when they come for care. For better prognosis, lower mortality, and fewer aftereffects, early diagnosis is crucial. First, we provide a brief overview of the diagnostics for STEC-HUS in this review, covering the history-taking process, clinical manifestations, fecal and serological methods for STEC detection, and complement activation monitoring. We also provide an overview of protective measures and treatment approaches against STEC-HUS, including immunizations, volume increase, renal replacement therapy (RRT), antibiotics, plasma exchange, antibodies, and inhibitors that block receptor binding in addition to the intracellular trafficking of the Shiga toxin.(12) It revealed that two pathotypes Enteroaggregative *E.coli*,,Enteroinvasive *E.coli* (EAEC, EIEC) were the most prevalent among NLF *E. coli*, and they played a crucial role not only in causing diarrhea in children but also in adults.(13) *E. coli* is a bacteria that can be found in the intestinal tract of humans and animals. Most *E. coli* are harmless and are an important part of a healthy human intestinal tract. Some kinds of *E. coli* cause disease by making a toxin called Shiga toxin. The bacteria that make these toxins are called "Shiga toxin-producing" *E. coli*, or STEC. Around 5-10% of STEC cases may develop a sometimes fatal condition called haemolytic uraemic syndrome (HUS), characterised by acute kidney failure, low platelets and anaemia. (14)

There have only been a few descriptive studies on non-lactose-fermenting *Escherichia coli* (NLFEC) in humans. Non-lactose-fermenting *E. coli* bacteria were assigned to clade I *E. coli*, while lactose-fermenting strains were assigned to phylogroup B2. All strains exhibited adhesion, iron acquisition, toxins, colicin and cytotoxin synthesis, and biofilm regulation pathogenicity indicators, when isolated from animal. Regarding the Clermont phylogenetic classification (15) All NLFEC strains were assigned to *Escherichia cryptic clade I*, and all LFECs were classified as *B2.E.coli clade I* is rare among extraintestinal *E.coli* strains. (16)*E. coli* is initially identified biochemically based on its capacity to ferment lactose. Some *E. coli* strains, however, are unable to metabolize this sugar due to a lack of lactose permease encoded by the lacY gene (17,18,19) thus, these bacteria are referred to as non-lactose-fermenting *E. coli* (NLFEC. Non-lactose-fermenting) *E. coli* have a low detection rate, with few descriptions and little information in the literature, and are only associated with human UTI and sepsis. (20)

Isolates showed tiny colonies on MacConkey agar plates that were brilliant and transparent, but lacked the ability to ferment lactose. On MacConkey agar, the remaining isolates formed vivid and pink colonies (indicating lactose fermentation). The isolates were identified as *E. coli* based on preliminary biochemical testing (SIM, Simmon's citrate, and TSI, triple sugar iron). The non-lactose-fermenting isolates, on the other hand, were able to metabolize three sugars (adonitol, dulcitol, and D-sorbitol), in contrast to *E. coli* ATCC 25922, which fermented both dulcitol and D-sorbitol.(21) The study of resistance profiles between NLFEC and LFEC strains shows that NLFEC strains have a greater spectrum of resistance. Studying shows that the NLFEC germs were resistant to doxycycline and ciprofloxacin, while the LFEC strains were resistant to streptomycin and gentamicin.(22)The assignment of NLFEC strains to *E.coli clade I* indicates that the gene evolutionary history of nonfermenting *E.coli* may be different from that of typical fermenting *E.coli.* Therefore, non-lactose-fermenting *E.coli* is a potentially toxic pathogen in animal infections and behaves similarly to lactose-fermenting *E.coli*.(23)

Diarrhoea *Escherichia coli* DEC continues to be a significant contributor to the occurrence of acute diarrhoea in developing nations. In these countries, DEC is responsible for approximately 30-40% of cases of acute diarrhoea. It has been observed that around 10% of *E. coli* isolates obtained from stool samples do not ferment lactose. However, there is limited information available in the literature regarding the pathogenicity of non-lactose-fermenting *E. coli* strains that cause infectious diarrhoea. DEC, the main cause of infectious diarrhea in developing nations, encompasses various pathotypes categorized based on their virulence and phenotypic characteristics. Each pathotype exhibits distinct pathogenesis and geographical distribution. Therefore, the effective management of the disease relies on the prompt and accurate identification of DEC pathotypes.(24)

Our objective was to clarify the significance of NLF *E. coli* in the development of diarrhea in both adults and children by identifying various DEC pathotypes among NLF *E. coli* in stool samples collected from individuals with gastroenteritis. From previous studying, out of the 300 samples analyzed, *E. coli* was detected in 198 (66%) samples through culture, and in 170 (56.6%) samples through polymerase chain reaction. Based on the presence of virulence genes, enteropathogenic *E. coli* (33.8%) was the most common pathotype, followed by Shiga toxin-producing *E. coli* (STEC, 23.2%), enterotoxigenic *E. coli* (ETEC, 13.6%), enteroinvasive *E. coli* (5.5%).(25) The gold standard for diagnosing STEC infections is bacterial investigation. It is important to rule out other intestinal pathogens that can cause diarrhea using fecal culture, including *Shigella, Campylobacter,Yersinia, Salmonella*, and*Clostridioides difficile* In actuality polymerase chain reaction (PCR) must be used in conjunction with fecal culture to identify the Stx-encoding gene, increase the detection rate, and further differentiate O157 infections from non-O157 infections When a patient is extremely sick, it is best to take stool samples of diarrhea as soon as possible, preferably before starting antibiotic treatment (26)

Shiga toxin-producing *Escherichia coli*, despite notable progress in the past decade, the precise mechanism by which Shiga toxin-producing *E. coli* triggers hemolytic uremic syndrome remains uncertain. Various factors, including Shiga toxin, lipopolysaccharide, adhesins such as intimin, and *E. coli*-secreted proteins A, B, and D, as well as the 60-MD plasmid and enterohemolysin, are likely to contribute to the pathogenesis.(Shiga toxin's)Stx has been shown to be toxic to human endothelial cells, causing cell death and potentially inducing apoptosis. Additionally, when endothelial cells are pre-stimulated with TNF-α, IL-1β, or sodium butyrate, the number of Gb3 receptors on the cells increases, making them more susceptible to the cytotoxic effects of Stx. Stx can also directly activate endothelial cells, leading to changes in the expression of vasomediators derived from the endothelium. Both Stx1 and Stx2 have been found to increase the production of prepro-endothelin mRNA transcript levels in bovine endothelial cells, without affecting nitric oxide levels. However, this effect was not observed with the receptor-binding B subunit of Stx, which

lacks the N-glycosidase enzyme. Interestingly, purified Stx was found to increase the release of nitric oxide from murine macrophages. Furthermore, aside from endothelial cells, Stx has been observed to have a harmful impact on various other cell types, such as renal glomerular and tubular epithelial cells It was discovered that cells lacking the toxin receptor were resistant to the toxic effect Stx has the ability to trigger apoptosis in renal tubular epithelial cells Burkitt lymphoma cells intestinal epithelial cells pulmonary epithelium-derived cells and Vero cells. The holotoxin and high doses of the B subunit were both found to induce cell death, suggesting that this effect may be independent of the impact on protein synthesis. Apoptosis was observed in the kidneys of patients with HUS and in mice inoculated with Stx-positive strains, but not Stx-negative strains. These findings, combined with the fact that Stx can induce apoptosis in renal tubular epithelial cells, suggest that Stx-induced apoptosis may contribute to renal injury during HUS. Studies have shown that apoptosis is a causal mechanism in the pathogenesis of renal tubular injury and glomerular sclerosis (27)

Shiga toxins (Stxs), which are some of the major virulence factors of enterohemorrhagic *Escherichia coli* (EHEC) O157: H7, are induced and released by various environmental stimuli, such as DNA damage responses and stress-inducing chemicals. To investigate the possible influence of growth medium on Stxs expression, we investigated the growth kinetics and Stx (Stx1 and 2) of cells grown in commonly used Luria-Bertani medium (LB) and *E.coli* medium (EC).) was investigated.For EHEC) expression was analyzedGrowth of EHEC O157: H7 in EC medium by direct plating and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) significantly reduced total bacterial numbers and Stx1 transcription.It was found that it is increasing. A period of more steady growth than pound. Here, we report that bile salts and lactose, the only two components of EC medium not present in LB, function as negative and positive regulatory signals for stx1 and stx2 transcription, respectively. Indeed, in EC medium without bile salts, Stx transcription was significantly increased compared to normal EC (~5.7-fold and ~21.8-fold for stx1 and stx2. In contrast, EHEC O157: H7 grown in lactose-free EC medium significantly reduced these transcriptions (~93.5-fold and ~4.3-fold for stx1 and stx2. Consistently, Stx transcription increased dramatically in LB medium supplemented with lactose, suggesting that lactose may be an environmental trigger of her Stx expression (28) Many selective plating media for different microorganisms are commercially available. All media are suitable for both routine analysis and research, but selectivity and sensitivity are his two most important factors in selection. Choosing the optimal medium is important, especially when the number of bacteria being tested is very small compared to the roommate's microbiome. In general, the "best medium" is determined primarily by time and other factors. For *E.coli* O157: researchers have compared many selective media over the years for the isolation of H7. Although(Sorbitol MacConkey agar) SMAC agar is very widely used in traditional culture techniques, SMAC agar plus BCIG (16), SMAC agar plus CR (12) and SMAC agar plus *E.coli* serotype O157 (21) Excellent. In this study, SMAC agar was found to be superior to his F-O157 agar.(29)

E.coli O157: H7 ferments sorbitol slowly or not at all and does not produce functional β-glucuronidase, whereas most other *E.coli* strains are positive in both tests. Additionally, *E.coli* strain O157 does not ferment rhamnose on agar plates, while 60% of sorbitol nonfermenting *E.coli* do not ferment rhamnose. Other serogroups of *E.coli* ferment rhamnose on agar plates. When testing for *E.coli* O157: H7 in clinical laboratories, stool samples are typically swabbed onto plates containing sorbitol MacConkey agar (SMAC) (containing sorbitol instead of lactose). (30,31). Nonfermented sorbitol colonies are selected and tested serologically for O157 and H7 antigens. Positive colonies should then be tested for Shiga toxin production. For example, most O157 strains do not ferment sorbitol (i.e., sorbitol negative). On the other hand, most non-O157 strains ferment sorbitol (32,33) resulting in sorbitol negative results.We successfully detected *E.coli* strain O157

(34,35,36) using MacConkey (SMAC) agar. However, SMAC agar could not successfully support the growth of stressed *E.coli* O157 cells (37,38)

Despite the fact that *E. coli* is often used as a model organism. The characteristics of this organism are short bacterial, nonsporeforming, facultative anaerobic, gramnegative bacteria, belonging to the Enterobacteriaceae family, and *E.coli*. It's made from a basic media. *E. coli* is an important part of common gut flora in humans. Enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli*, enterohemorrhagic *E.coli* (EHEC) enteroinvasive *E.coli*, enteraggregative *E.coli*, and diffusely adherent *E.coli* are six groups of intestinal pathogens that have been well documented. Pilli, enterotoxins (LT, ST), endotoxins (lipopolysaccharide), Shiga-like toxins, hemolysin, intimin, aerobactin, cytonecrotizing factor, and biofilm development are some of the virulence factors that contribute to the pathogenicity of *E.coli*. (39)

Conclusion

Diarrheagenic *Escherichia coli* (DEC) is still one of the leading causes of acute diarrhea episodes in impoverished nations. DEC accounts for 30-40% of severe diarrhea cases in these countries. Approximately 10% of *E. coli* isolates from stool samples have been found to be non-lactose fermenting (NLF). There is less literature on the pathogenicity of NLF *E. coli* producing infectious diarrhea.

However, the literature contains little information on the pathogenicity of non-lactose-fermenting *E. coli* strains that cause infectious diarrhea. We wanted to determine the role of NLF *E. coli* in causing diarrhoea in both adults and children by finding different DEC pathotypes in stool samples from gastroenteritis patients. In our investigation, two pathotypes (EAEC and EIEC) were common among NLF *E. coli*, and they were key aetiological agents in both children and adults. Our findings also offer light on the epidemiology of EIEC, which is one of the most underappreciated DEC pathotypes, as few microbiological facilities test NLF *E. coli* for EIEC.

The DEC is classified into multiple pathotypes on the basis of its virulence and phenotypic characteristics. There are different pathogenesis and geographical distribution patterns for each pathotype. Rapid and precise identification of the DEC pathotypes is therefore a prerequisite for effective disease management. In stool samples collected from individuals with gastroenteritis, our aim was to clarify the significance of NLF *E. coli* for development of diarrhoea in adults and children by identifying various DEC pathotypes among these bacteria. According to our research, the most common type of NLF *E. coli* is the two pathotypes, EAEC and EIEC, which play an important role not only in causing diarrhoea in children, but also in adults.

Moreover, due to limited testing in microbiological laboratories of NLF *E. coli* for EIEC, the epidemiology of EESC is highlighted in our study and often overlooked as a DEC pathotype. Different pathotypes classified according to their virulence and phenotypic characteristics are included in the DEC, which is a major cause of infectious diarrhoea in developing countries. The different pathogenesis and geographic distribution of each pathotype is apparent. Consequently, rapid and accurate identification of the DEC pathotype is essential to effective disease control. It is likely that the hybrid DEC will be more virulent in comparison to typical pathotypes. Pathotyping in clinical settings for the proper management of DEC associated diarrhoea should be included. The purpose of this study was to examine the prevalence of DEC pathotypes and findings: It seems that the hybrid DEC pathotype is more virulent compared to its basic counterparts. Therefore, pathotyping for the effective management associated diarrhoea should be made available in clinical settings.

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