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Review

OVERVIEW OF ESCHERICHIA COLI O157:H7: CURRENT STATUS AND PROMISSING THERAPY

Submitted to the Council of the College of Medicine, Diyala University in Partial Fulfillment of Requirements for the Bachelor Degree in medicine and general surgery

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Abstract

Escherichia coli (*E. coli*) are normal inhabitants of the human large intestine. Most strains are harmless, but some strains acquire bacteriophage or plasmid DNA-encoding enterotoxins or invasion factors and become pathogenic**.** *Escherichia coli* O157: H7 is a chemoorganotrophic facultative anaerobe with both respiratory and fermentative metabolisms identified by biochemical tests and selective/chromogenic media. Cells are Gram-negative straight rods about 1 μm2–6 μm and typically motile via peritrichous flagella**.** Zoonotic transmission of *E. coli* 0157:H7 occurs after consumption of undercooked meat or deficiently pasteurized dairy products or contact with contaminated fomites laden with Shiga toxin enterohemorrhagic *E. coli*. Zoonotic transmission of *E. coli* 0157:H7 occurs after consumption of undercooked meat or deficiently pasteurized dairy products or contact with contaminated fomites laden with Shiga toxin enterohemorrhagic *E. coli*. Its importance in medicine is due its potential association with hemolytic uremic syndrome which is serious medical condition**.**

Keywords: *Escherichia coli*, serotype, hemolytic uremic syndrome

Introduction

Escherichia coli (*E. coli*) are normal inhabitants of the human large intestine. Most strains are harmless, but some strains acquire bacteriophage or plasmid DNA-encoding enterotoxins or invasion factors and become pathogenic [30]. Despite the fact that *E. coli* as a commensal bacterium can be found in intestinal microflora of a variety of animals including man, not all the strains are harmless, and some can cause debilitating and sometimes fatal diseases in humans as well as mammals and birds [5]. Pathogenic strains are divided into intestinal pathogens causing diarrhea and extraintestinal *E. coli* (ExPEC) causing a variety of infections in both

humans and animals including urinary tract infections (UTI), meningitis and septicemia [21].

Diarrheagenic *E. coli* (DEC) strains are among the most common etiologic agents of diarrhea. Based on their specific virulence factors and phenotypic traits, it can be divided into enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Vero toxin-producing/Shiga toxin-producing *E. coli* (VTEC/STEC) which include its well-known subgroup enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) [18-20]. EHEC serotype O157:H7 was first recognized in 1982 as a human pathogen associated with outbreaks of bloody diarrhea in Oregon and Michigan, U.S.A. [28] and is also linked to sporadic cases of **Hemolytic uremic syndrome** (**HUS**) in 1983. Since then, many outbreaks associated with EHEC have been reported in the United States and E. coli O157:H7 has become one of the most important foodborne pathogens [1]. It acquired its importance as a culprit in causing Hemolytic uremic syndrome (HUS) in children.

AIM OF STUDY

To highlight the importance of the serotype *E. coli* O157:H7, clinical features of HUS, current status and future perspective of different immunization strategies and promising novel therapy

Escherichia coli O157: H7 is a chemoorganotrophic facultative anaerobe with both respiratory and fermentative metabolisms identified by biochemical tests and selective/chromogenic media. Cells are Gram-negative straight rods about 1 μm2–6 μm and typically motile via peritrichous flagella [11].

The enterohaemorrhagic *E. coli* (EHEC) pathotype describes strains that can express a type 3 secretion system (T3SS) and produce Shiga toxin

subtypes (Stx 1a, 2a, 2c, etc.), although the original definition also required an association with human disease [17] . most cases and outbreaks historically have involved the serotype O157:H7. However, with the development of better diagnostic tools, other serotypes and pathogenic groups of *E. coli* are now also becoming increasingly linked to sporadic cases and outbreaks [7]. *E. coli* O157:H7 became recognized as a human pathogen and a cause of foodborne disease in 1982, following two outbreaks of hemorrhagic colitis linked to the consumption of hamburgers [37] .

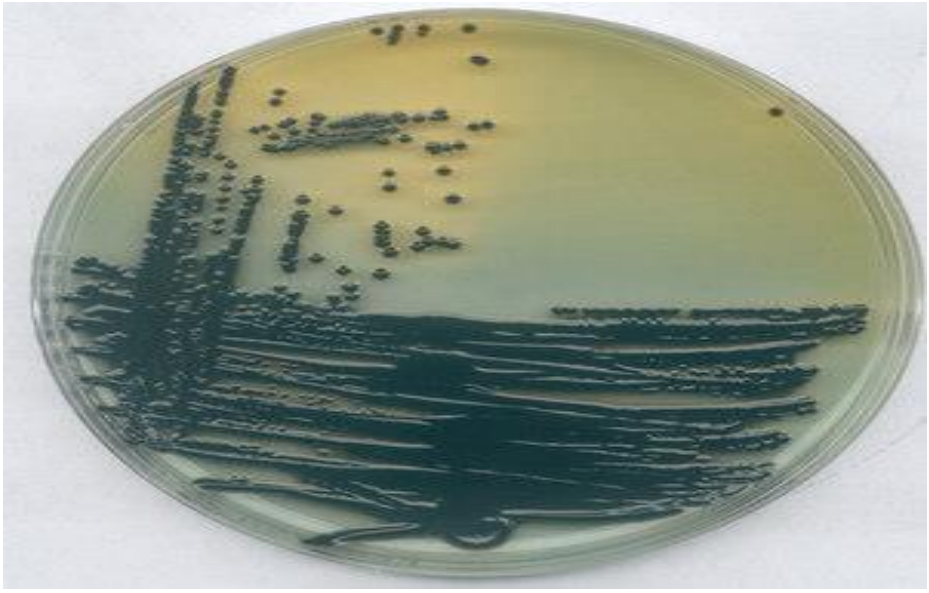

Figure 1. E. coli O157:H7 culture on agar [46]

Transmission

Zoonotic transmission of *E. coli* 0157:H7 occurs after consumption of undercooked meat or deficiently pasteurized dairy products or contact with contaminated fomites laden with Shiga toxin enterohemorrhagic *E. coli*. Other causal etiologies of Shiga toxin enterohemorrhagic E. coli include exposure to contaminated water from potable drinking sources, swimming pools and lakes, contaminated food such as insufficiently cooked meats, inadequately washed leafy greens and fruits, unpasteurized

drinks including apple juice, and direct contact with contaminated animals in petting farms [20] .

Outbreaks

The epidemiology of *E. coli*—associated infections varies widely depending on the type of strain involved. In the last years in Europe, *E. coli* outbreaks were mainly caused by various EHEC strains. STEC *E. coli* O104:H4 has been responsible for a large number of outbreaks in the recent years. During the spring of 2011, a novel *E. coli* O104:H4 serotype infected about 4,000 individuals in Central Europe, mainly in Germany, provoking more than 900 cases of HUS [25] .This particular pathogen demonstrated a combination of virulence factors from both EAEC and EHEC strains. A strain similar to the current outbreak strain had been previously isolated and characterized in Republic of Georgia. HUS cases were reported in several European countries (Data 2010). The prevalent serogroups identified are O157 (EHEC O157:H7 serotype is the predominant cause of HUS) and O26. The highly virulent EHEC O26:H11/H- serotype is emerging in Europe. *E. coli* O25b:H4/ST131 (sequence type 131) is an emerging disseminated multidrug-resistant ExPEC strain, causing a broad spectrum of diseases, mainly urinary tract infections. *E coli* O25b:H4/ST131 is widely distributed in Europe, with Spain and Italy most prominently affected [31] .

Virulence factors

The ability to produce one or more shiga toxins is a hallmark *E. coli* O157:H7 infection. However, toxin production is not sufficient to cause disease. Two other factors are indicted in contributing to the virulence of *E. coli* O157:H7. The first of these two factors is harboring a 60 MDa

virulence plasmid (pO157), which encodes a hemolysin. The other factor is the locus of enterocyte effacement (LEE) [36].

Shiga toxin

The Shiga toxin family comprises three members. Shiga toxin, produced by *Shigella dysenteriae* type 1, is the prototype Shiga toxin. On the other hand, Stx1 and Stx2 are produced by the EHEC. Several variants of Stx2 have been identified as well and these include Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g. These share 84–99% of the amino-acid sequence of Stx2 but differ in some of its biological characteristics [44] . Shiga toxins induce an increase in chemokine synthesis from intestinal epithelial cells. This augments host mucosal inflammatory responses with release of interleukins, such as IL-8 and IL-1, in addition to Tumor Necrosis Factor (TNF). Activation of human endothelium by TNF or IL-1 leads to an increase in toxin receptor synthesis and hence increased sensitivity of the cell leading to increased cell death after exposure to the toxins [32].

Plasmid (pO157)

All isolates of *E. coli* O157:H7 harbor the 60 MDa pO157 plasmid. This plasmid contains the *hly* operon encoding an enterohemolysin. This hemolysin, with the aid of specialized transport systems, may allow the bacterium to utilize the blood released into the intestine as a source of iron [28] .

The locus of enterocyte effacement

The locus of enterocyte effacement (LEE) is a 35.6 kb pathogenicity island inserted in the genome of some bacteria such as enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, *Citrobacter rodentium*, and *Escherichia albertii*. LEE comprises the genes responsible for causing attaching and effacing lesions, a characteristic lesion that involves intimate adherence of

bacteria to enterocytes, a signaling cascade leading to brush border and microvilli destruction, and loss of ions, causing severe diarrhea [15] .

Mechanism of resistance

Antimicrobial resistance is a major and increasing global healthcare problem. Since the introduction of the penicillin, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antimicrobial resistance to other species [42] .

As in humans, the use of antimicrobials leads to an increased incidence of resistance in both pathogenic and endogenous bacteria**.** *E. coli* is intrinsically resistant to therapeutic levels of penicillin G, the first β-lactam introduced into clinical practice, because of its outer membrane barrier. *E. coli* is also resistant to several different classes of antibiotics with distinct mechanisms of action [22] . In *E. coli*, β-lactamase production is the most important mediator of resistance to broad spectrum of β-lactams. β-lactamases constitute a wide class of enzymes, which are often encoded on plasmids, and are most commonly produced by *Enterobacteriaceae* in general and by *E. coli* in particular. β-lactamases confer resistance to penicillins and cephalosporins and are an emerging cause of multidrug resistance in Gram-negative bacteria [35].

Clinical features of infection

Symptoms of the diseases caused by STEC include abdominal cramps and diarrhoea that may in some cases progress to bloody diarrhoea (haemorrhagic colitis). Fever and vomiting may also occur. The incubation period can range from 3 to 8 days, with a median of 3 to 4 days. Most patients recover within 10 days, but in a small proportion of patients (particularly young children and the elderly), the infection may lead to a life-threatening disease, such as haemolytic uraemic syndrome (HUS). HUS is characterized by acute renal failure, haemolytic anaemia and

thrombocytopenia (low blood platelets) **[42].** HUS is defined by The leading cause of HUS in children is STEC infection with a bloody diarrhea episode preceding HUS, while in adults HUS is mainly related to genetic complement abnormalities or to underlying morbid conditions. Up to the mid-2000s, STEC O157:H7 serotype appeared as the most common cause of post-diarrheal HUS irrespective of the context (sporadic case patients or outbreak), while non O157:H7 serotypes were occasionally isolated from patients presenting with HUS **[16].**

All presentations of HUS share endothelial cell lesions leading to the common spectrum of thrombotic microangiopathy (TMA). TMA is defined by the presence, mostly in the kidney, of vascular endothelial cell damage: endothelial cell swelling and detachment from the basement membrane, splitting of the glomerular basement membrane with "double contours" aspect, fibrin and platelet thrombi in the glomerular capillaries and renal arterioles. These lesions are responsible for the triad of mechanical hemolytic anemia, platelet activation/aggregation leading to thrombi and thrombocytopenia, and kidney failure [6] . The HUS manifestations don't only involve kidneys and blood, they involve the CNS, GIT system, liver involvement and pancreatic complication and others [26].

Detection of the microorganism

The primary evaluation is by clinical examination and stool or blood cultures. In recent years, ELISA test has been designed to detect shiga toxins directly in stool samples. The test is rapid and has a good potential for shiga toxin detection since it can detect the presence of shiga toxin-producing *E. coli* (STEC) or other shiga toxin-producing bacteria. Since shiga toxin type differentiation requires high cost monoclonal and polyclonal antibodies it is not widely used. Hybridization method is an effective, highly sensitive and specific molecular method for precise detection of shiga toxins, and uses non-radioactive substances [2] .

In contrast to serological and microbiological tests, PCR provides a rapid and sensitive alternative. This technique, first developed by Karch and Meyer, includes a primer pair from a conserved region of stx1 and stx2 in homologous genes whose main defects were low Tm and ineffectiveness in different types of shiga toxins. In that regard, to detect different types of shiga toxins it is necessary to design a multiplex PCR with at least two pairs of primers to detect shiga toxin gene [24].

Treatment

Treatment of EHEC-associated hemorrhagic colitis is supportive, with measures such as fluids and a bland diet. Antibiotics do not seem to reduce symptoms, prevent complications or decrease shedding, and they appear to increase the risk of HUS [23] . While the effects of specific antibiotics are still incompletely understood, current recommendations suggest that these drugs should be avoided if possible (although there may be some situations, such as complications, where this is not feasible). The use of antimotility agents in hemorrhagic colitis also seems to increase the risk for developing HUS **[10] .**

The use of antibiotics in STEC infections has also been addressed in the Infectious Disease Society of America (IDSA) guidelines for the management of infectious diarrhea. Their previous edition, published in 2001, stated that antibiotic administration should be avoided in suspected STEC infections, as their role remained unclear [27]. However, the recommendation does not appear to be widely implemented, as the rate of antibiotic administration in this setting remains high. The latest edition of the IDSA guidelines, published in October of 2017, strongly recommends against the use of antibiotics in infections caused by Stx2 producing STEC [39].

Treatment of STEC-HUS is mainly symptomatic. Patients require hospitalization in specialized departments familiar with the management of acute kidney injury (vascular access insertion by trained physicians, initiation and modality of dialysis, adapted to body weight in children) and HUS (packed blood red cell transfusions; detection, monitoring, and treatment of hypertension, neurological manifestations, intestinal complications, pancreatitis, ischemic cardiomyopathy) [4] .

Specific treatments of STEC-HUS are lacking. Randomized trials did not show the benefit of anti-thrombotic/fibrinolytic or Stx binding agents, or plasma infusions. The benefit of plasma exchanges over supportive treatment was also most uncertain in large series of patients during the German STEC O104:H4 outbreak [21].

A study support the use of Eculizumab in the treatment of severe pediatric STEC associated HUS but need more trials to confirm [34].

Alternative therapies

The worldwide emergence of multidrug-resistant bacteria has dramatically limited the number of antibiotics that retain activity against these pathogens. This problem has been further amplified by the dearth of novel classes of antibiotics. Therefore, development of novel therapeutic strategies for infectious diseases is high demand. In response, several new therapies have been developed, such as phage therapy, antimicrobial peptide therapy and combinations of two or more antibiotics **[43].**

The potential use of bacteriophages as therapeutic agents was recognized from the 1900s. However, this therapeutic approach was eclipsed by the discovery and use of antibiotics. Nevertheless, phage therapy was used for the treatment of human bacterial infections, mainly in Eastern Europe. Phages have a number of advantages that make them attractive for therapeutic use against bacteria. First, they are highly specific

and can be very effective in lysing bacteria. Second, phages are safe as underscored by several clinical studies, and third, they can be readily modified to fight the emergence of new multiresistant bacterial strains. Many studies characterizing lytic phages specific for different *E. coli* strains have been published demonstrating their potential therapeutic value **[40].**

In addition to therapeutic use of lytic phages, phage-encoded enzymes can be potentially used as an effective antibacterials against pathogens. Endolysins are hydrolase enzymes produced by phages at the end of their replication cycle to digest the bacterial cell wall for the release of progeny virions. Endolysins work equally well when applied exogenously to bacterial cells and thus these enzymes are potentials candidates as new antibacterial agents **[14].**

Antimicrobial peptides (AMPs) are an abundant and diverse group of molecules that are produced by eukaryotic and prokaryotic organisms or encoded by phages. In eukaryotes, AMPs contribute to innate immune responses and defend organisms against potentially harmful microbes **[33]** Several AMPs are being developed as drugs. They are able to act against antibiotic-resistant pathogens and are less susceptible to bacterial resistance than conventional antibiotics. Synthetic AMPs have been also developed, with designs based on common structural elements in natural peptides**.** Numerous natural and synthetic AMPs have direct activity against wide range of microorganisms including Gram-positive and Gram-negative. There are also several reports in the literature regarding activity of AMPs against *E. coli* strains. Taken together, the results obtained so far highlight that AMPs represent a new promising therapeutic option for the treatment of bacterial diseases, including infections due to multidrug-resistant strains [30].

An alternative therapeutic strategy against multi-resistant bacteria could be the use of efflux pump inhibitors. Efflux is a well-known antibiotic resistance mechanism, bacteria being capable to export actively molecules from the cell using efflux pumps. Although not used in the clinical practice yet, the high therapeutic potential of the combination of efflux pumps inhibitors with antibiotics has been clearly demonstrated. Furthermore, this co-therapy would allow for the use of antibiotics normally compromised by efflux pump activity **[38].**

Prevention

People with higher chances for foodborne illness are pregnant women, newborns, children, older adults, and those with weak immune systems, such as people with cancer, diabetes, or HIV/AIDS [8] . Washing hands, food and cooking tool, keeping proper hygiene, cooking meat properly and buying from trusted stores are the main personal methods of prevention of the infection **[30] .**

There are many industrial methods of disinfection and prevention include disinfection with certain chemicals and treating the suspected animals with antibiotics. A study conducted by de Oliveira *et al*., [32] demonstrated the direct and indirect application of ozone produced significant reductions in the counts of *E. coli* O157:H7, proving its efficiency in controlling this pathogen, where the maximum reduction was obtained under the conditions evaluated, allows for recommending its use in the cleaning processes of equipment and utensils in the food industry [13] .

Ultrasonic treatment is a promising alternative for thermal sterilization in food industry, the US treatment had a good antibacterial effect on *E. coli* O157:H7. After the US treatment, the cell membrane of bacteria was destroyed, leading to the leakage of intracellular material.

Meanwhile, The US treatment also caused the inhibition of the Hexose Monophosphate Pathway of the bacteria. Finally, the loss of *E. coli* O157:H7 cell viability in vegetable juices was observed under the optimal US treatment condition (exposure time of 7 min, ultrasonic power of 100 W and ultrasonic intensity of 50 W/cm2) [29].

Vaccinating the host animal is an important topic in prevention. In theory, the benefit of vaccination within discrete populations (e.g., pens or herds of cattle) is reduced fecal-oral transmission within cattle environments, less contamination of cattle hides, and fewer pathogens carried into the abattoir at harvest. For vaccination to be useful as a preharvest intervention, the benefits must not be undone during subsequent management practices, such as transportation to the abattoir or during holding in lairage [12].

E. coli as Biological weapons

Escherichia coli is present in the Centers for Disease Control and Prevention (CDC) list of biological agents potentially threat to public health and safety. Several microorganisms or their products can be used as biological weapon for warfare and bioterrorism. The CDC classifies potential agents as biological weapon in three categories. In Category A agents which can be easily disseminated or spread from person to person, resulting in high mortality rate and impact on public health are listed. Category B lists pathogens moderately easy to disseminate, resulting in moderate morbidity rates and low mortality rates. Category C lists emerging pathogens with potentially high morbidity and mortality and which can be engineered for mass dissemination [9] . *E. coli* O157:H7 strain is present in Category B as “food safety threat”. Even though less dangerous than Category A agents, Category B agents are easier to produce and handle, and the use of such agents against civilian populations by terrorists might well cause considerable panic [3] .

Conclusion

E. coli infection and food poisoning represent a major health issue in both developed and developing countries and the need for urgent solutions and advance research in prevention, management and minimizing the post infection complication, is crucial.

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