

REVIEW ARTICLE

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General considerations regarding the infections with the *Escherichia coli* pathogen

Marius Necşulescu¹, Bogdan-Ioan Coculescu^{1,2}, Simona Bicheru¹, Diana Popescu¹, Lucia Ionescu¹, Gabriela Dumitrescu¹, Viorel Ordeanu^{1,2}

Abstract: *Escherichia coli* is the species of the genus *Escherichia* with the greatest epidemiological impact. *Escherichia coli* infections are found mainly in places with poor hygiene; the infants with ages between 1 and 3 years old are included in the category with the highest risk. It is a "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce and insufficiently washed vegetables. The disease has been reported worldwide, being described numerous episodes of infection with *Escherichia coli* that caused multiple illnesses and deaths. *Escherichia coli* has three types of antigens: antigen "O" (somatic), antigen "H" (flagella) and antigen "K" (capsular). Clinical manifestations are present in the form of non-specific diarrhea, a dysentery form of enteritis, choleraform enteritis, hemorrhagic colitis and hemolytic uremic syndrome (HUS). The *Escherichia coli* infection diagnosis is made by identifying the etiologic agent and/or by highlighting the VTEC toxin in the feces. The treatment consists in precautionary antibiotherapy, hydrodynamics and electrolyte rebalancing, blood transfusions and dialysis, if in the case of renal failure. The prevention of infections with *Escherichia coli* is achieved by personal hygiene, food hygiene and work hygiene.

Keywords: *Escherichia coli*, infection, toxin, treatment, hygiene

INTRODUCTION

Escherichia coli is the species of Enterobacteriaceae, of the genus *Escherichia*, with the greatest epidemiological impact. *Escherichia coli* infections are found mainly in places with poor hygiene; infants are included in the category with the highest risk. Also, adults can develop severe forms of infections, especially in regions where underdevelopment, poverty and lack of hygiene are the dominant picture.

The disease can occur in particular through the "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce

¹ Military Medical Research Center

² Titu Maiorescu University, Bucharest

and insufficiently washed vegetables. Processing raw food can contaminate kitchen utensils, especially if these are not separately used for each food category and are not cleaned between uses. Contamination can occur through contact with carrier animals, if the basic rules of personal hygiene are not complied with, such as washing hands with soap and water.

In recent years, in nearly all parts of the world have been reported numerous episodes of infection with *Escherichia coli*, resulting in numerous illnesses and even deaths. We recall the tragedy of Walkerton (Ontario/Canada), when approximately 5,000 people from the community showed symptoms specific to the digestive infection, the etiological agent being *Escherichia coli* [1]. In 2011, in Germany broke one of the largest outbreaks of acute gastroenteritis with hemolytic uremic syndrome (HUS) caused by the *E. coli* O104: H4 strain (German hemolytic uremic syndrome/enterohemorrhagic *E. coli* - GHUSEC). The outbreak has had an unusual epidemiological origin only partially resolved. Investigations conducted by authorities showed that the illnesses occurred amid the consumption of contaminated water by agricultural runoff into a water well and subsequently there was the groundwater contamination [2].

The latest outbreak of the infection with *Escherichia coli* is evolving in Romania with this disease being contracted by 12 children (so far), aged between 1 and 3 years, all coming from localities in Argeş County.

The particularly severe infection resulted in the death of 3 children and even though the competent bodies through their health authorities have sought to find the source of the outbreak, they have not been able until now to find the source of the infection.

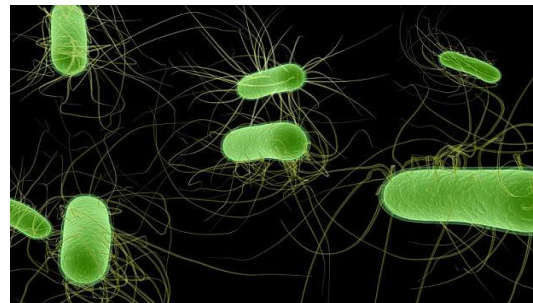
After the latest information, this seems to have been about a false "outbreak of diarrheal disease with uremic syndrome"; in reality the infectious etiology is varied, as confirmed by the Cantacuzino Institute.

In conclusion, due to the lack of a common source for the contagion, there cannot be a question of an epidemic or outbreak as the cases are isolated; however, due to the media pressure the incident could be termed as a "media outbreak".

ETIOLOGY

The genus *Escherichia* is of the Enterobacteriaceae family which comprises of 44 genera, 25 of which are found in human pathology. Most of these genres include commensal bacteria very abundant in nature (soil, water, etc.) [3], their natural habitat being the gastrointestinal tract of humans and warm-blooded animals, their feces containing more than 1010 enterobacteria/gram [4]. The constant presence of *Escherichia coli* strains in human and animal intestines and implicitly in the feces, determined that this bacterium represents the indicator of fecal pollution of the environment, especially of the water. Within the genus, are known 6 species: *Escherichia coli*, *Escherichia fergusonii*, *Escherichia hermannii*, *Escherichia vulneris*, *Escherichia albertii* and *Escherichia blattae*, the latter being isolated due to the fact that it only comes from specific species of insects.

Figure 1: *Escherichia coli*
(<http://www.telegraph.co.uk> 620 × 387)



The most important representative of the genus is *Escherichia coli*, bacterium discovered by Austrian pediatrician Theodor Escherich. In 1886, after a hard clinical and laboratory activity he has presented at the Faculty of Medicine in Munich and subsequently published in Stuttgart, a monograph on the link between intestinal bacteria and the physiology of digestion in children entitled "Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung" [5]. In 1919, Castellani and Chalmers proposed that the name of the isolated bacillus from the colon (*Bacterium coli commune*), by the renowned Austrian pediatrician Theodor Escherich be changed in *Escherichia coli*, in his honor, a name that was officially approved in 1958 [6]. Bacteria are present in the form of straight or slightly curved bacilli with rounded ends,

the presence of flagella and therefore the mobility being constant in most species (Figure 1). It has a size between 2-5 μm /0.5-1 μm and from a tinctorial point of view is Gram-negative.

It grows on simple culture media (simple glucose broth, nutrient agar and blood agar) developing specific Type S colonies, round, domed, shiny with a smooth surface (Figure 2) and regular edges or with dry R colonies with irregular edges.

Figure 2: *Escherichia coli* culture on blood agar
(<http://www.wzzm13.com> 534 × 401)



Biochemically, is fermenting the glucose with the production of gas, it produces indol, β -galactosidase and ferments lactose, characteristic that is allowing the differentiation from other enterobacteriaceae. *Escherichia coli* has an aerobic or optionally anaerobic metabolism and develops at an optimum temperature of 37°C and pH between 7.2-7.8 [7], but differs from other coliform bacteria by increasing to 44°C.

THE ANTIGENIC STRUCTURE

Escherichia coli has three types of antigens that can be highlighted by serologic antigen-antibody reactions:

- The O antigen (somatic) represented by a lipopolysaccharide surface antigen, thermostable, common to all S forms of the Enterobacteriaceae family;
- The H antigen (flagella) is a proteinaceous antigen (flagellin), heat-labile present in bacteria flagella;
- The K antigen (capsular) which may be of a polysaccharide or protein (fimbriae) nature. The O and H antigens are transmitted via plasmids and can be found together with the K antigens that are chromosomally determined.

Up until now, have been identified more than 170 types of the O antigen, more than 50 types of H antigen, 90 types of K antigen and several types of fimbriae antigens (F antigens). Combining all these antigenic variants, results in more than 1,000 antigenic types of *Escherichia coli*.

CHARACTERISTICS OF PATHOGENICITY

Colonic microflora in healthy persons (normal intestinal microbiota) is dominated by strains of *Escherichia coli* that live in a symbiotic relationship with the host. These infections may occur only if the host is immunocompromised or when it crosses the intestinal barrier and reaches areas which would normally be sterile. There have also been described pathogenic strains of *Escherichia coli* which have an exacerbated virulence with the possibility of being able to multiply, triggering infectious processes in immunocompetent individuals. Currently, have been described 6 intestinal *Escherichia coli* pathotypes involved in the EPEC diarrheal syndrome (intestinal pathotypes) and a pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC [8]. It is the most common etiologic agent in urinary infections, being incriminated in over 80% of urinary tract infections (UTI).

The health of the host (diabetes, immunodeficiency, colon malformations, obstructive uropathies, etc) is predisposing factors for infection with *Escherichia coli*.

Intestinal pathotypes are: enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), enteroinvasive *Escherichia coli* (EIEC), verotoxin producing *Escherichia coli* (VTEC), enteroaggregative *Escherichia coli* (EAEC) and diffuse adhesion *Escherichia coli* (DAEC). Within the VTEC pathotype stands out, mainly to highlight the association with severe clinical manifestations (haemorrhagic colitis), the subset of strains of the enterohaemorrhagic *Escherichia coli* (EHEC), whose prototype is the O157: H7 serotype. The pathogenic *Escherichia coli* strains produce virulence factors that contribute and affect the processes undertaken at cell level in the host (the protein synthesis the cytoskeleton's function, the cell division, ion secretion,

the mitochondrial function, apoptosis). Adhesins are adhesion structures located on the fimbriae/pili (P fimbriae from the uropathogenic strains of *Escherichia coli*, the CFA colonization factors from the ETEC strains) or on the surface of the outer bacteria membrane (Afa afimbriae adhesins from DAEC strains). They are important virulence factors that allow bacteria to colonize areas of the human body (the small intestine, the urinary tract, the vagina), in which it does not normally reside. Toxins are another category of virulence factors. Endotoxins are part of the structure of the outer membrane (the A lipid from the LPZ complex) and are present in all Gram-negative bacteria. Released upon the elimination of the bacterial cell wall, they manifest their toxic effects being involved in the occurrence of the fever and the toxic shock from septicemia. Exotoxins are synthesized in the processes of the bacterial metabolism, being specific to the various pathotypes of *Escherichia coli*. Currently, are known the structure and the functions of many toxins responsible for the pathogenicity of strains of *Escherichia coli* (e.g., heat-labile enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by the VTEC strains, enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 of EAEC strains etc.). Another important component of the pathogenicity of the species is the capsule. The main role of the capsule is to inhibit the action of the serum complement and the phagocytosis, thus imparting bacterial resistance. The K1 capsular polysaccharide antigen is present in 80% of *Escherichia coli* strains, isolated from the cases of neonatal meningitis.

THE DISEASE

Most of the *Escherichia coli* outbreaks were associated with the presence of the O15: H7 (VTEC) serotype. The incubation period of the disease varies between 1 and 9 days, averaging 3-5 days. Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleraform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS). The hemolytic uremic syndrome is characterized by acute renal failure, thrombocytopenia and angiopathic hemolytic anemia. The most common form of HUS is

associated with diarrhea and has the highest incidence in children, being the major cause of acute renal failure in children [9, 10]. The factor responsible in HUS manifestations occurred after the VTEC infection and the Shiga dysentery is a protein enterotoxin called the Shiga toxin or VTI. Initially, the hemolytic uremic syndrome mortality rate reached to 50%, but the evolution of medicine and modern treatment techniques have reduced the mortality rate to 3-5%, indicating that approximately 30% of the healed patients remain with long-term sequelae, such as acute kidney failure [8]. *Escherichia coli* is the cause in 80% (ExPEC pathotype) of ITU (urethral cystitis, cystitis, nephritis, pyelonephritis, etc.). Contamination may be downward (blood-borne) or upward, this route being the most common. Infection is favored by the existence of obstructive congenital lesions, post gonococcal infection strictures, urinary stasis via decubitus or pregnancy. Once the bladder mucosa is invaded, subsequently occurs the upward progression to the kidney and the infection stabilizes at this level as a result of slow mobilization of the phagocytes [11].

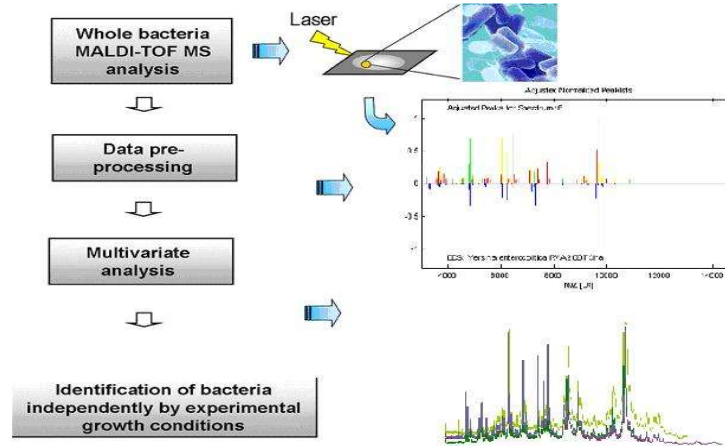
Lab diagnosis

The diagnosis of the *Escherichia coli* infection is made by addressing two strategies namely, identifying and highlighting the etiological agent of the VTI toxin. The usual method used by most diagnostic laboratories is the selective isolation of the etiologic agent (*Escherichia coli* O157H7; O157H), on the MacConkey with sorbitol culture media from stool [12]. The method's principle is that the strains of *Escherichia coli* O157: H7 do not ferment sorbitol in 24 hours and the colonies appear colored on the culture, characteristic that differentiates them from the other strains of *Escherichia coli*. If the isolated strains are suspected as belonging to the serotype O157, but ferment the sorbitol in 24 hours, then will be used the specific antisera for titration and the agglutination tests [13]. The detection of the VTI toxin can be achieved through the cytotoxicity method, cultivation of isolated strains from feces on Vero or HeLa cell lines. The genetic methods (PCR) are based on the detection of specific sequences of polynucleotides that encode the synthesis of VTI. A new and very precise method is MALDI TOF, namely the proteomics analysis through

mass spectrometry of the specific proteins extracted from the ribosomes [14]. The principle of the method consists in measuring the mass of the weights of the polypeptides from the sample to be examined,

generating and comparing its spectrum with the spectrum from the data base of the mass spectrometer (figure 3).

Figure 3: Maldi TOF operating flow (after Bruker Daltonix)



Other lab diagnostic techniques that are being used are the ELISA, for the detection of the O 157 antigen from feces and the serological methods which, however, are of limited value because *Escherichia coli* does not always develop serum antibodies against the VTI toxin. Also, the LPS antigen used may develop cross-reactions with other biological agents, such as *Brucella abortus*.

Treatment and Prevention

The treatment of the infection with *Escherichia coli* O157:H7 is often symptomatic and consists in the treatment of the complications arising due to massive fluid loss, and consequently of the acute diarrhea condition. As a result of dehydration and permeabilization of the blood vessels, pathological changes take place in the kidney function with renal impairment and severe anemia, that may become complicated with hemolytic uremic syndrome (HUS), as apparently is the case in the current outbreak in Romania, since winter-spring 2016. The therapeutic management must take into account the avoidance of the abuse of antibiotics that can foster the development of strains of *Escherichia coli*, avoiding the administration of antispasmodic drugs with effect in slowing bowel, thus maintaining more toxins in the digestive tract and amplifying its harmful effects. It

recommended avoiding administering products based on salicylates and ibuprofen that can increase digestive bleeding. The symptomatic treatment must include hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis. Prevention of disease is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources; washing utensils that came in contact with meat or raw meat products; avoiding the use of unpasteurized juices, in particular for children, etc. In serious cases, potentially fatal, especially in young children, may be needed the treatment from the Intensive Care with perfusions, resuscitation and even peritoneal dialysis.

CONCLUSIONS

- *Escherichia coli* is from the Enterobacteriaceae species present in the colonic microflora of a healthy human, which has the largest epidemiological impact.
- The transmission mechanism of the infection is

"fecal-oral" as a result of the consumption of contaminated food or water, or by "dirty hands".

- The disease has been reported worldwide, being described numerous episodes of infection with *Escherichia coli* that caused multiple illnesses and sometimes even deaths (Walkerton Ontario/Canada, GHUSEC - Germany).
- *Escherichia coli* has three types of antigens: antigen "O" (somatic) antigen "H" (flagella) and antigen "K" (capsular) that can be highlighted by serologic antigen-antibody reactions. Combining all these antigenic variants theoretically result in 1,000 antigenic types of *Escherichia coli*.
- There are six known pathotypes of *Escherichia coli* involved in the EPEC diarrheal syndrome (intestinal pathotypes) and one pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC.
- Toxins are the most important category of virulence factors in *Escherichia coli* (heat-labile and heat-stable enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by VTEC strains, enteroaggregative heat-stable enterotoxin of EAEC strains etc.).

• Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleraform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS).

- The *Escherichia coli* infection diagnosis is made by identifying the etiologic agent (biochemical tests, agglutination reactions, PCR, mass spectrometry) and by highlighting the VT toxin (cell culture cytotoxicity assay, immuno-chromatographic methods, ELISA).
- The treatment is symptomatic and includes hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis.
- The prevention of infections with *Escherichia coli* is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources.

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