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Escherichia coli: Germ Theory, A Bacterial Killer Mechanism, Virulence, Pathogenicity Islands (PAIs), Pathogenesis, Secretion Systems

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ABSTRACT

Why do bacteria damage their hosts? After bacteria bypass the immune system, bacterial virulence enables a host to replicate and propagate within a host in part by demolishing or escaping host defenses. Bacterial pathogens possess an array of specific killer mechanisms that submit virulence and the capacity to intercept host defence mechanisms. Mechanisms of virulence are often mediated by the subversion of normal aspects of host biology. Also, recently, three novels but wide themes have emerged in the field of bacterial virulence: a bacterial killing mechanism, secretion systems and pathogenicity islands. So, pathogen changes the host function so as to support the pathogen's survival or multiplication. Such subversion is often mediated by the specific interaction of bacterial effector molecules with host-encoded proteins and other molecules. Escherichia coli is a considerable and diverse micro alive. E. coli needs only to acquire a mix of mobile genetic elements to become a pathogen capable of causing diseases. The worldwide burden of these diseases is staggering, with hundreds of millions alive affected annually. E. coli strains have been well a bacteria model, and each uses an arsenal of virulence and toxin to subvert host cellular functions to reenforce its virulence. This review focuses on the drastic and different pathogenic mechanisms that are used by various E. coli strains.

INTRODUCTION

A bacterial killer mechanism, virulence, pathogenicity islands, pathogenesis, secretion systems are the pathogenicity of a microbe, that is, its ability to cause disease. The term pathogenicity is used in absolute terms for its ability to cause disease, while virulence is used to express the degree to which a pathogen is capable of causing disease (A Latin Dictionary, 2009). The factors that determine the ability of bacteria to cause disease are the number of infecting bacteria, way of entry into body, effect of defense mechanisms of host organism and unique characteristics of bacteria called virulence. Host-mediated pathogenesis may also be important, as host organism's defense mechanisms can sometimes damage host tissues while preventing infection. Disease mechanisms: Adhesion. First stage of most bacterial diseases is attachment of bacteria to cell surface. Many bacterial and host molecules associated with the attachment of bacteria to host cells have been identified. Receptor to which most bacteria bind is

essential for the normal functions of the host cell. colonization. Some virulent bacteria secrete special proteins to invade certain parts of the host's body. Infestation (first action to attack and deconstruct the host cell). Some virulent bacteria disrupt the host cell membrane or cause the bacteria to be taken up into host cell by endocytosis. This virulence allows bacteria to enter host cells, so that it is possible for bacteria to enter body through epithelial tissue on body surface. Immune response inhibitors. Bacteria produce virulence that disrupt functioning of host organism's immune system. toxins. Virulence is proteins that poison host cells and cause tissue damage (Levin & Bergstrom, 2000; Freeman et al., 2007).

Escherichia coli is a Gram negative (Gram$^-$) bacillus known to be a part of normal intestinal flora but can also be the cause of intestinal and extraintestinal illness in humans. Its virulence lends to *E. coli*'s ability to evade host defenses and develop resistance to common antibiotics. Actually, 171 somatic (O), 55 flagellar (H) and 80 capsular (K) antigens have been identified, and there are over 160 serological types of *E. coli* (Gambushe et al., 2022).

Bacterial genomes generally consist of stable regions termed core genome, and variable regions that form the so-called flexible gene pool. Flexible part is composed of bacteriophages, plasmids, transposons as well as unstable large regions that have been termed genomic islands. Genomic islands encoding virulence of pathogenic bacteria have been designated pathogenicity islands. Pathogenicity islands were first discovered in uropathogenic *E. coli* and presently more than 30 bacterial species carrying pathogenicity islands have been described (Hacker et al., 2003).

Diseases will be described by the causative *E. coli* subtypes, including enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), which is also known as Shiga toxin-producing *E. coli* (STEC) and will be referred to as *E. coli* EHEC/STEC, enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC) (Manatsathit et al., 2002; Yang et al., 2023).

This review summarizes current knowledge on *E. coli* pathogenesis and their general features, and discusses their putative role in toxicology of *E. coli* in light of virulence of *E. coli*. Also, this review will discuss *E. coli* infections into those causing diseases.

Escherichia coli

Escherichia coli is Gram$^-$ scilicet its cell wall is composed of a thin peptidoglycan layer and an outer membrane. During the staining process, *E. coli* picks up the color of the counterstain safranin and stains pink at Gram-stained smear under microscope (Amanze et al., 2022). Facultative anaerobic, rod-shaped, coliform bacteria that is commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless. However, some serotypes (EPEC, ETEC etc.) can cause severe food poisoning, and are occasionally responsible for contamination (Basavaraju & Gunashree, 2022).

Bacterial conjugation, genetic recombination, operon concepts were first discovered in *E. coli*, many important molecular biology mechanisms (DNA replication, RNA transcription, protein synthesis). At least ten Nobel Prizes are based on research in *E. coli* (Peng et al., 2006). *E. coli* was determined to divide into 2 in 20 minutes (Clark & Maaløe, 1967).

The bacteria are 1-2 μm long and 0.1-0.5 μm in diameter (Yang & Li, 2005). It does not form endospores, it dies by pasteurization and boiling. It reproduces best at body temperature, as it is adapted to grow in the intestines of mammals.

Colonies of *E. coli* on MacConkey agar plate are pink to dark pink, dry and donut-shaped, surrounded by a dark pink area of precipitated bile salts (Onyeberechiya et al., 2021). *E. coli* perform dark pink colony on Flexicult Vet Agar (Cugmas et al., 2021), dark yellow colony on Sorbitol-MacConkey (Schuetz, 2019) and navy blue pigment colony on chromogenic coliform agar (Sawicka et al., 2022). In addition, *E. coli* that is labelled with DsRed-Express2, red fluorescent protein (Smith & Schuster, 2021). *E. coli* O157 on Biosynth™ Agar which contains tellurite at 0.1 μg ml^{-1} (Taylor, 1999). Lactose fermentation produces acids, which lower the pH. This encourages dye absorption by the colonies, which are now colored purple-black. Lactose non-fermenters may increase the pH by deamination of proteins. This ensures that the dye is not absorbed. The colonies will be colorless.

If *E. coli* is grown in Eosin methylene blue (EMB, alos know as ''evine's formulation'') it will give a distinctive metallic green sheen (due to metachromatic properties of the dyes, *E. coli* movement using flagella, and strong acid end-products of fermentation) (Oh & Eom, 2021).

E. coli (STEC/VTEC), which produces Shiga or Vega toxin, is bacteria that can cause serious diseases. *E. coli* STEC is heat sensitive so that be sure to follow basic food hygiene rules when preparing meals. Adhering to WHO "Five keys to safer food" is virtually measure to prevent infections with foodborne pathogens such as *E. coli* STEC (WHO, 2018). *E. coli* is bacteria widespread found in the guts of humans and warm-blooded animals. It is transmitted to humans primarily through the consumption of contaminated foods such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts. *E. coli* STEC grow for optimum temperature is 37°C and so can grow at temperatures between 7°C and 50°C. *E. coli* STEC is destroyed by thoroughly cooking all parts of food until it reaches temperature of 70°C or higher. *E. coli* O157:H7 is virtually health *E. coli* STEC serotype; but other serotypes have often been implicated in sporadic cases and outbreaks (WHO, 2018).

Transmission: most available information on *E. coli* STEC relates to serotype O157:H7, since it is easily differentiated biochemically from other *E. coli* strains. *E. coli* O157:H7 is transmitted to humans primarily through consumption of contaminated foods. Fecal contamination of water and foods, as well as cross-contamination during food preparation, will also lead to infection. Examples of foods implicated in outbreaks of *E. coli* O157:H7 include undercooked hamburgers (WHO, 2018).

E. coli Structure

Flagella is peritrich feature. It is controlled by 20 genes. *hag* gene determines primary sequence of flagellin, *fla* gene encodes other flagella consist of 10 protein, and *mot* gene is responsible for flagella function (Samiei et al., 2023). Cell-wall of *E. coli* consist of lipopolysaccharide (endotoxin) structures. This endotoxin to induce pyrogenic response and stimulate intravascular coagulation is important in symptomatology of *E. coli*-induced septicemic diseases. It also plays role in resistance to phagocytosis. Capsule of *E. coli* produce polysaccharides. Polysaccharide is produced both virulence and *in vivo*. It increases invasiveness, increases resistance to serum neutralization and polymorph phagocytosis (Rathore et al., 2022). Pilus are heat-sensitive, surface-associated proteins that are antigenically unrelated to "O" and "H" antigens which are thin protein filaments. It has adhesive properties. Type 1 battery forms most of pathogenic and apathogenic *E. coli.*

E. coli ETEC has specialized pilus that is antigenically unrelated to common pilus (type 1 pili), which acts as ligand for bacterial cell binding to specific complex carbohydrate receptors on small intestinal epithelial cell surfaces, and this interaction results in colonization of intestine. These pili are called Colonization Factor Antigens (CFA). While most ETEC isolates produce both CFA/I, CFA/II and CFA/IV, CFA/III and a number of other unidentified CFAs occur on other specific serotypes. The CFA-type pili plays an important role in host specificity.

The genes encoding CFA production are found on ETEC virulence plasmids and usually on the same plasmids carrying one or both types of *E. coli* enterotoxin (LT and ST) genes. Most *E. coli* ETEC diarrheas are caused by CFA and *E. coli* with both enterotoxins, less by *E. coli* with CFA and only one toxin (usually LT); and rarely by *E. coli* that do not have CFA but only ST. K99, K88, 987P, F41, colonization factor 1 and 2 are some important pili (Fındık, 2023). Virulence structures of *E. coli* such as flagella, cell wall, colicins, enterotoxins, capsule, cytotoxins, pili, hemolysin and aerobactin etc. have both apparent and potential effects on virulence in the tissues (Berne et al., 2015).

Germ Theory

The germ theory of disease — infectious disease is primarily caused by transmission of an organism from one host to another — is a gross oversimplification (Stewart, 1968). Germ theory of disease is currently accepted scientific theory for many diseases. It states that pathogens or germs can lead to disease. These

small organisms, too small to be seen without magnification, invade living hosts. Their growth and reproduction within their hosts can cause disease. Germ refers to not just bacteria but to any type of microorganism, such as protists or fungi, or even non-living pathogens that can cause disease, such as viruses, prions, or viroids (Oxford Dictionaries, 2016). Population size required by pathogen to infect host cell is called Median Infection Dose (ID). To measure this, method called ID_{50} is used. That is, what is number of bacteria that allows us to see signs of infection in at least 50% of subjects? The answer to this question determines ID_{50} value (Ramesh et al, 2020). In science, Median Lethal Dose (LD) of pathogen is measured by method called LD_{50}, just like ID_{50}. This is known as dose at which pathogen causes at least 50% of subjects to die. A critical point here is size of host organism. Naturally, larger numbers of pathogens may be needed to infect larger organisms. For this reason, sometimes ID_{50} and LD_{50} values are given based on measure known pathogen per fragment. This can be expressed micrograms/milligrams/nanograms per kilogram (LibreTexts, 2023).

Koch's Postulates and Molecular Koch's Postulates

Robert Koch published four criteria postulates that summarized his method for determining whether particular microorganism was cause of particular disease, in 1884. Each of Koch's postulates represents criteria that must be met before disease can be positively linked with pathogen. In order to determine whether criteria are met, tests are performed on laboratory animals and cultures from healthy and diseased animals are compared (LibreTexts, 2023). Koch postulates four criteria for establishing causal relationship between disease and microbe. Criteria, which were first put forward by Robert Koch and Friedrich Loeffler in 1884, were revised and published in 1890 (Koch, 1893).

Koch's Postulates: 1. Suspected pathogen must be found in every case of disease and not be found in healthy individuals. 2. Pathogen should be separated (isolated) from diseased organism and grown in pure culture. 3. Pathogen in culture should cause disease when transplanted into a healthy organism. 4. Pathogen must be re-isolated from vaccinated, diseased experimental host organism and found to be the same as original specific causative agent (Koch, 1893).

In 1988, Stanley Falkow (1934–) proposed revised form of Koch's postulates known as molecular Koch's postulates. The premise for molecular Koch's postulates is not in ability to isolate a particular pathogen but rather to identify gene that may cause organism to be pathogenic (LibreTexts, 2023).

Falkow's modifications to Koch's original postulates explain not only infections caused by intracellular pathogens but also existence of pathogenic strains of organisms that are usually nonpathogenic. Predominant *E. coli* is a member of normal microbiota of host intestine and is generally considered innocuous. However, there are pathogenic strains of *E. coli* such as enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (O157:H7) (EHEC). We now know ETEC and EHEC exist because of acquisition of new genes by once-harmless *E. coli*, which, in form of these pathogenic strains, is now capable of producing toxins and causing disease. Pathogenic state resulted from minor genetic changes (LibreTexts, 2023).

Pathogenicity Islands (PAIs)

Pathogenicity islands (PAIs), as distinct type of genetic element, were described for first time for pathogenic *E. coli* strain (O6:K15:H31) (Blum et al., 1994; Hacker et al., 1983), which is the model organisms of extraintestinal pathogenic *E. coli* (ExPEC) used for studies on ExPEC pathogenesis and evolution of bacterial pathogens. The PAIs type of genetic elements is characterized by large size (>10 kb), presence of virulence-associated genes, frequent association with tRNA-encoding genes or other *att* sites for temperate bacteriophages, and G+C content different from that of rest of chromosome. These elements are frequently flanked by repeat structures and carry many fragments of other mobile and accessory genetic elements, such as Insertion Sequence (IS) elements, bacteriophages, and plasmids. Some PAIs are unstable regions and can spontaneously disappear from chromosome. Therefore, PAIs are considered to have evolved from mobile genetic elements by gene transfer. It can also

be assumed that these DNA regions, since their acquisition, underwent and will continue to undergo further evolutionary changes, resulting in an ongoing evolution of bacterial pathogens (Hacker & Kaper, 1999; Hacker et al., 1999; Dobrindt et al., 2002).

Secretion Systems

Bacterial secretion systems are protein complexes present on cell membranes of bacteria for secretion of substances. Specifically, they are cellular devices used by pathogenic bacteria to secrete their virulence to invade host cells (Trivedi et al., 2022). They can be classified into different types based on their specific structure, composition and activity. Generally, proteins can be secreted through two different processes. One process is one-step mechanism in which proteins from cytoplasm of bacteria are transported and delivered directly through cell membrane into host cell. Another involves two-step activity in which proteins are first transported out of inner cell membrane, then deposited in periplasm, and finally through outer cell membrane into host cell (Bocian-Ostrzycka et al., 2017).

E. coli on lettuce leaves congregate at the stomata. As a result of the consumption of these leaves, the number of bacteria constantly increasing, escapes from the immune system and creates a basis for the disease. Their presence may constitute a health hazard and cause severe food poisoning, particularly if the bacteria are toxigenic (Kotzekidou, 2016). They may get onto the vegetables if the water used for irrigation is contaminated with fecal material. Although thorough washing is recommended, Scanning Electron Microscopy (SEM) shows that the bacteria enter the leaves through the stomata and would resist being removed. Similar bacterial contamination was also found with spinach and green onions (Saldaña et al., 2011).

A Bacterial Killer Mechanism of *E. coli*

There is great diversity within the *E. coli* species, and it has even been shown by modern biotechniques that members of *Shigella* and *Salmonella* families are actually subtypes of *E. coli*. Within the *E. coli* strains have different characteristics. There may be small mutations that make them different from each other, or presence or absence of all gene, or even many genes. These genes are found in bacteriophages, transposons, or plasmids, and they are transmitted from other bacterial species to *E. coli* (Peng et al., 2006).

Among genes that differentiate strains are disease virulence. Shiga toxin gene carried by strain O157:H7 was passed on to *E. coli* from *Shigella*. Some of features that cause *E. coli* to cause disease are listed below. Not all of them go together, certain *E. coli* strains have certain combinations of these factors (Peng et al., 2006).

Pilus or fimbriae are hair-like structures on bacteria that allow it to start on certain surfaces. Although harmless *E. coli* also have pili, specialized pili found in *E. coli* ETEC types enable them to attach to small intestinal epithelial cells. In this way, bacteria are not excreted in stool, but settles in small intestine and can multiply there. That's why these types of pili are called colonization factors (Colonization Factor Antigen, CFA). These factors are specific to the host organism and determine in which animals the bacteria can multiply. Other types of pili connect to urinary tract cells or bladder cells, so they cause urinary tract infections. Exotoxins, ST exotoxin, which causes diarrhea caused by ETEC types, prevents epithelial cells from absorbing water, while LT exotoxin causes cells to secrete water and electrolytes. EHEC type bacteria do not have ST and LT exotoxins, they secrete Shiga toxin, this toxin leads to the death of intestinal epithelial cells, so the intestine loses its ability to absorb water, result is a bloody diarrhea. Capsule is additional protective layer outside cell, preventing body's protection mechanisms from recognizing and destroying the bacteria. Hemolysis enables the breakdown of red blood cells; the released iron is a food source for bacteria. Siderophores serve to collect iron in blood. It provides the iron that bacteria need to grow in bleeding diarrhea and systemic infections. The K1 antigen prevents phagocytosis of bacteria. Endotoxin is glycolipid found in cell membrane, body's strong response to it plays virtually role in inflammation (Peng et al., 2006; Pokharel et al., 2023).

Septicemic *E. coli* (SEPEC) strains are responsible for septicemia in hosts. It has been determined that there are alternative virulence factors in every step of

the disease process and that they can use combinations of these virulence especially for the pathogenicity of invasive strains. The first step in the invasion process is attachment to the intestinal surface. Adhesion may be mediated by fimbrial adhesives. Like F5 in ETEC strains, on the other hand, long polar fimbriae or non-fimbrial attachment is an example. Septicemic strains carry Col V plasmid encoding Colicin V. This plasmid encodes type IV pilus, which has been shown to be important for invasion and attachment in *Salmonella typhi*. In addition, this plasmid regulates serum resistance and the aerobactin iron uptake system, both of which play important roles for the survival of *E. coli* strains (Moulin-Schouleur et al., 2007).

DISCUSSION

These disciplines, in conjunction with advances in molecular biologic techniques, have led to striking advances in our understanding of molecular pathogenesis of infection and role of an ever-widening array of potential bacterial virulence. In their comprehensive review in this topic of Critical Care, Webb and Kahler, 2008 speculate that targeting virulence may be an attractive therapeutic strategy. Leaving aside the issues of drug formulation and delivery, what is required for identification of 'drugable' virulence target?

The observation that important virulence is present in very similar forms in different bacteria may be explained by horizontal gene transfer. Different scenarios can be considered to explain the transfer between bacterial strains and species.

Washing hands, paying attention to cleaning rules while preparing food, choosing well-cooked meat, pasteurized milk and dairy products, well-washed vegetables, drinking clean water, taking a shower before and after entering pool, ensuring that children do not swallow water in pool, these bacteria can on food to grow at 70°C conditions. So, it can be destroyed by cooking at this temperature and above, clean toilets and washing hands with soap at entrance and exit are important in protection from pathogens.

A piece portraits of *E. coli* being infected by T4 bacteriophages. These T4 bacteriophages viruses use a machinery to inject their acid nucleic content into the host, where it will be replicated and transcripted. Eventually, this results in the reproduction of phages and lysis of the cell host. Thus, the host cell structure is disrupted and disease occurs (Hampton et al., 2020).

E. coli plays such an influential role in the world of microbiology that it is a model strain and also a model is on display. A model of *E. coli* at ''The Bacterial World'' exhibition a few years ago at the Oxford Natural History Museum (Figure 1). This 28-metre-long inflatable *E. coli* sculpture was created by artist Luke Jerram in collaboration with researchers from the University of Sheffield, who loaned it to the Museum for the Bacterial World exhibition. Although it has a bad reputation for making people ill, there are millions of *E. coli* living harmlessly in your gut right now, keeping more dangerous bacteria at bay. *E. coli* are also vital in medical research. Described as the 'workhorse' of biomedicine, these bacteria are used by scientists as tiny bio-factories, making useful products for research, medicine and industry (Oumnh.ox.ac.uk 2023).

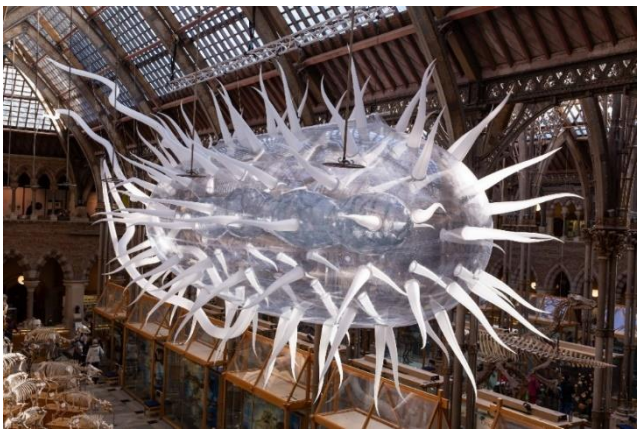

Figure 1. 28-metre-long inflatable *E. coli* sculpture that was created by artist Luke Jerram in collaboration with researchers from the University of Sheffield, who loaned it to the Museum for the Bacterial World exhibition (Oumnh.ox.ac.uk, 2023).

E. coli is one of the three living things that are the top models of science most commonly used in the laboratory (Blount, 2015).

It is essential to know all lethal effect mechanisms of such an effective and strong pathogen and to investigate this pathogen, which is increasing its pathogenicity day by day with genetic instruments, in

terms of reducing its role in infection world. The ability to obtain complex virulence traits in one genetic event, rather than by undergoing natural selection for many generations, provides mechanism for sudden radical changes in bacterial-host interactions. Secretion systems, a bacterial killing mechanism and PIs must have played critical roles in evolution of known pathogens and are likely to lead to emergence of novel infectious diseases in future.

CONCLUSION

E. coli innocuous bacteria can obtain a mixture of wide mobile genetic elements, virulence, toxin, secretion, pathogenic island becoming an emerging living creature pathogen capable of causing a broad spectrum of intestinal and extraintestinal diseases. E. coli pathotypes have been well characterized, causing important diseases. *E. coli* operate many effectors that overthrow cells. Synthesis, specific gene transcription, secretion of diverse micro/macro-molecules and ions, cytoskeleton rearrangement, apoptosis, autophagy, mitochondrial activities, cell division, and signal transduction in epithelial intestinal and extraintestinal host cells are affected by *E. coli*.

Compliance with Ethical Standards

Authors' Contributions

NF: Manuscript design, Writing.

FF: Draft checking, Reading, Editing.

Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

Data availability is not applicable to this article as no new data were created or analyzed in this study.

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