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THE VERSATILE STRATEGIES OF *Escherichia coli* PATHOTYPES: A MINI REVIEW

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ABSTRACT: The widespread species *Escherichia coli* includes a broad variety of different types, ranging from highly pathogenic strains to avirulent isolates. Few microorganisms are as versatile as *E. coli*. Pathogenic strains remain a leading cause of severe and persistent infant diarrhea in developing countries. They may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infection. The human gastrointestinal tract is susceptible to diarrheagenic *E. coli* infections. *Escherichia coli* have effectively managed to subvert the host cytoskeleton for their own purposes causing substantial diarrheal disease, a major public health problem worldwide. This review deals with the different strategies regarding *E. coli* as a pathogen and the virulence traits of its pathotypes highlighting the species as a commensal, opportunistic and specialized pathogen.

KEY WORDS: pathogenicity, virulence, *Escherichia coli* pathotypes, versatile strategies, diarrheagenic *E. coli* categories, public health.

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INTRODUCTION

A bacterial pathogen is a highly adapted microorganism, which has the capability to cause disease. The strategies used by these bacteria to cause infection and disease generally include an interactive group of virulence determinants, sometimes coregulated, which are suited for the interaction of a particular bacterium with a specific host. Bacterial pathogens have evolved numerous strategies to exploit their host's cellular processes so that they can survive and persist. The widespread species *Escherichia coli* includes a broad variety of different types, ranging from: i) highly specialized pathogenic strains causing worldwide outbreaks of severe diseases (10) to ii) opportunistic pathogens which have the potential to cause disease if the human host defenses are compromised (16) and to iii) avirulent isolates which are part of the normal intestinal microbiota, or which are well-characterized and safe laboratory strains (10). The species includes intestinal diarrheagenic *E. coli* and extraintestinal pathogens (18, 23, 24, 27).

The gastrointestinal tract of most warm-blooded animals is colonized by *E. coli* within a couple of hours or a few days after birth. The bacterium is ingested with foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. *Escherichia coli* can adhere to the mucus overlying the large intestine. An important member of the normal intestinal microbiota of humans and other mammals, *E. coli* has also been widely exploited as a cloning host in recombinant DNA technology (10). But *E. coli* is more than a harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen (10). Several different *E. coli* strains cause diverse intestinal (26) and extraintestinal (9, 18, 27) diseases by means of virulence factors that affect a wide range of cellular processes. Once established, an *E. coli* strain may persist for months or years. Resident strains shift over a long period, and more rapidly after enteric infection or antimicrobial chemotherapy that perturbs the normal microbiota. The basis for these shifts and the ecology of *E. coli* in the intestine of humans are poorly understood despite the vast amount of information on almost every other aspect of the organism's existence.

Pathogenic *E. coli* strains may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infection (19). The human gastrointestinal tract is susceptible to diarrheagenic *E. coli* infections. Several *E. coli* pathotypes have

been implicated in diarrheal illness, a major public health problem worldwide, with over two million deaths occurring each year (14).

Escherichia coli **as a commensal organism**

The human colon maintains a microbial density approaching 10^{12} organisms per gram of feces, representing a perfectly balanced ecosystem. The commensal microbiota, derived from the Latin *com mensa* meaning sharing a table, consists of more than 400 species and lives in perfect harmony with the human intestine (7).

Escherichia coli is the head of the large bacterial family, Enterobacteriaceae, the enteric bacteria, which are facultative anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. They are among the most important bacteria. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists (7) of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with human diseases.

Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. It can grow in media with glucose as the sole organic constituent. Wild-type *E. coli* has no growth factor requirements, and metabolically it can transform glucose into all of the macromolecular components that make up the cell (7). The bacterium can grow in the presence or absence of O_2. Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is capable of utilizing NO_3, NO_2 or fumarate as final electron acceptors for respiratory electron transport processes (16). In part, this adapts *E. coli* to its intestinal (anaerobic) and its extraintestinal (aerobic or anaerobic) habitats (7, 16).

Escherichia coli can respond to environmental signals such as chemicals, pH, temperature, osmolarity, and other stimulants in a number of very remarkable ways considering it is a single-celled organism. For example, it can sense the presence or absence of chemicals and gases in its environment and swim towards or away from them. Or it can stop swimming and grow fimbriae that will specifically attach it to a cell or surface receptor. In response to change in temperature and osmolarity, it can vary the pore diameter of its outer membrane porins to accommodate larger molecules (nutrients) or to exclude inhibitory substances (7). With its complex mechanisms for regulation of metabolism, the bacterium can survey the chemical

contents of its environment in advance of synthesizing any enzymes necessary to use these compounds. It does not wastefully produce enzymes for degradation of carbon sources unless they are available, and it does not produce enzymes for synthesis of metabolites if they are available as nutrients in the environment (7, 16).

Escherichia coli is a consistent inhabitant of the human intestinal tract, and it is the predominant facultative organism in the human gastrointestinal tract; however, it makes up a very small proportion of the total bacterial content. The anaerobic *Bacteroides* species in the bowel outnumber *E. coli* by at least 20:1. However, the regular presence of *E. coli* in the human intestine and feces has led to tracking of the bacterium in nature as an indicator of fecal pollution and water contamination (7). Thus, it means that, wherever *E. coli* is found, there may be fecal contamination by intestinal parasites of humans. Since this microorganism is a typical component of the fecal microbiota, its detection may indicate the potential occurrence of other microorganisms which could be even more pathogenic to man and both domestic and wild animals (23).

Escherichia coli as an opportunistic organism

Bacterial pathogens can be divided into pathogens that can and those that cannot invade and survive within host eukaryotic cells. Belonging to these categories is uropathogenic *E. coli* (UPEC), which can cause urinary tract infections, but its classification as opportunistic or specialized pathogen remains unclear (17). Uropathogenic *E. coli* has special features that allow it to take advantage of the bladder environment. There, UPEC can grow to substantial numbers in pure culture that are shed frequently into the environment and have a high probability of transmission to other hosts (6). Entry into host cells is required for many bacterial pathogens to effectively disseminate within a host, avoid immune detection and cause disease. In recent years, many extracellular bacteria have been shown to act as opportunistic intracellular pathogens. Uropathogenic *E. coli* is capable of transiently invading, surviving and multiplying within the host cells and tissues of the urinary tract. Host cell invasion and intracellular bacterial replication are proposed to contribute to the capability of these pathogens to effectively colonize, disseminate and persist long-term within the urinary tract. Invasion of host cells by UPEC is independently promoted by distinct virulence factors, including cytotoxic necrotizing factors, Afa/Dr adhesins, and type-1 pili (2).

The human urinary tract is normally a sterile environment, protected from pathogens by the shear flow of urine, secreted and tissue-associated antibacterial factors, and the bactericidal activities of effector immune cells (2). However, even in populations with these natural defenses seemingly intact, and despite the increasing use of antibiotics, bacterial infection of the urinary tract is an exceedingly common problem. The vast majority of urinary tract infections are caused by UPEC, a surprisingly heterogeneous group of pathogens. It is believed that most UPEC are found initially in the gut and are later introduced through the urethra to the bladder and kidneys.

Escherichia coli as a specialized pathogen

Pathogenicity indicates the capability of one microorganism to cause disease in a susceptible host. Virulence is determined by sophisticated mechanisms for gaining access to environment and to the nutrients sequestered within them, for releasing these nutrients in a usable form, and finally for moving to new hosts when these nutrients are expended (23).

Although most *E. coli* are harmless commensals of the human and animal intestine, certain specific, highly-adapted *E. coli* strains are capable of causing a variety of different diseases. They can also be highly versatile, and frequently deadly, pathogens. Several different *E. coli* strains cause diverse intestinal and extraintestinal diseases by means of virulence factors that affect a wide range of cellular processes (10). Infections due to pathogenic *E. coli* may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infection (19). There are at least seven distinct diarrheagenic *E. coli* categories or pathotypes which include:

i) EPEC (Enteropathogenic *E. coli*), an established etiological agent of human infantile diarrhea, is a pathogen that subverts intestinal epithelial cell function to produce distinctive “attaching and effacing” (A/E) lesions. These lesions are characterized by localized destruction (effacement) of brush border microvilli, intimate bacterial attachment to the host-cell membrane and formation of an actin-rich cytoskeletal structure beneath intimately attached bacteria (12). Enteropathogenic *E. coli* is a leading cause of infantile diarrhea in developing countries (26), where it is one of the most common pathogens. In Brazil, for example, EPEC could be isolated from

stools of over 40% infants with acute diarrhea and was associated with a mortality of 7% (5). From the end of the nineties, there have been changes in these numbers. In industrialized countries, the frequency of these organisms has decreased, but they continue to be an important cause of diarrhea.

The pathogenesis of EPEC is in some way unique for enteric bacterial pathogens since it is essentially noninvasive and produces no toxins. The attachment of EPEC to the epithelial cell, described as localized adherence, results in the so-called A/E lesions (3). Enteropathogenic *E. coli* also uses its type-III secretion system to deliver bacterial effector proteins like EspA and EspB into the host cell to alter the cytoskeleton (13). However, the most fascinating aspect of EPEC pathogenesis is that it inserts, through the type-III secretion system, its own receptor into the host cell. Rather than searching for a receptor it provides its own receptor and uses it when needed. Thus, EPEC is capable of inserting the Tir receptor into the host cell membrane where it serves as the receptor for the bacterial protein intimin after it is phosphorylated on tyrosine by the host cell (4). With few exceptions, typical strains produce only the virulence factors encoded by the LEE (locus of enterocyte effacement) region and the EAF (EPEC adherence factor) plasmid (26).

ii) A-EPEC (Atypical Enteropathogenic *E. coli*) is EPEC that have lost the EAF plasmid. This group is not well studied and apparently includes some clones of heterogeneous *E. coli* pathotypes. Some studies (21, 25) have shown that, probably, A-EPEC is another EPEC category associated with diarrhea of clinical importance. Atypical EPEC strains frequently express EAST1 toxin and other potential virulence factors not encoded in the LEE region (26). The atypical EPEC strains may be less virulent than the typical ones. One reason may be the lack of the EAF plasmid; Levine *et al*. (15) showed that an O127:H6 strain without plasmid was less virulent for adult volunteers than the wildtype strain. However, A-EPEC strains have not been proven to be less pathogenic, and these organisms have other virulence factors that may compensate for the absence of the EAF plasmid. More studies are necessary to resolve this issue.

iii) ETEC (Enterotoxigenic *E. coli*) strains are a major cause of secretory diarrhea in both humans and animals (1). They produce toxins which are heat-labile (LT) and/or heat-stable (STa and STb) and also cause diarrhea.

iv) EHEC (Enterohemorragic *E. coli*) strains are implicated in foodborne diseases principally due to ingestion of uncooked minced meat and raw milk. These strains produce shiga-like toxin 1 (stx1), shiga-like toxin 2 (stx2) and variants thereof. They are involved in episodes of diarrhea with complications. Serotype O157:H7 is the prototype of increasing importance and is associated with hemorrhagic colitis, bloody diarrhea and the hemolytic uremic syndrome (HUS). Enterohemorragic *E. coli* typically causes an afebrile bloody colitis and, in approximately 10% of patients, this infection can be followed by HUS (20). Like EPEC, EHEC elicits an A/E lesion of the intestinal mucosa, a phenotype that requires a functional *eaeA* chromosomal gene.

v) EIEC (Enteroinvasive *E. coli*) causes a broad spectrum of human's diseases. It is biochemically, genetically and pathogenetically closely related to *Shigella* spp. Both characteristically cause an invasive inflammatory colitis, but may also elicit a watery diarrhea syndrome indistinguishable from that caused by other *E. coli* pathogens. The pathogenesis of disease caused by EIEC and *Shigella* involves cellular invasion and spread, and requires specific chromosomal and plasmid-borne virulence genes (19).

vi) DAEC (Diffusely Adherent *E. coli*) strains are defined by the presence of the diffusely adherent pattern in the HEp-2 adherence assay, and cause a watery diarrhea syndrome in adults and children. The pathogenesis of DAEC diarrhea has not been elucidated yet, but several virulence-related characteristics have been identified (19, 22). Most DAEC strains express a surface fimbria designated F1845 that may be encoded either by the chromosome or a plasmid. It was shown that DAEC could induce characteristic elongated projections from the surface of epithelial cells in culture.

vii) EAEC (Enteroaggregative *E. coli*) strains are defined by their distinctive adherence pattern on HEp-2 cells in culture (19). The essential element of the aggregative phenotype is the stacked brick pattern by lying side-by-side with an appreciable distinction of where one bacterium begins and another

ends. The EAEC are a heterogeneous group of bacteria that display a wide array of virulence factors (25). Enteroaggregative *E. coli* is a pathogen associated with persistent diarrhea in the developing world and has recently been implicated in the developed world as cause of both outbreaks and sporadic diarrhea among AIDS patients.

Besides these categories we can find others that have importance in developing countries, like STEC and ExPEC.

i) STEC (Shiga toxin-producing *Escherichia coli*) infections depend, in large part, on the STX genotype of the infecting strain (11). Control of Stx expression appears to be during transcription. Injury to microvascular endothelial cells is the key event underlying the pathogenesis of HUS (11). A large number of STEC strains have caused major outbreaks and sporadic cases of human illnesses, including mild diarrhea, bloody diarrhea, hemorrhagic colitis, and the life-threatening hemolytic uremic syndrome. These illnesses have been traced to both O157 and non-O157 STEC. In a large number of STEC-associated outbreaks, the infections were attributed to consumption of ground beef or other beef products contaminated with cattle feces (8).

ii) ExPEC (Extraintestinal Pathogenic *Escherichia coli*) strains are the cause of a diverse spectrum of invasive human and animal infections, often leading to septicemia (18). Uropathogenic *E. coli* strains are included in the recently defined pathotype ExPEC together with other *E. coli* isolates involved in various extraintestinal infections. These strains possess specific virulence factors such as adherence systems, toxins, iron uptake systems that distinguish them from the commensal strains (27). The expression of bacterial adherence structures requires a complex organization which involves a different number of proteins depending upon the assembly in fimbrial protruding or afimbrial aggregates. Apparently, there are a few virulence factors, which are conserved in the septicemic strains, implying that they are essential for the infection. For the other virulence-related genes a high level of diversity is observed, demonstrating that all stages of the infection can be mediated by a number of alternative virulence factors. The variable profile of virulence genes in septicemic *E. coli* strains as well as a

prevalence of mobility-related sequences point out the existence of a "mix and match" combinatorial system (18). The toxins synthesized by ExPEC strains, mainly represented by hemolysin and cytotoxic necrotizing factor, contribute to destruction of eukaryotic cells. Such siderophores as aerobactin confer the strains the advantage of capturing iron from the environment in which they must survive and replicate. Co-expression of virulence factors contributes to the host's defense system defeat and onset of infection (27). Although the reasons for their evolution remain mysterious, by virtue of their numerous virulence traits ExPEC clearly possesses a unique capability to cause disease outside the host intestinal tract (9).

FUTURE PROSPECTS

Knowledge of the pathogenic mechanisms of *E. coli* pathotypes has led to the development of rational interventions for the treatment and prevention of *E. coli*-induced diseases. Continuous research and investigations into *E. coli* virulence are providing us with useful insights into the origins and evolution of this versatile bacterial pathogen.

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