Alfredo G. Torres *Editor*

Escherichia coli in the Americas

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Foreword

In 1885, a German pediatrician, Theodor Escherich, first described the bacterium *Bacterium coli commune* as a normal intestinal inhabitant of healthy children. Although his research on this organism, subsequently named *Escherichia coli* in his honor, is largely remembered for the description of this species as a nonpathogenic, commensal intestinal inhabitant, he also reported in 1894 that *B. coli* was present in the urine of young girls suffering from urinary tract infections and suggested that it reached the bladder by the ascending route. This was the first description of *B.* (*E.*) *coli* as a potential cause of disease and was followed by Escherich's 1899 report that *B. coli* was the cause of dysentery. The latter report was preceded by Kiyoshi Shiga's 1898 report that the cause of dysentery was a bacterium that he called *Bacillus dysenteriae*, which was subsequently named *Shigella dysenteriae* in his honor. Conradi described a neurotoxin from lysates of this organism in 1903 which was later called Shiga toxin.

For several decades thereafter, no major advances were made in the study of pathogenic *E. coli* until the 1944 proposal by Kauffman of a scheme for serological classification of *E. coli* based on the O (somatic) antigen (a component of the lipopolysaccharide), the H (flagellar protein) antigen, and the K (acid polysaccharide capsular) antigen. The importance of this typing scheme cannot be exaggerated because it allowed the various strains of *E. coli* to be differentiated from one another. The development of this scheme allowed a British pediatrician, John Bray, to report in 1945 that antigenically homogeneous strains of *Bacterium coli neopolitanum* were associated with summer diarrhea in infants.

Although the serotyping scheme of Kauffman facilitated the differentiation of *E. coli* strains associated with disease from those strains not associated with disease, the virulence mechanisms remained unknown for many years. In 1956, De and colleagues in India demonstrated that *E. coli* isolated from adults and children with cholera-like illness caused fluid accumulation in ligated rabbit ileal loops. In England, Smith and Hall showed in 1967 that *E. coli* strains isolated from the stool of young animals with severe diarrhea could produce a heat labile (LT) and a heat stable (ST) enterotoxin and demonstrated that these traits were genetically controlled by plasmids. The development of tissue culture assays for LT and suckling mouse assays for ST eventually allowed the identification of these strains, which

were ultimately called enterotoxigenic *E. coli* (ETEC). Epidemiological studies in the 1960s and early 1970s in Brazil by Trabulsi and colleagues and in Bengal by Gorbach and Sack solidified the link between such strains and human diarrhea. Definitive proof that such strains were human pathogens was derived from volunteer challenge studies in the 1970s. ETEC is now known to be among the most common causes of infant diarrhea in developing countries and is the dominant cause of traveler's diarrhea. In the late 1970s, the Falkow laboratory ushered in the era of molecular pathogenesis by cloning the genes encoding LT and ST and developing DNA probes to diagnose strains possessing these genes. These studies reported the first virulence factor genes and diagnostic DNA probes for any microbial pathogen. Factors responsible for adherence to the small intestinal mucosa were discovered and characterized as fimbrial or fibrillar colonization factors (CFs or CFAs). More recent studies have identified additional accessory virulence factors for ETEC, and this continues to be an area of active research.

The serotypes recognized as ETEC differed from the first diarrheagenic *E. coli* serotypes recognized by Bray. Because the LT and ST toxins were plasmid-mediated, controversy arose in the field when some investigators claimed that strains belonging to the "classic" serotypes but lacking LT and ST had simply lost the plasmid-encoded virulence factors. This controversy was definitely resolved by challenge studies conducted by Levine and colleagues in which strains belonging to the classic diarrheagenic *E. coli* strains lacking LT and ST caused diarrhea in adult volunteers. The mechanisms by which these classic diarrheagenic *E. coli* strains, termed enteropathogenic *E. coli* (EPEC), caused diarrhea began to be elucidated by Kaper and colleagues who described a package of plasmid- and chromosomally encoded virulence factors that conspired to induce a so-called attaching-and-effacing lesion of the small intestine. A key set of virulence factors, including a type III secretion system, were shown to be encoded on a pathogenicity island called locus of enterocyte effacement (LEE). The aggressive outbreaks among infants in industrialized countries caused by EPEC have disappeared, but the pathogen continues to be an important cause of infant diarrhea in developing countries, particularly in sub-Saharan Africa. Questions that continue to confront EPEC research include the mechanism of its striking age-related pathogenicity, the contributions of its multiple virulence factors toward secretory diarrhea, and which of its factors may contribute to effective vaccine development.

In 1983, a new class of pathogenic *E. coli* was recognized from two landmarks but at first seemingly unrelated, epidemiological reports. Karmali and colleagues investigated an outbreak of hemolytic uremic syndrome (HUS) in Canada and implicated *E. coli* strains of various serotypes that produced a cytotoxin active on Vero cells. Concurrently, investigators from the CDC reported an outbreak of bloody diarrhea (called hemorrhagic colitis) due to *E. coli* of an unusual serotype, O157:H7, that was linked to consumption of fast-food hamburgers in the USA. O'Brien and colleagues showed that such strains produced a phage-encoded Shiga-like toxin that was the same as the verocytotoxin. Studies by Tzipori and colleagues showed that O157:H7 strains produced intestinal attaching and effacing lesions in piglets that were similar to those produced by EPEC strains. O157:H7 and

similar Shiga toxin-producing strains were termed enterohemorrhagic *E. coli* (EHEC) or more broadly, STEC (Shiga toxin-producing *E. coli*) or VTEC (verocytotoxin-producing *E. coli*). EHEC have been responsible for numerous outbreaks of disease in industrialized countries including an outbreak involving more than 8000 victims in Japan in 1996.

Enteroaggregative *E. coli* (EAEC) was first described in the Kaper lab in the 1980s. The pathotype was first recognized by its distinctive auto-aggregating phenotype in the HEp-2 adherence assay, and this phenotype was associated with diarrheal disease in some early studies in India and Chile. Subsequent work over many years has implicated the organism as a cause of endemic diarrhea, traveler's diarrhea, and possibly persistent diarrhea and growth faltering. Although pathogenesis studies have described a large regulon of genes under the control of AraC/XylS family regulator AggR, the extreme mosaicism of the EAEC pan-genome has impeded efforts to generate a clear understanding of its role as a global pathogen. Future studies will need to yield a better definition of what gene complement comprises a true EAEC enteric pathogen.

Additional classes, or pathotypes, of diarrheagenic *E. coli* have been described. Strains that adhere to HEp-2 cells in a diffuse adherence pattern have been termed diffusely adherent *E. coli* (DAEC) and reported to be associated with diarrheal disease in some epidemiological studies but unassociated with disease in other studies. Adherent Invasive *E. coli* (AIEC) have been associated with Crohn's disease, but no unique virulence factors have yet been described for this pathotype. Host genetics, microflora, and chronic inflammation are hypothesized to be involved in disease associated with AIEC.

Enteroinvasive *E. coli* (EIEC) are taxonomically indistinguishable from *Shigella* at the species level, but owing to the clinical significance of *Shigella*, a nomenclature distinction is still maintained based on a few minor biochemical tests. Four *Shigella* species and EIEC cause varying degrees of dysentery, but in most cases, EIEC causes watery diarrhea that is indistinguishable from that due to other diarrheagenic *E. coli*. However, an important distinction is made with *S. dysenteriae* 1, which produces Shiga toxin, unlike other shigellae and EIEC.

EIEC and shigellae invade the intestinal epithelial cells by virtue of a plasmid-encoded type III secretion system and associated effector proteins, which allow the organism to counteract initial host immune responses, mediate invasions, escape the phagolysosome, rearrange host cytoskeleton, destabilize tight junctions, and spread laterally among epithelial cells via actin-based motility.

In 2011, a large multi-country outbreak of hemorrhagic colitis and hemolytic-uremic syndrome was caused by a strain of the unusual STEC serotype O104:H4. Molecular studies of the strain revealed that it was a typical EAEC strain indigenous to Africa but which had become lysogenized with a Shiga toxin-encoding phage. Retrospective analyses of strain collections revealed that this organism had been previously implicated in human infections, but had not been recognized as a lysogenized EAEC. Although the strain has not emerged as a global problem, it points up the remarkable plasticity of the *E. coli* genome and suggests that the complete story of *E. coli* epidemiology has yet to be written.

While the great majority of pathogenic *E. coli* strains have been associated with intestinal disease, *E. coli* also cause disease outside the intestinal tract, and such extraintestinal *E. coli* have been called ExPEC. ExPEC is the major cause of community-acquired urinary tract infections (UTI) and is the second most common cause of neonatal meningitis. It is also a leading cause of adult bacteremia. In animals, avian pathogenic *E. coli* is an important cause of respiratory infections, pericarditis, and septicemia in poultry. In extraintestinal infections, the distinction between pathogenic and nonpathogenic *E. coli* strains is not as clear as it is with diarrheagenic pathotypes, since in the appropriate circumstances nearly any *E. coli* strain may gain access to the bloodstream or the urinary tract. In addition to elucidating the pathogenesis of urinary tract infections, the study of uropathogenic *E. coli* (UPEC) has produced several paradigms of bacterial pathogenesis. The first demonstration of molecular Koch's postulates was reported with the cloning and mutation of hemolysin produced by UPEC. The concept of pathogenicity islands was first reported for UPEC, and classic studies of chaperone-usher assembly of fimbriae were performed with this pathotype. The determination of the genome sequence of UPEC strain CFT073 revealed the mosaic structure of pathogenic *E. coli* with only 39 % of predicted proteins shared by *E. coli* K-12, O157:H7, and UPEC.

Although the broad categories of pathogenic *E. coli* have provided a useful framework to guide investigations, the sheer diversity of virulence factors and the substantial variation within each pathotype greatly complicates the establishment of "hard and fast" rules about this species. A sampling of pathogenic *E. coli* virulence factor activities includes ADP ribosylation of Gs to activate adenylate cyclase and ion secretion, depurination of 28S rRNA to inhibit protein synthesis, DNase I activity to block mitosis in the G2/M phase, disruption of mitochondrial membrane potential, activation of guanylate cyclase resulting in ion secretion, activation of Cdc42 and Rac thereby modulating actin cytoskeleton structure, and microtubule destabilization. These virulence factors are frequently encoded on mobile genetic elements such as plasmids, phage, transposons, and pathogenicity islands. Further details and primary literature citations on these virulence factors and the history of the discovery and recognition of the various *E. coli* pathotypes can be found in several comprehensive reviews (see different chapters in the book).

The breadth of activities of virulence factors and the substantial genetic variation demonstrated by genome sequence studies greatly complicates the task of determining which strains of *E. coli* may be pathogens and which are non-pathogens. The ongoing evolution of pathogenic *E. coli*, as demonstrated by the 2011 O104:H4 outbreak in Europe, makes it very difficult to have a static definition of pathotypes. Future research efforts should more fully characterize the role of coinfections and host factors to gain a more comprehensive picture of disease due to pathogenic *E. coli*.

In this book, *Escherichia coli in the Americas*, members of the Latin American Coalition for *E. coli* Research (LACER) provide a comprehensive review of the different categories of *E. coli* including aspects such as virulence mechanisms, environmental niche, host reservoir, disease outcomes, diagnosis, treatment, and vaccine development. Over the past 50 years, several landmark studies in Latin America have

yielded important insights into pathogenic *E. coli* such as the classic epidemiological studies in Brazil by Trabulsi and colleagues and the studies in Chile that identified EAEC as an important diarrheal pathogen. The lessons learned in Latin America have widespread significance for the study of *E. coli* throughout the world, and the information contained in this volume will be of value for a wide audience, from students to experts, from molecular biologist to epidemiologist.

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Key Review References

Friedmann HC (2014) Escherich and Escherichia. In: EcoSal Plus. 6(1). doi:10.1128/ecosalplus.ESP-0025-2013

Nataro JP, Kaper JB (1998) Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11:142–201

Kaper JB, Nataro JP, Mobley HLT (2004) Pathogenic *Escherichia coli*. Nat Rev Microbiol 2:123–140

Kaper JB, O'Brien AD (2014) Overview and historical perspectives. In: Sperandio V, Hovde CJ (eds) Enterohemorrhagic and other shiga toxin-producing *Escherichia coli*. American Society for Microbiology Press, Washington, DC. Microbiol Spectr. 2(6). doi: 10.1128/microbiolspec.EHEC-0028-2014

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013) Recent advances in understanding enteric pathogenic *Escherichia coli.* Clin Microbiol Rev 26:822–880

Preface

In an interconnected and interdependent world, an outbreak caused by an infectious agent in a country is of significant concern because it could result in a sanitary emergency affecting other countries. Globalization has greatly impacted the American continent at different levels, including opening markets and aiding nations to sell their products outside of the country, increasing the real-time communication abilities and allowing an emphasis on international cooperation, as well as many other financial, cultural, and touristic benefits. However, this increased connectivity also potentiates the risk of dissemination for emerging or reemerging infectious diseases. In the case of *Escherichia coli*, a bacterium that is considered a benign as well as a pathogenic organism, globalization has created a scenario in which a pathogenic *E. coli* causes regional outbreaks that can quickly disseminate to other countries. If such bacterial strains are resistant to one or more antibiotics, this becomes a global health threat and alerts have to be in place to notify the authorities and the health providers about such incidents. Globalization in the food chain supply and the distribution of food products to different markets and populations increases the possibility of a rapid spread of an infection caused by *E. coli* and other pathogenic organisms. So there is need for rapid response and effort of the scientific community to identify, diagnose, and understand the pathogenic *E. coli* responsible for the disease.

As such, the Latin American Coalition for *E. coli* Research (LACER) was created in 2009, to promote and expand research efforts in the American continent, to support and expand the best science, to prepare the next generation of scientist-physicians and research investigators, and to work together with the community to translate scientific findings into products improving the well-being of the population. In 2016, the LACER group consist of a multidisciplinary network of more than 60 international research groups working on different aspects of pathogenic *E. coli*, including but not restricted to epidemiology, pathogenesis, vaccine and therapeutic design and testing, public health, surveillance, and clinical identification and treatment.

One major goal of the educational mission of LACER is to advance our understanding about this pathogen and disseminate such knowledge to the region and to the world. Since its inception, some of the educational activities of LACER have included workshops, symposiums, and minicourses for students, scientists, professors, and the

public in general, in different Latin American countries and the USA. In 2010, the members of LACER decided to produce a book entitled *Pathogenic Escherichia coli in Latin America*, which allowed leading investigators in the Latin American region to discuss the mechanisms of *E. coli* pathogenesis as well as the methods of diagnosis, clinical management, host immune responses, animal reservoirs, and epidemiology. In addition, the book discussed epidemiological and public health issues regarding pathogenic *E. coli* in representative Latin American countries.

As the LACER science grew strong and contributions started getting recognized in different forums and social media platforms, the membership, which has expanded significantly, decided to produce a new book in which broader aspects of the pathogens' lifestyles and the diseases they produce were discussed. The current book entitled *Escherichia coli in the Americas* is a compilation of chapters by a large number of *E. coli* experts in Latin America, the USA, and Canada. The book is divided into three major areas: The first includes chapters describing individual pathogenic *E. coli* strains and their different virulence mechanisms used to cause disease. The second includes common mechanisms used by this bacterium to interact with animal or plant hosts (human, animals, and food products) and to resist killing by antibiotics, etc. The third includes chapters devoted to the diagnostics, therapeutic interventions, and vaccine design.

Through the years, LACER members have created a special bond, and this group has become more than just some people working together. It has resulted in unique combination of talent, expertise, and collaborative attitudes that makes the group stronger together than apart. Everyone involved in collaborative research at LACER has a role to play in building our understanding about the always evolving *E. coli*, and advancing technologies and methodologies to diagnose, treat, and prevent such infections have a shared goal of protecting the public health.

Galveston, TX, USA Alfredo G. Torres, MS, PhD

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Chapter 1
Enterotoxigenic *Escherichia coli*

Roberto M. Vidal, Nayaret L. Chamorro, and Jorge A. Girón

Summary Pediatric diarrheal diseases continue to represent a significant health burden in areas of the world with poor sanitation. Hundreds of thousands of deaths, mostly in young children occur each year due to severe acute gastrointestinal disease caused by diverse enteric pathogens. Enterotoxigenic *Escherichia coli* (ETEC) is a leading cause of childhood gut illness and death in endemic areas. While the epidemiology of ETEC infections is known for some regions of North, Central, and South American countries, the actual impact in most of the continent is unknown. Despite much research efforts of many investigators, safe and effective vaccines against diarrheal disease caused by ETEC are not yet available. The major challenges in developing such vaccines are the poor immunogenicity of the heat-stable enterotoxin produced by the majority of strains and the array of antigenically diverse colonization factors (CFs) that mediate gut colonization; however, while inducing protective antibodies, they only protect against homologous strains. The use of new multi-epitope fusion antigens consisting of chimeric CFs-toxin fusions lacking toxicity has shown important and promising immunogenicity and protection. We review here the current knowledge on ETEC, its epidemiology in the Americas, and the most important vaccine strategies available.

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1 Diarrheagenic *Escherichia coli*

In the 1940s, it was established that some *E. coli* strains caused intestinal infections and so were designated as enteropathogenic *E. coli* to distinguish them from fecal commensal strains. *E. coli* is a Gram negative, facultative anaerobic bacterium that lives in the human gastrointestinal tract as a member of the gut microbiota (Nataro and Kaper 1998; Dubreuil 2012). *E. coli* is a beneficial organism that protects the epithelium of other harmful bacteria by producing an acidic niche through the metabolism of nutrients, provides the host a source of vitamins B and K, and constantly activates the immune system. However, some strains have acquired mobile genetic elements (e.g., plasmids, pathogenicity islands, transposons, bacteriophages) that code for a myriad of virulence factors that allow bacteria to cause a variety of diseases in healthy individuals, including watery diarrhea, dysentery, sepsis and meningitis, the hemolytic uremic syndrome, and urinary tract infections (Kaper et al. 2004). As new information of novel virulence determinants and serotypes identified in epidemic strains became available, it was then possible to start separating diarrheagenic *E. coli* (DEC) strains into different classes. Currently, based on the presence of defined virulence factors, their epidemiology, and clinical manifestations of the disease DEC strains are classified into six pathogroups: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) or Shiga-toxigenic *E. coli* (STEC), diffuse-adhering *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC) (Nataro and Kaper 1998; Qadri et al. 2005; Dubreuil 2012). The plasticity of the *E. coli* genomes and the ability of these organisms to mobilize and acquire foreign genetic elements allows the emergence of new epidemic strains, sometimes hybrid strains, with hyper-virulent attributes. This was exemplified by the epidemics of hemolytic uremic syndrome occurred in Northern European countries in the summer of 2011 by an EAEC O104:H4 carrying the Shiga toxin genes (Rasko et al. 2011).

2 Global Significance of ETEC Infections

It is estimated that about 280 million cases of diarrhea occur annually in the world, producing approximately 400,000 deaths, the majority of them in young children (Steffen et al. 2005). For many decades, ETEC has led, along with *Shigella*, *Salmonella*, and Rotavirus, the list of the most frequent diarrheal microorganisms in endemic areas worldwide. In the realm of infectious diarrheal diseases, ETEC is responsible for the highest attack rates of morbidity and lethality mainly in children under 5 years of age, living under poor sanitation conditions in the developing world. Recent data provided by the Global Enteric Multi-Center Study (GEMS) showed that ETEC is one of the four main etiologic agents of moderate-to-severe diarrhea in regions of sub-Saharan Africa and South Asia (Kotloff et al. 2013). ETEC is also responsible for diarrhea in travelers ("the Moctezuma's revenge") and military personnel deployed to endemic areas (Diemert 2006; Rodas et al. 2011a).

Since the early 1970s, ETEC infections have been monitored in many countries in the Americas, Southeast Asia, and the Indian subcontinent, particularly in those countries visited by travelers and military personnel who are easy targets of ETEC. The interest for studying the epidemiology of ETEC infections appeared to have dimmed or perhaps shifted to other pathogens in the 1990s, particularly due to the emergence of other DECs such as EPEC, STEC, and more recently EAEC. The advent of more sophisticated molecular techniques accelerated the study of the mechanisms of virulence of these *E. coli* pathogroups, gathering highlights and shifted the interests of many researchers to these pathogens. Nevertheless, new and old die-hard *E. coli*/ETEC researchers continued to study the prevalence of ETEC infections among diarrheal cases in several countries of the American continent and other regions of the world. While in some geographic areas ETEC has been displaced by other DEC, such as EPEC or EAEC, it is clear that children continue to die due to watery diarrhea-causing ETEC. Much has been learned from ETEC research in the last 45 years or so however, children continue to die from ETEC infections due to the lack of safe and effective vaccines against the diarrheal disease caused by this important pathogen.

ETEC is a health burden associated mainly with poverty, and the lack of sanitation, potable water, and sewage treatment in developing countries. The consumption of contaminated food and water, and possibly person-to-person contact account for the transmission and elevated number of diarrheal cases. The ability of the organism to survive at room temperature for extended periods of time in cooked food, raw vegetables, and in drinking and nondrinking water is an important factor in the prevalence of this organism in the communities with poor sanitation.

The bacteria are transmitted by ingestion of contaminated food and water (Curtis et al. 2000) with an infective dose is 10^6–10^{10} colony-forming units (CFU) (Nataro and Kaper 1998). Within a period of 14–50 h after ingestion, these bacteria colonize the epithelial mucosa of the small intestine, producing secretory diarrhea without obvious signs of destroying or invading the epithelium or causing inflammation. In addition to diarrhea, some patients may manifest other symptoms such as headache, fever, nausea, and vomiting. The symptoms usually disappear within the first 5 days without the need for antibiotic treatment. The lethal cases are almost exclusively associated with children, due to severe dehydration and lack of protective immunity.

3 Pathogenicity Mechanisms

3.1 Toxins and Adhesins

The main feature of the pathogenesis of diarrheal disease caused by ETEC is the successful colonization of the surface of the intestinal mucosa and the hypersecretion of water and electrolytes due to enterotoxic activity. ETEC overcomes the nonspecific immunological barriers present in the digestive tract and once on the