Extraintestinal Pathogenic *Escherichia coli*

Dvora Biran and Eliora Z. Ron

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Abstract Extraintestinal pathogenic *E. coli* (ExPEC) present a major clinical problem that has emerged in the past years. Most of the infections are hospital or community-acquired and involve patients with a compromised immune system. The infective agents belong to a large number of strains of different serotypes that do not cross react. The seriousness of the infection is due to the fact that most of the infecting bacteria are highly antibiotic resistant. Here, we discuss the bacterial factors responsible for pathogenesis and potential means to combat the infections.

D. Biran · E. Z. Ron (✉)
Department of Molecular Microbiology and Biotechnology, Faculty of Life Sciences, Tel Aviv University, 39978 Tel Aviv, Israel
e-mail: eliora@post.tau.ac.il

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1 Introduction

Although most strains of *Escherichia coli* are commensals and abundant, many strains are virulent. In addition to the well-established role of *E. coli* as the causative agent of intestinal infections, many virulent strains cause extraintestinal infections.

The importance of ExPEC is increasing rapidly because they are abundant and are highly resistant to antibiotics. Many of the ExPEC infections are associated with immunodeficiency due to very young age (neonatal), old age, chemotherapy, or diseases that weaken the immune system, such as HIV. Thus, as a human pathogen, ExPEC are the leading causative agents in hospital- and community-acquired infections (healthcare-associated infections). According to the WHO (Healthcare-associated infections FACT SHEET), "hundreds of millions of patients are affected by health care-associated infections worldwide each year, leading to significant mortality and financial losses for health systems. Of every 100 hospitalized patients at any given time, 7 in developed and 10 in developing countries will acquire at least one health care-associated infection." The estimated cost of treating healthcare-associated infections is about 20 billion US$ a year.

ExPEC bacteria are involved in infections of humans and farm animals. They are often classified as APEC (avian pathogenic *E. coli*), UPEC (*E. coli* causing urinary tract infections = UTI), NMEC (neonatal meningitis-causing *E. coli*), or septicemic. However, although this classification is sometimes convenient, it is actually meaningless because there is much overlap between the groups (Ron 2006). Several examples include APEC strains, such as *E. coli* serotype O2 which are a frequent cause of UTI; *E. coli* serotype O18 that is involved in avian colisepticemia and human newborn meningitis (Ewers et al. 2007; Krishnan et al. 2015; Nicholson et al. 2016; Tivendale et al. 2010) and UPEC strains often become septicemic. The similarities between the human and animal strains can also be characterized at the genomic level (Bauchart et al. 2010; Maluta et al. 2014; Zhu Ge et al. 2014) and multilocus sequence typing (MLST) of *E. coli* O78 strains indicate that several isolates from newborn meningitis cluster with avian septicemic isolates (Adiri et al. 2003). The similarity between ExPEC strains involved in animal infections and human infections raises the possibility of zoonosis. This possibility is difficult to prove, but it should certainly be considered especially for the transfer of antimicrobial-resistant ExPEC through contaminated food (Manges 2016).

In a few cases where host specificity was documented, it appears to involve specificity of adherence. Such specificity can be shown in clinical isolates of *E. coli* serogroup O78—human intestinal strains produce the human-specific adherence fimbria CFA/I that bind specifically to intestinal epithelia (Buhler et al. 1991; Cheney and Boedeker 1983), isolates from septicaemia of lambs produce the P, S, and F1C adhesins (Dozois et al. 1997) or the K99 fimbriae (E. Z. Ron. Unpublished), and some O78 isolates from avian colispeticemia code for avian-specific fimbriae (AC/I pili, belonging to the group of S-fimbriae) (Babai et al. 1997, 2000; Dobrindt et al. 2001; Yerushalmi et al. 1990).

Here, we will discuss ExPEC strains and the genetic and physiological factors that promote the virulence.

2 Infections Involving ExPEC

2.1 Avian Colisepticemia

This is an important disease in poultry leading to losses of millions each year to the poultry industry. This disease is characteristic for birds under stress—high temperature, high humidity, or mild viral infections, even due to vaccinations. The disease starts from the upper respiratory tract and the bacteria enter the bloodstream, are dispersed in the body and infect vital organs. This infection involves high morbidity and mortality. The majority of infections (about 80%) are caused by *E. coli* serotypes O1, O2, and O78 but many additional serotypes were shown to be involved (Cordoni et al. 2016; Dho-Moulin and Fairbrother 1999; Dziva et al. 2013; Huja et al. 2015; Mangiamele et al. 2013; Mellata et al. 2009; Nicholson et al. 2016; Rodriguez-Siek et al. 2005; Sola-Gines et al. 2015).

2.2 Veterinary Infections

ExPEC are the cause of several diseases of calves and lambs. The bacteria infect the newborns and cause a lethal septicemia (Ansari et al. 1978; Duff and Hunt, 1989; Kjelstrup et al. 2013). Apparently, these diseases are not of major veterinary impact.

2.3 Neonatal Meningitis

NMEC (Neonatal meningitis-causing *E. coli*) are the major Gram-negative pathogens associated with meningitis in newborn infants (Czirok et al. 1977; Milch et al. 1977; Wijetunge et al. 2015a, b). This group includes several serotypes such as O1, O18 (Wijetunge et al. 2015a, b), and O78 (Czirok et al. 1977; Milch et al. 1977). Although quite rare (1 per 1000 births in developing countries and 1 per 10,000 in developed countries) it is severe, as it involves a very high mortality rate.

2.4 Urinary Tract Infections (UTI)

UTI is the most common ExPEC infection (Ejrnaes 2011; Ena et al. 2006; Foxman 2010, 2014; Jacobsen et al. 2008; Marrs et al. 2005; Zhang and Foxman 2003). In

2007, there were in the US about 10 million ambulatory visits and about 2 million admissions to hospital emergency departments (Foxman 2010, 2014). It is very common in young women, where the infection can become recurrent, and in older patients following catheterization. UTIs can get complicated and cause kidney failure and quite often, especially in the elderly, lead to bloodstream infections such as sepsis.

2.5 Blood Stream Infections/Septicemia/Sepsis

This ExPEC infection is the most serious one in terms of severity as well as an economic burden. Every year there are more than a million cases of sepsis in the US and the estimate is that about 30% of them die. This number is higher than deaths in the US due to prostate cancer, breast cancer, and HIV combined (Sepsis Fact Sheet, CDC, 2016). In 2011, the US spent $20.3 on hospital care for sepsis patients—about 55 million US$ a day and the cost per patient can be as high as 56,000 US$. Sepsis is clearly an emerging disease as the number of cases per year increases rapidly. There are several reasons for this escalation such as the increased longevity of people, the broader use of invasive procedures, immunosuppressors, and chemotherapy. But probably the most important reason for the current situation is the fast spread of antibiotic-resistant *E. coli*, the major cause of sepsis.

3 Virulence Factors

A general feature of ExPEC is that production of exotoxins is not a major factor in their virulence, in contrast to many intestinal strains. There is evidence for production of cytotoxin by ExPEC, but it is not clear if they are important for pathogenicity (De Rycke and Oswald 2001; Peres et al. 1997; Taieb et al. 2016). The virulence of ExPEC strains appears to depend on their ability to survive in host tissues, especially in serum. Many of the genes involved in virulence are present on large plasmids, most frequently on a ColV plasmid (Huja et al. 2015; Milch et al. 1984; Waters and Crosa, 1991; Wijetunge et al. 2014). The ColV plasmids are a family of related plasmids that encode a broad spectrum of iron uptake systems and genes for increased serum survival.

In general, there is an extensive variability in virulence-associated genes of ExPEC (Mokady et al. 2005a, b; Ron 2006, 2010). There appears to be a large "pool" of such genes and much overlap between them. For example—there are several genetic systems for iron acquisition and an ExPEC strain can carry one or more of them, the same for genes coding for fimbriae or adherence factors, etc. It is clear that many of the virulence factors were obtained by lateral gene transfer, such as the gene coding for Yersiniabactin, the Yersinia iron uptake system (Gophna et al. 2001; Huja et al. 2015). However, all the ExPEC strains carry at least one

adherence system and septicemic strains carry at least one efficient iron-binding system and genes for serum survival (ISS—increased serum survival).

3.1 Adherence

Adherence to host cells is the initial step of an *E. coli* infection and is essential for invasion and infection. Adherence also influences host specificity and even tissue specificity. Thus, intestinal pathogens adhere preferentially to gut epithelium while bacteria involved in UTI adhere to bladder epithelium (Kalita et al. 2014).

Adherence depends mainly by specific organelles—pili, or fimbriae—that recognize specific ligands on the epithel. Infections of mammalian farm animals (cattle, sheep, pigs, etc.) begin by intestinal colonization of newborn and often involve K99 and K88 pili and AC/I pili were found only in APEC and show specificity to chicken tracheal epithelium (Babai et al. 2000; Yerushalmi et al. 1990). The most common fimbriae in strains involved in UTI/sepsis are the P-fimbriae that bind glycolipids containing a-D-Gal-1,4-b-D-Gal (Korhonen et al. 1982; Lane and Mobley 2007; Lund et al. 1988; Stromberg et al. 1990), F1C fimbriae, which bind b-GalNac-1,4-bGal (Khan et al. 2000; van Die et al. 1991) and fimbriae of the S-family. The S-family includes the SfaI, SfaII, Foc, and AC/I fimbriae. The Sfa fimbrial adhesins are produced by strains involved in sepsis and newborn meningitis and interact with glycoproteins containing sialic acid (Babai et al. 2000; Bauchart et al. 2010; Dobrindt et al. 2001; Hacker et al. 1985; Moch et al. 1987; Parkkinen et al. 1986). The group of S-fimbriae is interesting as there is evidence for horizontal gene transfer and combinatorial gene shuffling resulting in pili with different adherence specificities that are related to the clinical symptoms or the host. Thus, the *sfaAII* gens (from a NBM strain) is homologous to the *facA* gene of AC/I pili (APEC) while the *sfaIIS* gene—coding for the adhesion—is homologous to this of the *sfaI* cluster from a human sepsis strain (Babai et al. 2000). The combinatorial shuffling of fimbrial genes is probably of ecological and functional importance as it increases the fimrial diversity to improve adaptation to different hosts and resistance to the immune system of the host. Moreover, many of the ExPEC strains express more than one type of fimbriae and the expression of fimbrial genes appears to be coordinated, also important for diversity and increase the probability of survival under changing environmental conditions (Holden and Gally 2004).

3.2 Type Three Secretion Systems (TTSS)

Type three secretion systems are needle-like structures used to secrete effector proteins into host cells. The TTSS of intestinal pathogenic *E. coli*, especially the LEE system, have been well characterized. ExPEC strains do not have an LEE system but do have a homologous gene cluster—ETT2 = *E. coli* Type Three secretion system 2,

similar to the SPI1 pathogenicity island of Salmonella. It is present in the majority of ExPEC strain from humans and animal farms (Cheng et al. 2012; Hartleib et al. 2003; Ren et al. 2004; Wang et al. 2016b). However, the ETT2 gene clusters carry a large number of mutations and deletions and it is not even clear how many of the strains express the ETT2 genes (Ideses et al. 2005; Ren et al. 2004). So far, there is no evidence that the ETT2 system is a secretion system, as no secreted proteins have been detected (Hu et al. 2017). Yet, in *E. coli* O157:H7 it encodes regulators that affect expression of genes in the LEE gene cluster (Zhang et al. 2004), and in avian *E. coli* O78, the ETT2 system affects motility (Wang et al. 2016a). The ETT2 system of *E. coli* O78-9 is degenerate, as it carries a large deletion and several point mutations. Yet, it is critical for virulence and for serum resistance (Huja et al. 2015; Ideses et al. 2005; Wang et al. 2016a). Recently it was shown that ETT2 has a global effect on the cells surface and is involved in secretion of flagella and fimbriae, in production of outer membrane vesicles and multicellular behaviour (Shulman et al. 2018).

4 Avoiding the Immune Response

ExPEC strains are characterized by high resistance to serum, which contains antibodies and complement. The complement complex mediates direct killing by the formation of pores in the cell membrane. Pathogens evolved outer surface features that inhibit complement-dependent killing, such as lipopolysaccharides and capsules, which are the important factors involved in serum resistance (Phan et al. 2013)

4.1 Lipopolysaccharides—LPS

Complete lipopolysaccharides are essential for serum survival and pathogenicity of ExPEC (Hammond 1992; Kusecek et al. 1984). However, because a very large number of LPS serotypes are involved in septicemia, there does not appear to be an advantage for specific serotypes. An important factor is the length of the O-antigen chain, which also influences the level of serum resistance (Grozdanov et al. 2002)

4.2 Capsules

The capsules produced by *E. coli* strains are divided into four groups according to their composition and biosynthesis (Whitfield and Roberts 1999). Capsules of group 1, 2, and 3 have been extensively studied, they are acidic polysaccharides composed of oligosaccharide repeating units and their role in virulence is well established. (Buckles et al. 2009; Goller and Seed 2010; Hafez et al. 2009; Kim et al. 2003; Sarkar et al. 2014). Capsules belonging to group 4—also called

"O-antigen capsules" have only recently been studied and shown to contribute to enteropathogenic *E. coli* resistance to human alpha-defensin 5 (Thomassin et al. 2013) to shield intimin and the type three secretion system of intestinal pathogenic *E. coli* (Shifrin et al. 2008) and to facilitate spreading of *Shigella sonnei* to peripheral organs (Caboni et al. 2015). Its essential role for virulence was shown in avian ExPEC strain serotype O78 when a transposition that abolished capsule synthesis resulted in reduced virulence (Dziva et al. 2013). Moreover, a precise deletion of the *etp* gene involved in the biosynthesis of the group 4 capsule resulted in serum sensitivity (Biran and Ron 2017). Thus, it is clear that O-antigen/group 4 capsule is also a critical virulence factor for the spread of bacteria in the blood-stream and for septicemia.

4.3 ISS—Increased Serum Survival

Studies in avian pathogenic *E. coli* indicated that a gene present in the ColV plasmid confers serum resistance (Binns et al. 1979). This gene—called *iss* for increased serum survival—encodes a small membrane protein (Binns et al. 1982; Horne et al. 2000; Nolan et al. 2002, 2003). This gene is homologous to the *bor* gene of *E. coli* K-12 that originated from bacteriophage λ (Johnson et al. 2008; Lynne et al. 2007). It is clear that the *iss* gene is a major factor in serum survival (Binns et al. 1982; Huja et al. 2015; Nolan et al. 2002, 2003). Yet, the molecular basis for its role in serum survival is not clear. Moreover, a deletion of the *iss* gene from the ColV plasmid results in serum sensitivity and is not complemented by the chromosomal *iss* (*bor*) gene (Huja et al. 2015). This finding is difficult to explain, as the chromosomal gene codes for the homologous protein as the plasmid gene.

5 Avoiding Metabolic Immunity

As already noted, to survive in serum, bacteria must overcome the innate immunity of the host, mainly the effect of the complement system. However, another obstacle is the nutritional immunity of the serum caused by the fact that nutrients are bound in storage molecules and are unavailable to the bacteria (Weinberg 2009). Most significant is the limitation in iron, which is bound in the blood to human proteins (such as ferritin, hemosiderin) Therefore, most of the ExPEC strains contain genes involved in iron sequestering and it is clear that iron acquisition systems and receptors play a pivotal role in the virulence of septicemic pathogens. Indeed, systems-wide analyses of the response of septicemic bacteria to serum show an induction of the genes involved in iron metabolism and controlled by the iron homeostasis regulator Fur (Huja et al. 2014). It appears that the presence of multiple iron acquisition systems is essential, but just as important is their precise regulation upon exposure to serum. Thus, the nonpathogenic *E. coli* K-12 grows poorly even

in serum in which the complement system has been heat inactivated, and its iron metabolism is not induced upon exposure to serum (Otto et al. 2016). Furthermore, these bacteria grow much better in the presence of serum (inactivate) upon introduction of the *fur* gene from septicemic strains (Otto et al. 2016).

Functional genomic analyses indicate that exposure to serum changes the expression of a large number of genes, most of which are induced even in the absence of active complement (Huja et al. 2014). Therefore, it is clear that overcoming the nutritional immunity is an essential step for surviving serum and establishing a bloodstream infection.

6 Concluding remarks and future perspectives

ExPEC—Extraintestinal Pathogenic *E. coli* constitute a clinical problem of increasing importance. Yet, our understanding of the pathogenesis of these bacteria is quite limited. As they do not appear to produce potent secreted toxins, their ability to cause infection depends on their ability to survive and multiply in the host. In order to overcome hostile environments, such as the urinary tract or even blood where they are exposed to innate immunity and nutritional immunity, a whole series of functions and regulatory mechanisms were evolved. The role of most of these functions and regulations in infection is not clear yet, but it is evident that the majority of these is important for overcoming the nutritional immunity and not only the innate immunity.

Why are ExPEC strains so difficult to combat? There are several major reasons, which are as follows:

1. The extraintestinal infections involve a very large number of serotypes that do not cross react. Therefore, simple vaccines comprising several strains are not feasible. In addition, if there is a vaccine—who should be vaccinated? As in most cases, the infection is opportunistic, often following a medical intervention, it is difficult to define the population at risk.
2. ExPEC carry a variety of genes coding for drug resistance, which are often on conjugative plasmids that easily spread in the whole bacterial population. Moreover, ExPEC are present in large number in the intestine, where they encounter bacteria, such as Klebsiella and Acinetobacter from which they can get resistance genes by horizontal gene transfer.
3. The search for new anti-ExPEC targets is a real challenge, as many of the genes involved in pathogenesis have overlapping activities, and inhibiting one of them will probably be insufficient to prevent the infection. For example—in order to overcome the deprivation of iron in serum, ExPEC strains code for several efficient iron binding systems, most of which were obtained by horizontal gene transfer. In order to prevent ExPEC from resisting serum, it should probably be necessary to inhibit all of these iron acquisition systems.

4. Once the bacteria enter the bloodstream the infections progress very quickly, with the bacteria getting to the vital organs and reaching high numbers. As *E. coli* contains the endotoxic cell envelope of lipopolysaccharides, the patients are exposed to critical danger even only from the endotoxin of dead bacteria.

In conclusion—it is essential to identify new targets for developing drugs or vaccines and, in parallel, to develop means that can constitute early warning systems, especially in hospital and community institutions.

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