Extra-intestinal pathogenic *Escherichia coli* – threat connected with food-borne infections

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**Abstract**

Infections caused by extra-intestinal pathogenic *Escherichia coli* (ExPEC) are a serious public health problem worldwide. The most troublesome are urinary tract infections, severe neonatal meningitis, serious intraabdominal infections, and more rarely, pneumonia, intravascular-device infections, osteomyelitis, soft-tissue infections or sometimes bacteremia. These strains are also able cause significant economic losses in animal husbandry. A thorough understanding of ExPEC ecology, reservoirs, chains and dynamics of transmission can greatly contribute to the burden of ExPEC-associated disease. The ability of *E. coli* (including ExPEC) to exist and survive in various ecological niches impedes the precise recognition and indication of transmission routes most important for individual infections cases. Among many identified ExPEC reservoirs, animal companion and animals providing food seem to be important sources of infection for human; however, the real level of risk connected with potential transmission of these bacteria remains unclear. Food is indicated as one of potential ways of transmission. Despite a quite high number of reports, many of the uncertainties are expected to be reliably elucidated. This review presents most important data on the current state of knowledge concerning the potential role of food in ExPEC transmission. The possible consequences of ExPEC infections in human and animals are briefly described.

**Key words**

food, food-borne pathogens, extra-intestinal pathogenic *escherichia coli*, ExPEC

**INTRODUCTION**

Extra-intestinal pathogenic *Escherichia coli* (ExPEC), recognized recently as the most common Gram-negative pathogen in humans [1], is a widely diverse *E. coli* pathotype able to colonize various, often highly specialized, and ecological niches [2]. The facultative pathogen strains can reside in the gastro-intestinal tract where they usually do not cause disease [3, 4]. Outside the gastro-intestinal tract, however, they can cause infections of tissues or organs vulnerable to the virulence factors possessed by colonizing strains [2, 4].

The most important features differentiating the ExPEC from commensal and enteric *E. coli* (helpful also during ExPEC classification) are virulence traits that allow their successful colonization [2]. Among the virulence factors, adhesins (e.g. fimbriae), iron-acquisition systems, capsules and toxins (e.g. haemolysin) are indicated as distinctive traits for ExPEC [4, 5]. The efforts aimed at defining ExPEC have been based on available methods of phenotyping and genotyping [6]. The earliest classification system based on serological typing uses O, H and K surface antigens [1]. Currently, phylogenetic grouping is a common classification system which enables the distinguishing of *E. coli* into one of eight groups: A, B1, B2, C, D, E, F and cryptic clade 1 [7, 8]. The majority of ExPEC are classified into B2 and D phylogroup [4]. Another classification system, based on the multilocus sequence typing (MLST) method, utilizes known sequence variation within a set of housekeeping genes to assign a sequence type (ST). For consistency, current nomenclature describes ExPEC strains using combination of Serotype-Phylogroup-Sequence Type, e.g. O15:K52:H1-D-ST393 or O11:K52:H18-D-ST69 [4]. Genotyping based on the virulence gene profile is also a commonly used method for classifying *E. coli*, including ExPEC.

In contrast to enteric *E. coli* pathotypes, ExPEC can mostly affect some specific subgroups of the population, such as neonates, the elderly or immunocompromised patients [9, 10]. Infections caused by ExPEC are noted in all age groups of humans and animals. In neonates, ExPEC is the leading cause of meningitis. Severe neurological lesions induced by these pathogens lead to the death of 20–40% of infected newborns [11]. In adults, ExPEC is identified as one of most common causes of urinary tract infections, and less often is recognized as the cause of diverse intraabdominal infections, pneumonia, intravascular-device infections, osteomyelitis, soft-tissue infections among others [5]. The consequence of infection at any of mentioned sites can be bacteremia [5].

Food animals and pets are considered as potential reservoirs of ExPEC; however, some uncertainties and doubts concerning this issue are still emphasized. They arise from problems connected with the precise investigation of the transmission of these pathogens among animals, and their dissemination via food [4]. Infections caused by ExPEC in animals can induce severe losses, e.g. in the poultry industry. Avian pathogenic *E. coli* (APEC) invading the respiratory tract of poultry cause inflammation of air sacs, which can lead to the development of septicemia and generalized infection. The losses connected with these outbreaks result from a reduced growth rate, high mortality (even 20%), and expenses connected with condemnation of carcasses at the slaughterhouse and elsewhere [9].
Avian pathogenic *E. coli* can also cause inflammation of the oviduct (salpingitis) in laying hens and broiler breeders which can result in reduced egg production and increased embryonic mortality. Another quite frequently described problem among chickens, induced by ExPEC, is cellulites, characterized as a subcutaneous inflammation in the lower abdomen and thigh [12].

In cattle, mastitis belongs to the most important problems for which ExPEC are responsible as one of the etiological agents. Apart from economic losses connected with decreased milk production, sporadic cases of death of cows can occur after peracute course of mastitis. A less common, however annoying, problem caused by ExPEC in cattle are urinary tract infections (UTIs) [9].

In horses, bronchopneumonia associated with extra-intestinal-pathogenic *E. coli* has been described [15]. Cases of haemorrhagic and necrotizing pneumonia induced by ExPEC have also been noted in dogs and cats [16, 17, 18, 19].

The human-to-human transmission of ExPEC has been unambiguously demonstrated by several investigators [20, 21, 22, 23]. Evidence of resembling ExPEC environmental *E. coli* in domestic animals and various environmental samples suggests multiple non-human reservoirs for human ExPEC. The dissemination of these pathogens along various transmission routes is not called in question; however, the importance of particular routes remains unclear [4]. A number of investigations concerning the food-borne transmission of food animals or retail meat associated ExPEC have been conducted recently in many countries [6]. Although special attention was focused on poultry products [24, 25, 26, 27, 28], other types of meat were also widely investigated [29, 30, 31, 32]. The suspicion concerning the crucial role of food as a reservoir and transmission route of human ExPEC derives, in part, from the confirmed contribution of food in the transmission of enteric *E. coli*. However, evidences of genetic relationships between APEC and human ExPEC, or evidences of the pathogenic potential of APEC for mammals or human-derived ExPEC for birds [4], additionally support the suspicion.

The aim of this review is the presentation of the current state of knowledge concerning the role of food in ExPEC transmission.

**Poultry meat.** Suspicions concerning the principal role of poultry retail meat products as a vehicle of ExPEC causing diseases in humans, are based on quite numerous, however still indirect, evidences. Considerable similarity between genomes, virulence factors profiles and antimicrobial resistance patterns of APEC and human ExPEC strains, have been observed by many investigators [31, 33, 34, 35, 36, 37, 38, 39, 40, 41]. These observations contributed to the emergence of an hypothesis that some human-associated ExPEC evolved from, or are the same as, APEC causing extraintestinal infections in poultry [42]. Recent findings indicate large plasmids of APEC as a potential source of virulence genes for other ExPEC strains [12, 37, 43]. Additional evidences suggesting that the zoonotic potential of APEC originate from experiments demonstrating the pathogenicity of these strains. In one experiment, *B2 E. coli* isolated from meat and from the intestines of healthy chickens, caused infection in the urine model of human UTI [29]. More recently, *B2 E. coli* from UTI patients, poultry meat and healthy chickens were shown as being virulent in a mouse model of UTI [12, 44]. Mellata et al. [45] in their findings established that chicken-derived food products contain *E. coli* strains that, in rodent models of multiple human-associated ExPEC infections, are able to cause disease comparable to human-source *E. coli* clinical isolates, which suggests that they may pose a significant food safety threat.

Research teams investigating the role of retail meat in human ExPEC infections in various countries point to large proportion of chicken meat contaminated with *E. coli* including often ExPEC strains. The authors from the USA and Canada reported a higher number ExPEC isolations from poultry retail meat versus pork and beef [31, 46, 47]. Research in Sweden identified as belonging to ExPEC, lineages ST69, ST10, and ST117, about 50% of the extended-spectrum beta-lactamases (ESBL) producing *E. coli* isolated from retail domestic chicken meat [4]. Considerably high contamination with ExPEC was also found in chicken meat imported into Sweden [48, 49]. In Finland, the investigation of 291 poultry meat products from retail market indicated the presence of potential ExPEC strains in 22% of samples [50]. A study conducted in the Czech Republic showed a 23% prevalence of ExPEC in meat of broilers [51]. Reports showing considerable (however, not always high) levels of ExPEC prevalence in retail chicken meat also come also from Asia, South America and the Middle East [52, 53, 54, 55].

**Pork.** Retail pork is also mentioned as a potential way of the transfer of ExPEC strains from animals to humans; but not as frequent as poultry meat products. Strains recognized as human ExPEC were found on pig farms [35], in pigs [56] and in retail pork meat [29, 30, 31, 46, 57]. Some investigators note a possible connection between certain UTI cases in women with frequent pork consumption reported by the patients [30]. A study conducted in Denmark and Norway identified *E. coli* ST131 among strains isolated from UTI cases and from pork [58]. In another study, ST10 strains were detected among isolates from human clinical samples, from pig faeces and from retail pork [6, 35]. The porcine isolates, however, are regarded as being less virulent than poultry strains [59], which could be connected with the lower overall numbers of virulent genes found in porcine isolates, compared with those derived from poultry [35].

**Beef.** Cattle and beef products are regarded as not significant sources of ExPEC [4, 60]; however, some authors [61] count ground beef among dangerous meat products. Xia et al. [32] found that 3.4% of 239 isolates from retail ground beef met the molecular criteria for ExPEC. Schmidt et al. [60], during investigation of *E. coli* isolates collected from 103 strip loins samples, did not find strains presenting virulence-associated markers of human ExPEC. Nevertheless, in the same study, two of the *E. coli* isolates obtained from the hides of slaughtered cows were identified as ExPEC [60]. In another study, a single isolate obtained from a cow was similar by PPE to human O11/O17/O77:K52:H18-D-ST69 isolate [62]. Brazilian investigators tested ground beef samples (*n* = 23), swabs from grinding machines (*n* = 23) and swabs from the hands of meat handlers collected in 23 butcheries [63]. Among *E. coli* isolates obtained from ground beef samples,
three were identified as ExPEC. Additionally, two ExPEC were isolated from swabs from grinding machines.

The mentioned results seem to indicate the presence of ExPEC in more than 10% of ground beef samples, but because of the relatively low number of tested samples the conclusion concerning as high prevalence of ExPEC in ground beef could be erroneous. The studies conducted on more numerous samples indicated usually lower prevalence of ExPEC in beef.

The reason for the limited prevalence of ExPEC in healthy beef cattle is not known [6].

Eggs. The high prevalence of ExPEC in chicken and in poultry meat suggests a potential threat connected with the consumption of eggs. Studies concerning this problem, infrequent until now, showed a low number of egg-source isolates identified as ExPEC. A survey of 108 E. coli isolates collected from eggs by investigators from the USA, indicated five (4.8%) strains qualified molecularly as ExPEC [64]. Pathotypes of two of these isolates were recognized as APEC, one isolate contained traits of neonatal meningitis E. coli (NMEC) and sepsis-associated E. coli (SEPEC), and the remaining two strains did not fit into any pathotype groups. In further investigation [45], four of the mentioned isolates showed in virulence tests in rodent models (mice), comparable lethality to ExPEC isolated from chicken meat.

The low prevalence of ExPEC in eggs, in contrast to chicken meat, is explained as a possible consequence of the washing process which can eliminate most chicken-sources E. coli isolates from the egg surface, leaving only those that were resistant to the washing process, thereby possibly favouring non-ExPEC over ExPEC [64].

Milk. The contribution of ExPEC in the etiology of mastitis suggest the role of raw milk as a vector of the mentioned type of germs. To date, very limited data are available to describe this route of transmission; however, some studies provide preliminary information concerning raw milk products. Reports from various countries of Latin America, show the results of surveys involving the prevalence of ExPEC in cheeses traditionally made from unpasteurized milk [65, 66, 67]. Investigators from Mexico surveyed 52 samples of five types of fresh unpasteurized cheese [66]. A total of 31 potentially uropathogenic E. coli (UPEC) were isolated from 15 (29%) of samples. In Brazil, during a survey of 147 raw milk cheese samples, only two strains carrying ExPEC gens were identified [65]. However, in earlier findings involving a total of 83 cheese samples collected from three provinces, 17 isolates were identified as potential ExPEC [67]. Results of the mentioned studies are incompatible and further investigations are necessary to assess the degree of danger connected with the possible transfer of such strains via milk.

Plants. In spite of traits which facilitate the spread of ExPEC in the environment (e.g. ability for long survival in water) and the confirmed presence of strains resembling ExPEC in soil, the data on the contamination of edible plants by this pathotype of E. coli are scant. Available reports usually focus on the general level of contamination caused by E. coli and analysis of the phylogenetic status or virulence potential of isolated strains, rarely regard the virulence factors typical for ExPEC. Nevertheless, a research group from Minnesota in the USA, in addition to meat samples, investigated microbiologically 222 vegetable items and 74 fruit items [25]. The samples were purchased from various retail markets (traditional supermarkets, natural foods markets and farmers’ markets). The authors defined ExPEC as E. coli strains carrying at least two of the following genes papA and/or papC, siaf, fopA, dom, kpsM II, and utiA. During the study, no strains able meet these criteria were found in vegetable and fruit samples [25].

Investigators from Pennsylvania in the USA surveyed 150 samples of leafy greens (50 samples each of lettuce, spinach and kale) [68]. All E. coli isolated from these vegetables (15 isolates from lettuce, 15 from kale and eight from spinach) carried the fimH gene encoding fimbriae FimH, which enable the binding of bacteria to the uroplakin 1A receptor (UP1a) of bladder epithelial cells [69]. FimH adhesin is typical for UPEC; however, the fimH gene is common in commensal strains of E. coli and other bacteria found in the environment [68]. Next to fimH gene, one isolate from kale and one from lettuce possessed the ExPEC-associated hlyD gene encoding haemolysin secretion protein D. Moreover, one isolate from spinach, except to fimH, carried the ironV gene encoding siderophore receptor protein [68].

The results described in both mentioned reports seem to indicate a low risk of ExPEC transmission by edible plants; however, for more precise determination of the degree, further investigations are necessary.

CHALLENGES AND DOUBTS

Many studies considering the possible transmission of ExPEC from animals to humans via food are based on simply finding similarities (e.g. virulence factors or antimicrobial resistance profiles) between animal and human isolates. This can also concern other potential sources of infections. Singer [2] indicates this approach as inadequate for establishing transmission events of ExPEC, and emphasizes the need to quantify the frequency and directionality of these events, and points to particular difficulties in documenting the routes of transmission because of the wide range of potential ExPEC sources (human intestinal tract, animals, food animals, retail meat products, environment, etc.).

Although there are no doubts concerning the potential of ExPEC transfer to humans as a result of the mishandling and/or undercooking of meats, some specific traits of ExPEC infections, e.g. the extended period between gastrointestinal colonization and subsequent extraintestinal infection (up to six months) makes any sound establishment of a source of the infection very challenging [2]. Some studies assume that meat as a source of human ExPEC can lead, for example, to consider comparisons of ExPEC in meat eaters and vegetarians as being unambiguously correct. Partisanship of this comparison relies on the assumption that differences in ExPEC presence in the mentioned two groups can be attributed to the meat consumption only. However, these differences can be additionally connected with many other factors, such as health status, age, hospitalization, etc. [2].

Another challenge is the determination of the directionality of ExPEC transmission. Although most often the unidirectional transfer from animals to human (via, e.g. meat) is considered the important one, in opposite or bidirectional transfer, as well as human-to-human transmission, the entire complex of environmental dissemination routes should be
more intensive investigated. In many studies, the restriction of sampling schemes to very few sources forecloses estimation of the possible sources attribution and the real estimation of their importance.

Whole genome sequencing (WGS), used commonly in epidemiological investigations, showed a more than 95% sequence identity between selected APEC and human ExPEC strains [37]. Despite the high similarity, differences on the level of 5% could suggest a not recent transmission event [2].

Singer emphasizes, that for analysis of ExPEC isolates collected from multiple sources in a contemporaneous and spatially overlapping sampling frame, a better understanding is needed regarding the expected number of single nucleotide polymorphisms (SNPs) if the isolates were, in fact, transferred from one source to another [2].

A more general problem which still remains unclear is the definition of specific extraintestinal pathotypes of *E. coli*. Various approaches are used to determine whether a given *E. coli* isolate is ExPEC. The simplest and widely used, although least reliable approach, is estimation of the clinical context and source of isolation. A more reliable way is characterization of the isolate for phylogenetic background and virulence factor profile. The next, more direct approach, is challenge animals with the isolate in an experimental infection model [70].

Although organisms of particular phylogroups are more or less prone to evolve by acquisition of particular virulence traits, the diversity of ExPEC strains is definitely lower than in the *E. coli* species as a whole [70].

The ambiguities and data gaps mentioned, as well as needs concerning the improvement of ExPEC study designs, can indicate certain directions for further research. They do not, however, diminish the potential for ExPEC to be transferred to humans via food, especially via meat. The quite complex ecology of ExPEC demands particular diligence during study and sampling design and recognition of the broad range of potential sources and routes of transmission [2]. Improvement of the mentioned elements would enable a scientifically-sound estimation of the importance of food as one route of ExPEC transmission to humans. A precise assessment of the risk connected with this route could be one of the key data during development of the most effective prevention strategy.

REFERENCES


