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Review

Extraintestinal Pathogenic *Escherichia coli*

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) possesses virulence traits that allow it to invade, colonize, and induce disease in bodily sites outside of the gastrointestinal tract. Human diseases caused by ExPEC include urinary tract infections, neonatal meningitis, sepsis, pneumonia, surgical site infections, as well as infections in other extraintestinal locations. ExPEC-induced diseases represent a large burden in terms of medical costs and productivity losses. In addition to human illnesses, ExPEC strains also cause extraintestinal infections in domestic animals and pets. A commonality of virulence factors has been demonstrated between human and animal ExPEC, suggesting that the organisms are zoonotic pathogens. ExPEC strains have been isolated from food products, in particular from raw meats and poultry, indicating that these organisms potentially represent a new class of foodborne pathogens. This review discusses various aspects of ExPEC, including its presence in food products, in animals used for food or as companion pets; the diseases ExPEC can cause; and the virulence factors and virulence mechanisms that cause disease.

INTRODUCTION

PATHOGENIC STRAINS OF *ESCHERICHIA COLI* have long been recognized as agents of foodborne diarrhea. It is not always appreciated that*E. coli* is an important cause of extraintestinal diseases—diseases that occur in bodily sites outside the gastrointestinal tract (Johnson and Russo, 2002). These include the urinary tract, central nervous system, circulatory system, and respiratory system (Russo and Johnson, 2003). Diarrheic strains of *E. coli* do not generally cause extraintestinal diseases, and those that cause extraintestinal illnesses do not normally induce diarrhea (Russo and Johnson, 2003). *E. coli* strains that induce extraintestinal diseases are termed extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson, 2000). In terms of morbidity and mortality, ExPEC has a great impact on public health, with an economic cost of several billion dollars annually (Russo and Johnson, 2003).

CLINICAL GROUPINGS OF HUMAN *E. COLI* STRAINS

E. coli strains of significance to humans can be classified according to genetic and clinical criteria into three groups: commensal strains, pathogenic intestinal (enteric or diarrheagenic) strains, and pathogenic extraintestinal strains (Russo and Johnson, 2000). Using the *E. coli* reference strains of Ochman and Selander (1984), Goullet and Picard (1989) found that the reference strains formed six phylogenetic groups based on the electrophoretic polymorphism of esterases and other enzymes. These groups were designated A, B_1, B_2, C, D, and E.

The majority of the normal facultative fecal bacterial strains found in healthy humans, mammals, and birds are commensal *E. coli* strains. The commensal strains are generally benign, do not cause intestinal tract disease, and can be beneficial to the host (Neill et al., 1994; Russo and Johnson, 2003). However,

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commensal strains may cause illness if the host is compromised immunologically or medically (Picard et al., 1999; Russo and Johnson, 2003). Generally, human commensal *E. coli* strains derive from phylogenetic groups A and B_1 and typically lack the specialized virulence determinants found in pathogenic strains that cause intestinal or extraintestinal diseases (Picard et al., 1999; Russo and Johnson, 2000).

Intestinal pathogenic strains of *E. coli* are seldom found in the fecal flora of healthy individuals and are rarely a cause of extraintestinal disease. These obligate pathogens induce colitis or gastroenteritis if contaminated food or water is ingested (Guerrant and Thielman, 1995; Russo and Johnson, 2000). The diarrhea-inducing strains include the enterotoxigenic, enterohemorrhagic, enteroinvasive, enteropathogenic, enteroaggregative, diffusely adherent, and cell-detaching *E. coli* pathotypes (Fratamico and Smith, 2006; Guerrant and Thielman, 1995; Nataro and Kaper, 1998). Each pathotype possesses a characteristic combination of virulence traits, resulting in a unique diarrheal syndrome. Strains within each pathotype include a diversity of phylogenetic groupings and are associated mainly with the A, B_1, or D phylogenetic groups. For example, studying Shiga-toxin producing *E. coli* (STEC) (274 of 287 strains were non-O157 serotypes), Giradeau et al. (2005) found that 201 (70.0%) belonged to phylogenetic group B_1, whereas 53 (18.5%) and 29 (10.1%) belonged to groups A and D, respectively. Therefore, within each diarrheic pathotype, the underlying unifying theme is not membership in a common phylogenetic group, but rather possession of a distinctive combination of virulence traits. These virulence traits were acquired through horizontal transfer of plasmids or lysogenic bacteriophages from other bacterial genera (Russo and Johnson, 2000).

ExPEC strains of *E. coli* are phylogenetically and epidemiologically distinct from commensal and intestinal pathogenic strains. They do not produce enteric disease; however, they can asymptomatically colonize the human intestinal tract and may be the predominant strain in ~20% of normal individuals (Johnson and Russo, 2002; Russo and Johnson, 2000, 2003). Asymptomatic colonization of the intestinal tract occurs with both commensal and ExPEC strains of *E. coli*, but not with the intestinal pathogenic strains. Currently, only the intestinal pathogenic *E. coli* strains induce diarrhea, and only the commensal and ExPEC strains can cause extraintestinal diseases (Johnson and Russo, 2002).

The ExPEC strains can cause disease at a number of anatomical locations through entry into a sterile extraintestinal site from their locus of colonization (colon, vagina, oropharynx) (Russo and Johnson, 2003). Most of the ExPEC strains are found in the B_2 and D phylogenetic groups and have acquired various virulence genes that allow them to induce extraintestinal infections in both normal and compromised hosts. The majority of the virulence factors present in the ExPEC strains are distinct from those found in the intestinal pathogenic strains (Picard et al., 1999; Russo and Johnson, 2000, 2003). The ExPEC strains express a variety of virulence-associated genes: instead of a common virulence mechanism, there are many different virulence factors present which cause disease (Brzuszkiewicz et al., 2006). The incidence of ExPEC-induced diseases increases with patient age; therefore, the demographic increase in the elderly population worldwide indicates that there may be a corresponding increase in the incidence of extraintestinal diseases induced by *E. coli* (Russo and Johnson, 2003).

ExPEC strains were defined by Johnson et al. (2003a) as *E. coli* isolates containing 2 or more of the following virulence markers as determined by multiplex PCR: *papA* (P fimbriae structural subunit) and/or *papC* (P fimbriae assembly), *sfa/foc* (S and F1C fimbriae subunits), *afa/dra* (Dr-antigen-binding adhesins), *kpsMT* II (group 2 capsular polysaccharide units), and *iutA* (aerobactin receptor). Other virulence markers that may be associated with ExPEC status are listed in Table 1. Johnson and Russo (2005) have prepared an extensive listing of potential virulence factors associated with ExPEC strains.

ANTIBIOTIC RESISTANCE IN *E. COLI*

Antibiotic resistance in *E. coli* strains from human, animal, and environmental sources is a major public health concern. For example,

Table. 1. Additional Virulence Factors That May Be Associated with Extraintestinal Pathogenic *Escherichia coli*[a]

Virulence genotype	*Gene encodes*
papG III	P-fimbrial adhesin unit, variant III
fimH	Type 1 fimbrial adhesin
gafD	G fimbriae
bmaE	M-agglutinin subunit
sfaS	N-acetyl-D-glucosamine specific fimbrial lectin
Iha	Iron-regulated adhesin
fyuA	Yersiniabactin receptor
ireA	Siderophore receptor
iron	Siderophore receptor
iutA	Aerobactin receptor
kpsMT II	Group 2 capsular polysaccharide subunit
K1	K1 (group 2) *kps* variant
ibeA	Invasion of brain endothelium
traT	Serum (complement) resistance
Iss	Increased serum survival
ompT	Outer membrane protease T subunit
cvaC	Colicin V structural subunit
malX	Pathogenicity-associated island (CFT073)
Hly	Hemolysin
Cnf1	Cytotoxic necrosis factor 1
cdtB	Cytolethal distending toxin protein B subunit

[a]Modified from Freitag et al., 2005.

extended spectrum β-lactamase (ESBL)-producing *E. coli* have spread as a major cause of hospital-acquired infections, as well as infections in outpatient settings (Oteo et al., 2005; Pitout et al., 2005). Genes that encode ESBL are often found on large plasmids that also carry genes for resistance to other antibiotics (Livermore and Woodford, 2006). Beginning in the 1990s, the frequency of resistance to fluoroquinolone antibiotics, including ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, and nalidixic acid in *E. coli* has increased worldwide (Goettsch et al., 2000; Hopkins et al., 2005). Administration of ciprofloxacin or other fluoroquinolones is a risk factor for isolation of resistant strains of *E. coli* from patients undergoing long-term hospital care, and resistance is associated with treatment failure. In addition, multiresistance, defined as resistance to norfloxacin in addition to two or three other antibiotics, has also increased (Goettsch et al., 2000).

Several reports have indicated that quinolone resistance in uropathogenic *E. coli* is associated with decreased prevalence or expression of virulence factors compared to quinolone-susceptible strains (Drews et al., 2005; Horcajada et al., 2005; Soto et al., 2006; Vila et al., 2002). Vila and coworkers (2002) suggested that a possible reason for this is that virulence genes could be lost concomitant with a mutation at codon 83 of the *gyrA* gene, which affects supercoiling of DNA, leading to changes in gene expression. Another reason is that with exposure to quinolones and development of resistance to these agents, there is a concomitant increase in the deletion and transposition of pathogenicity islands (PAIs). Soto and coworkers (2006) found that uropathogenic *E. coli* strains incubated with subinhibitory concentrations of ciprofloxacin showed partial or total loss of virulence genes encoded within PAIs.

PREVALENCE OF ExPEC IN FOOD PRODUCTS

In a study of 169 raw cut-up chicken parts obtained from retail grocery stores in the Minneapolis–St. Paul area during 2000, Johnson et al. (2003a) isolated *E. coli* from 150 (88.8%) samples. Strains resistant to nalidixic acid were found in 62/150 (41.3%) of the samples, and susceptible strains were present in 143/150 (95.3%) of the chicken samples. Fifty-five of the chicken samples contained both nalidixic acid–resistant and nalidixic acid–sensitive *E. coli*. One susceptible and one resistant strain from each sample (a total of 110 strains) were analyzed for their ExPEC status. Twenty-three of the 110 strains were identified as ExPEC by the presence of virulence markers; 10 were susceptible to nalidixic acid and 13 were resistant (Johnson et al., 2003a).

Of the 55 chicken samples assayed for ExPEC status, 21/55 (38.2%) chicken samples contained ExPEC. Most of the ExPEC strains (17/23; 73.9%) derived from phylogenetic groups B2 or D. O antigens were determined for 22 of the 23 ExPEC strains; 20 of 22 strains of ExPEC were O typeable and included 16 O types: the major O types included O7 (5 isolates), O78 (3 isolates), and O120 (3 isolates) (Johnson et al., 2003a). Johnson et al. (2003a) indicated that nalidixic acid–resistant *E. coli* and

ExPEC are common in retail chicken parts. There is the possibility that chicken-derived ExPEC is pathogenic for humans.

A survey of 346 food products (222 vegetable, 74 fruit, and 50 raw meat items) purchased in Minneapolis–St. Paul retail establishments in 1999–2000 revealed that ExPEC was only isolated from turkey (28/50 meat samples) (Johnson et al., 2005a). Only 35/222 (15.8%) of the vegetables and 4/74 (5.4%) of the fruit contained *E. coli*, whereas all of the meat samples were contaminated with *E. coli*. Only turkey contained ExPEC strains. Twelve ExPEC strains were isolated from 10 samples of turkey (Johnson et al., 2005a). Eight of the ExPEC strains derived from either phylogenetic group B_2 (5 strains) or D (3 strains); phylogenetic groups A and B_1 were represented by 2 strains each. Twenty-four of 28 (85.7%) turkey samples contained *E. coli* strains that were resistant to at least one of 10 antimicrobials tested, and 8 (28.6%) of the samples contained *E. coli* strains resistant to ≥4 antimicrobial compounds. The survey conducted by Johnson et al. (2005a) indicated that retail turkey is a source of antimicrobial-resistant *E. coli* and ExPEC.

Johnson et al. (2005b) also surveyed 1648 retail food items collected in the Minneapolis–St. Paul area during 2001–2003 for the presence of *E. coli*, resistant *E. coli*, ExPEC, and urinary tract infection (UTI)-causing *E. coli* (Table 2). The UTI-inducing strains of *E. coli* are also considered to be ExPEC strains (Russo and Johnson, 2003). The presence of *E. coli* in miscellaneous foods was quite low. Most (180/195) of the raw poultry samples were contaminated with *E. coli*. Only 25.6% of the miscellaneous food product samples contained resistant *E. coli*; however, resistant strains were present in most of the raw meat products. Approximately 17% of the resistant *E. coli* strains in beef and pork were resistant to ≥5 antimicrobials, whereas approximately 55% of the poultry samples contained strains resistant to ≥5 antimicrobials (Johnson et al., 2005b). The investigators suggest that the presence of resistant *E. coli* in these food items developed in the farm environment.

The presence of ExPEC strains was particularly high in poultry products, whereas O-UTI (UTI associated with specific *E. coli* O antigens) *E. coli* strains were more evenly distributed. Johnson et al. (2005b) did not discuss antimicrobial resistance among the ExPEC and O-UTI *E. coli*, but their data suggest that many of these extraintestinal pathogenic strains are also resistant. Seventeen of the food-derived ExPEC strains, from phylogenetic groups B_2 and D, exhibited virulence traits consistent with potential causation of human disease (Johnson et al., 2005b). The studies by Johnson et al. (2003a, 2005a, 2005b) indicate that meats, particularly poultry, can be an important source of resistant ExPEC strains.

ExPEC IN ANIMALS

ExPEC strains have been isolated from cases of extraintestinal disease in food animals and

TABLE 2. THE PRESENCE OF *ESCHERICHIA COLI* IN 1648 RETAIL FOOD ITEMS[a]

	Miscellaneous food items[b]	*Raw beef and pork*[c]	*Raw poultry*
Number of samples with *E. coli* (%)	121/1315 (9.2%)	95/138 (68.8%)	180/195 (92.3%)
Number of samples with antimicrobial-resistant *E. coli* (%)	31/121 (25.6%)	73/95 (76.8%)	165/180 (91.7%)
Number of samples with extraintestinal pathogenic *E. coli* (%)[d]	5/121 (4.1%)	18/95 (18.9%)	83/180 (46.1%)
Number of samples with O-UTI *E. coli* (%)[e]	12/121 (9.9%)	13/95 (13.7%)	28/180 (15.6%)

[a]Table modified from Johnson et al. (2005b).
[b]Fresh fruit and vegetables, cheese, dry salami, turkey franks, fish, crab, shrimp, delicatessen items, cream or custard pastries.
[c]Variably ground or frozen.
[d]Positive for >2 of: *papA* and/or *papC*, *sfa/foc*, *afa/dra*, *kpsM* II, and *iutA*.
[e]O-antigens associated with urinary infections: O1, O2, O4, O6, O7, O16, O18, O25, O75.

pets. Eighteen *E. coli* strains isolated from piglets or calves with septicemia showed a number of virulence factors also present in human ExPEC strains (Dezfulian et al., 2003). These animal strains belonged to phylogenetic groups B_1 or A. Experimental infection of piglets with the isolates of these animal ExPEC strains caused lethal infection in many cases (Dezfulian et al., 2003). In comparing ExPEC strains causing human UTI (18 strains) or bacteremia (14 strains) with 19 strains causing bacteremia in calves and piglets, Girardeau et al. (2003) showed that there were major similarities in virulence factors found in human and animal ExPEC; however, 26 diarrhea-associated bovine *E. coli* strains differed from human and animal ExPEC in lacking the typical ExPEC virulence traits.

Maynard et al. (2004) compared 39 resistant ExPEC strains from animals (15, 8, 8, and 8 from swine, cattle, chickens, and pets, respectively) with 70 resistant human ExPEC strains. Fifty-one percent of the animal isolates and 50% of the human isolates were resistant to more than 3 antimicrobial compounds. Most of the animal strains (67%) belonged to phylogenetic groups A and B_1; 77% of the human strains belonged to groups B_2 and D. Phylogenetic group B_2 made up 54% of the human ExPEC strains and 88% of the pet strains (Maynard et al., 2004). Of 61 ExPEC strains isolated from human clinical cases, Johnson et al. (2003b) demonstrated that 40 (65.6%) belonged to phylogenetic group B_2 and 21 (34.4%) to group D. However, 28 ExPEC strains isolated from cattle and swine clinical cases belonged mostly to group A (23/28; 82.1%) whereas B_1, B_2, and D groups represented only 3, 1, and 1 strain, respectively.

Avian pathogenic *E. coli* (APEC) infections of poultry result in significant morbidity and mortality, with resultant serious economic losses to the poultry industry. These pathogenic *E. coli* enter the respiratory tract and colonize the air sacs. Aerosacculitis is followed by extraintestinal infections including septicemia, pneumonia, pericarditis, perihepatitis, peritonitis, and death (Dho-Moulin and Fairbrother, 1999; Li et al., 2005). *E. coli* strains of serotypes O2 and O78 are responsible for 80% of avian cases worldwide. Rodriguez-Siek et al. (2005a) studied 451 isolates of APEC and found that most of the strains shared virulence traits associated with human ExPEC strains. In a comparison of 524 APEC isolates and 200 isolates of human uropathogenic *E. coli* (UPEC), Rodriguez-Siek et al. (2005b) found that the two groups showed considerable overlap of serogroups, phylogenetic groups, and virulence phenotypes. In the APEC strains, 199 (38.0%) of the isolates belonged to phylogenetic group A, 81 (15.5%) to group B_1, 97 (18.5%) to group B_2, and 147 (28.1%) belonged to group D. In the UPEC strains, 21 (10.5%) were members of phylogenetic group A, 12 (6.0%) were group B_1, 130 (65.0%) were group B_2, and 37 (18.5%) were group D (Rodriguez-Siek et al., 2005b). As would be expected of human ExPEC strains, the phylogenetic groups B_2 and D made up 83.5% of the human uropathogenic strains. Many of the virulence factors present in APEC strains are also present in human ExPEC strains that cause human neonatal meningitis and UTIs, as well as in animal disease-causing ExPEC strains (Schouler et al., 2004). Ron (2006) suggested that avian strains of ExPEC are zoonotic pathogens, and Rodriguez-Siek et al. (2005b) conjectured that certain strains of APEC have the potential to infect humans and/or poultry and can act as a reservoir for virulence genes for ExPEC.

Germon et al. (2005) detected *ibeA* (the gene encoding for invasion of brain endothelium in ExPEC strains, responsible for human neonatal meningitis) in 53/213 APEC strains; the gene was not present in 55 nonpathogenic avian *E. coli* strains. The *ibeA* gene was mainly associated with serotypes O2 (28/53), O18 (7/53), and O88 (7/53). An $ibeA^+$ APEC strain and its isogenic *ibeA* mutant were tested for virulence by Germon et al. (2005). The invasive capacity of the APEC *ibeA* mutant against human brain microvascular endothelial cells was reduced approximately 30% as compared to the parent strain. Challenge assays of 3-week-old chickens by inoculation of chicken air sacs with 10^7 CFU of the parent and the mutant *ibeA* indicated that the parent APEC killed 6 of 22 chickens, whereas only 1 of 22 was killed by the *ibeA* mutant. In addition, bacterial counts were reduced 7-fold in the liver and 17-fold in the blood of chickens inoculated with the mutant (Germon

et al., 2005). Thus, the *ibeA* gene is an important virulence factor in chicken ExPEC. Multiple antimicrobial resistance was common in APEC strains isolated from cases of avian colibacillosis in Georgia between 1996 and 2000 (Zhao et al., 2005). Resistance to ≥3 antimicrobials was found in 87/95 (91.6%) APEC strains, 67/95 (70.5%) strains were resistant to ≥5 antimicrobials, and 30/95 (31.6%) APEC strains were resistant to ≥8 antimicrobials (Zhao et al., 2005).

The *papG* allele III (tip protein subunit of P pili) is commonly present in *E. coli* strains isolated from dogs with ExPEC-induced UTI (Johnson et al., 2000). The PapG III peptide sequences from *E. coli* isolated from humans and dogs were highly homologous, and there were no host-species–specific differences in the predicted peptide sequences (Johnson et al., 2000). In addition, a number of human ExPEC-associated virulence genes were detected among the canine ExPEC strains, indicating a commonality between canine and human ExPEC strains expressing the *papG* allele III. This commonality suggests that dogs may be a reservoir of human ExPEC strains. In a further study, Johnson et al. (2001a) studied the incidence of the *papG* allele III in *E. coli* isolated from fresh canine fecal samples collected from sidewalks in the St. Paul, Minnesota area in 1996-1997. They found that 19/34 (55.9%) of *E. coli*-positive fecal samples contained *E. coli* with the *papG* allele III. Human clinical ExPEC strains isolated from patients with cystitis, pyelonephritis, bacteremia, or meningitis and *papG* allele III-positive canine strains of *E. coli* were similar in terms of their serotypes, virulence genotypes, and random polymorphic DNA profiles (Johnson et al., 2001a).

In a study of 37 dogs with *E. coli*-induced UTI, Johnson et al. (2003b) found that urinary isolates were derived predominantly from phylogenetic group B_2, possessed typical human UTI-associated O antigens (O4, O6), and exhibited several virulence genes that may contribute to urovirulence, including *papG* allele III, *sfa/foc*, *sfaS*, *hly*, *fyuA*, *iroN*, and *ompT*. These data indicated that canine UTIs were caused by ExPEC strains that closely resemble human uropathogens (Johnson et al., 2003b). Canine ExPEC that induce UTIs are not dog-specific and may have the capacity to induce extraintestinal diseases in humans.

The commonality between human and canine ExPEC strains implies that (1) humans may acquire ExPEC strains from dogs, (2) similar virulence genes are present in human and dog ExPEC strains, indicating that the pathogenic mechanisms are probably similar, and (3) if colonization of humans with canine-derived ExPEC strains occurs, then antimicrobials used in veterinary practice could lead to selection of antimicrobial resistance in the new human pathogens (Johnson et al., 2001b).

In a study of 38 ExPEC strains that caused UTIs (isolated from 15 dogs, 4 cats, and 19 humans), 29 of the strains (from 12 dogs, 4 cats, and 13 humans) tested positive for the *papG* allele III, with overlapping of a number of virulence genes (Johnson et al., 2001c). The animal and human ExPEC strains did not segregate according to host grouping, composite virulence gene-serotype profiles, or pulsed field gel electrophoresis profiles. Thus, certain pathogenic lineages of ExPEC cause disease in both animals and humans. While cross-species transmission of ExPEC has not been demonstrated, the commonality of dog, cat, and human ExPEC strains containing the *papG* allele III does suggest the possibility of cross-species infection (Johnson et al., 2001c). The demonstration of infection of dogs or cats by inoculation with human ExPEC would give an indication that cross-species transmission can occur. The studies of Johnson et al. (2000, 2001a, 2001b, 2001c, 2003d) indicate that ExPEC strains that cause canine (and probably feline) UTIs are similar to those ExPEC strains that cause human extraintestinal diseases.

COLONIZATION OF THE HUMAN GUT BY ExPEC

Johnson and Russo (2002) stated that B_2 may be the dominant fecal *E. coli* group in about 20% of normal healthy humans. For the majority of healthy individuals, most fecal strains of *E. coli* belong to phylogenetic groups A and B_1 (Russo and Johnson, 2000). Duriez et al. (2001), studying the phylogenetic grouping of *E. coli* strains isolated from the stools of 168 healthy human

subjects, found that 67 (39.9%) of the strains belonged to group A, 57 (33.9%) to group B_1, 26 (15.5%) to group D, and 18 (10.7%) to group B_2. However, determination of the grouping of 118 *E. coli* strains isolated from patients with extraintestinal diseases (not stool samples) indicated that 11 (9.3%) belonged to phylogenetic group A, 3 (2.5%) to group B_1, 19 (16.1%) to group D, and 85 (72.0%) to group B_2 (Duriez et al., 2001). Virulence factors were more frequently found in group B_2 strains present in both fecal and extraintestinal disease isolates as compared to the other phylogenetic groups (Duriez et al., 2001). However, Sannes et al. (2004) and Zhang et al. (2002) found that phylogenetic group B_2 strains from bacteremic or cystitis cases had almost twofold the number of virulence-associated genes compared to fecal B_2 strains.

In a group of 93 women (18 to 39 years of age) experiencing their first case of *E. coli* UTI, Zhang et al. (2002) found that 7 strains (7.5%) isolated from their urine were from phylogenetic group A, 3 strains (3.2%) were from group B_1, 19 strains (20.4%) were from group D, and 64 strains (68.8%) were from group B_2. In addition, these investigators found that group B_2 *E. coli* was common in rectal isolates from 88 healthy women who had never had a UTI. Group A was dominant in the rectal samples of 18/88 (20.5%), group B_1 in 11/88 (12.5%), group D in 17/88 (19.3), and group B_2 was the dominant rectal *E. coli* in 42/88 (47.7%) of the healthy women (Zhang et al., 2002). Obata-Yasuoka et al. (2002) compared *E. coli* strains isolated from Japanese women with vaginosis to strains of *E. coli* isolated from the stools of normal Japanese men and women. There were 88 isolates from vaginal samples: 7 (8.0%) belonged to phylogenetic group A, 67 (76.1%) to group B_2, and 14 (15.9%) to group D. Of 61 *E. coli* strains from the stool samples of healthy individuals, 17 (27.9%) were from group A, 27 (44.3%) from group B_2, and 17 (27.9%) from group D. Sannes et al. (2004) found that 66.6% (42/63) of the isolates from United States veterans with *E. coli*-induced bacteremia belonged to phylogenetic group B_2. More than half of rectal *E. coli* from control veteran patients not suffering from bacteremia belonged to group B_2 (38/71, 53.5%). While the dominant commensal strains of *E. coli* generally present in the human gut belong to group A (Russo and Johnson, 2000), the work of Sannes et al. (2004), Obata-Yasuoka et al. (2002), and Zhang et al. (2002) indicate that in some individuals, group B_2 may be the dominant fecal strain.

DISEASES INDUCED BY ExPEC

Neonatal meningitis

The neonatal period is defined as the first 28 days after birth. Bacterial neonatal meningitis is an inflammation of the membranes of the brain or spinal cord and consists of a purulent exudate of the membranes, perivascular inflammation, and brain edema. Group B β-hemolytic *Streptococcus*, gram-negative enteric bacteria, and *Listeria monocytogenes* account for most cases. The incidence of bacterial neonatal meningitis ranges from 2 to 5 cases per 10,000 live births in developed countries, but is approximately 10-fold higher in developing countries. Obstetric or perinatal complications, prematurity, and low birth weight increase the risk of morbidity and mortality (Barnett and Krishnamoorthy, 2006; Harvey et al., 1999; Kimberlin, 2002). Sequelae can include hydrocephalus, seizures, mental retardation, cerebral palsy, and hearing loss (Harvey et al., 1999; Pong and Bradley, 1999). While Holt et al. (2001) did not break down treatment of bacterial neonatal meningitis by the causative organism, they stated that the most common antibiotics used for treatment included cefotaxine, gentamycin, and/or penicillin. Third generation cephalosporins such as cefotaxine decreased mortality but not morbidity (Harvey et al., 1999).

In the United States, more than 50% of cases of neonatal meningitis caused by gram-negative enteric organisms were due to ExPEC strains, and approximately 80% of those cases were caused by strains carrying the K1 capsular antigen (Pong and Bradley, 1999). Bonacorsi et al. (2003) found that 118/132 (89.4%) of ExPEC that induced neonatal meningitis produced the K1 capsule, while in another study, this number was 57/70 (81.4%) (Johnson et al., 2002a). In addition, Johnson et al. (2002a) found that 54/57 (94.7%) of the K1 strains belonged

to group B_2. The most frequent serotype of ExPEC causing neonatal meningitis was O18:K1 (Bonacorsi et al., 2003; Johnson et al., 2002a), which has a world-wide distribution. The K1 capsule confers resistance to serum and opsonophagocytic killing (Xie et al., 2004). The K1 polysialic acid capsule may also act as a mimic of the polysialic acid (PSA) chains attached to human embryonic and neonatal neural cell adhesion molecules (NCAM) (Cieslewicz and Vimr, 1997). PSA-NCAM is abundant in the human embryonic and neonatal brain and is involved in the early development of the nervous system (Brusés and Rutishauser, 2001; Nakayama et al., 1995). The K1 polysialic acid may interfere with normal brain maturation by competing with neural PSA for sites on NCAM.

Between 20–40% of the estimated 400 annual cases of neonatal meningitis occurring in the United States are due to *E. coli* (Russo and Johnson, 2003). The mortality rate for *E. coli*–induced neonatal meningitis is approximately 8%, and most survivors show neurological or developmental deficits (Mylonakis and Go, 2006). The infection can be acquired during passage through the birth canal, antenatally from *E. coli* infections of umbilici or circumcision wounds, or from organisms colonizing the infant's upper respiratory or intestinal tract (Go and Cunha, 2004). The association of *E. coli* meningitis with the neonate is due to an immature immune system and not to greater susceptibility of the neonatal brain microvascular endothelial cells (BMECs) to *E. coli* binding, invasion, or transcytosis (Xie et al., 2004).

The phylogenetic grouping of a number of neonatal meningitis-inducing *E. coli* isolates is presented in Table 3. The majority of strains belong to phylogenetic group B_2 (Bonacorsi et al., 2003; Clermont et al., 2001; Johnson et al., 2002a). Strains belonging to groups A, B_1, or D had fewer virulence factors than group B_2 (Bonacorsi et al., 2003; Johnson et al., 2002a). While neonatal meningitis can be caused by ExPEC belonging to all four phylogenetic groups, group A strains were found only in those neonatal meningitis cases in which the neonate was a high-risk patient with an underlying immune or medical condition (Bingen et al., 1998). Detection of a group A (and perhaps a B_1) isolate in a putative normal-risk neonate suggests immune deficiency or other underlying medical problems (Bonacorsi et al., 2003).

Many of the virulence genes associated with ExPEC neonatal meningitis strains are present on PAIs (Bonacorsi et al., 2003). For example, the genes involved in facilitating blood-brain barrier penetration—*sfaS*, *ibeA*, and *cnf1*—are located on PAIs (Bonacorsi et al., 2003). PAIs are large chromosomal regions encoding virulence genes that were acquired from unrelated bacteria through horizontal gene transfer. There is a significant difference in the guanine + cytosine content of PAIs compared to the rest of the bacterial genome.

The blood-brain barrier separates the central nervous system (CNS) from the vascular compartment of the body anatomically and functionally, and maintains homeostasis of the CNS. The blood-brain barrier is composed of a layer of BMECs lining the lumen of the brain capillaries (Huang and Jong, 2001; Huang et al., 2000). The brain endothelium prevents the intracellular transition of certain macromolecules

TABLE 3. PHYLOGENETIC GROUPING OF NEONATAL MENINGITIS-INDUCING EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM PATIENTS

	Number of patients (%)		
Phylogenetic group	*Clermont et al., 2001 (n = 124)*[a]	*Bonacorsi et al., 2003 (n = 132)*[b]	*Johnson et al., 2002a (n = 70)*[c]
A	9 (7.3%)	11 (8.3%)	1 (1.4%)
B_1	4 (3.2%)	1 (1.5%)	7 (10.0%)
B_2	89 (71.8%)	99 (75.0%)	57 (81.4%)
D	22 (17.7%)	30 (15.2%)	5 (7.1%)

[a]Patients were from France.
[b]Patients were from France (n = 91) and North America (n = 41).
[c]Patients were from The Netherlands.

into the CNS and protects against fluctuations in the concentration of plasma solutes. In addition, the brain endothelium protects against the entry of microbes and toxins circulating in the blood (Huang and Jong, 2001; Huang et al., 2000).

The pathogenesis of ExPEC-induced neonatal meningitis occurs in several steps: bacteremia, binding of bacteria to the surface of BMECs, bacterial invasion of BMECs, and invasion of the meninges (membranes that surround the brain and spinal cord) and the CNS.

There is a correlation between the extent of bacteremia and the development of meningitis. In one study, there was a significant increase in *E. coli*-induced meningitis in neonates when the blood bacterial count was $>10^3$/mL compared to counts $<10^3$/mL (Kim, 2001). Outer membrane protein A (OmpA), K1 capsular polysaccharide antigen, and O-lipopolysaccharide (O-LPS) have been implicated in the survival and multiplication of *E. coli* K1 in the circulatory system. These determinants contribute to bacteremia by protecting *E. coli* against complement-mediated serum and opsonophagocytic killing (Xie et al., 2004). The salmochelin iron uptake system encoded by *iroN* is also a necessary virulence factor needed to induce a high level of *E. coli* K1 bacteremia in the neonatal rat (Négre et al., 2004).

E. coli must bind to the surface of BMECs in order to invade the blood-brain barrier, and the bacterial proteins FimH and OmpA play important roles in binding (Kim 2003; Xie et al., 2004). Electron microscopy indicated that *E. coli* K1 invades BMECs through a zipper-like mechanism and the internalized bacteria are found within BMEC membrane-bound vacuoles (Kim, 2003). Vacuoles containing K1-negative strains mature to fuse with lysosomes, and the bacteria are lysed, whereas the presence of the K1 capsule inhibits vacuole maturation and there is no fusion with lysosomes, allowing the bacteria to survive intracellularly and to traverse human BMECs as live bacteria. There is no multiplication of *E. coli* K1 in the vacuole (Kim, 2003). Free bacteria are not found in the cytoplasm, between adjacent BMECs, or in the intercellular junctions of BMECs (Xie et al., 2004). Therefore, *E. coli* invade BMECs via a transcellular (transcytotic) process. *E. coli* K1 invasion is specific to brain endothelial cells and the organism does not invade and survive in non-brain endothelial cells (Kim, 2003).

A number of bacterial invasins contribute to BMEC invasion by *E. coli* K1, including IbeA, IbeB, and IbeC proteins, AslA, OmpA, type 1 fimbriae (FimH), and cytotoxic necrotizing factor 1 (CNF1) (Kim, 2001; Xie et al., 2004). Mutations/ deletions in the *ibeA* locus (Prasadarao et al., 1999), *aslA* gene (Hoffman et al., 2000), *ompA* gene (Prasadarao et al., 1996a, 1996b), *cnf1* gene (Khan et al., 2002), and *fimH* gene (Teng et al., 2005) led to decreased invasive capacity by *E. coli* K1. Rearrangement of the actin cytoskeleton is an important part of the invasion of BMECs by *E. coli* K1, since F-actin condensation is associated with the invading organisms. Invasion is blocked by the use of actin microfilament inhibitors such as cytochalasin D or latrunculin A (Kim, 2001, 2003; Xie et al., 2004). The virulence factors OmpA and CNF1 are involved in cytoskeletal rearrangements during invasion of BMECs by *E. coli* K1 (Kim, 2001, 2003; Xie et al., 2004). RhoA is also involved in actin cytoskeletal rearrangements, and the invasion of BMECs by *E. coli* K1 requires RhoA activation (Kim, 2001, 2003; Xie et al., 2004). Deamidation of RhoA by CNF1 results in a constitutively activated RhoA protein (Barbieri et al., 2002). There was an approximately 3-fold increase in the invasion of BMECs and in the induction of meningitis in the neonatal rat using wild-type *E. coli* K1 compared with the *cnf1*-negative mutant of *E. coli* K1. In addition, the mutant was significantly less efficient in activating RhoA (Khan et al., 2002). The role of CNF1 in the invasion of BMECs by *E. coli* K1 is to ensure the continued activation of RhoA. An *ompA/cnf1* double-knockout mutant of *E. coli* K1 exhibited an approximately 30-fold decrease in invading capacity of human BMECs compared to the wild type (Khan et al., 2003). The *ompA* mutant demonstrated an approximately 12-fold decrease in invasion, whereas the *cnf1* mutant had an 8-fold decrease in invasive ability (Khan et al., 2003). Therefore, OmpA and CNF1 work together in the invasion of BMECs by *E. coli* K1, by inducing rearrangement of the actin cytoskeleton.

After the bacteria traverse the BMECs, they invade the meninges and CNS, multiply and induce the release of proinflammatory compounds (cytokines, chemokines, reactive oxygen species, nitric oxide), which leads to increased blood-brain barrier permeability and pleocytosis (increase in leukocytes in the spinal fluid) (Kim, 2003). With increased permeability, there is brain edema and increased intracranial pressure. The effects induced by *E. coli* K1 invasion of the BMECs ultimately lead to meningitis and neuronal injury (Kim, 2003).

UTIs

UTIs are among the most common bacterial infections found in humans. In the United States, uropathogenic *E. coli* cause 70–90% of community acquired UTIs and 50% of nosocomial UTIs (Kucheria et al., 2005). The virulence factors and clinical picture presented by uropathogenic *E. coli* infections indicate that these pathogens are ExPEC strains (Johnson and Russo, 2002, 2005).

An individual with UTI will have a significant number of pathogens in the urinary system. Microbial pathogens may be present in the bladder (cystitis), kidneys (pyelonephritis), urine (bacteriuria), or prostrate (prostatitis) (Foxman, 2002; Kucheria et al., 2005; Marrs et al., 2005). Cystitis in healthy individuals generally resolves without sequelae, but pyelonephritis can induce serious morbidity and may be fatal. Some individuals have high numbers of bacteria in the urine but show no symptoms (asymptomatic bacteriuria). UTIs occuring in the normal genitourinary tracts of immunocompetent individuals are called uncomplicated infections. UTIs diagnosed in individuals with genitourinary tracts that have structural or functional abnormalities, including indwelling urethral catheters, or in immunocompromised individuals, are labeled complicated infections.

Individuals with increased risk for UTIs include infants, pregnant women, and the elderly. Patients with spinal cord injuries, diabetes, multiple sclerosis, urinary catheters, HIV/AIDS, or underlying urologic abnormalities are also at risk (Foxman, 2002). Since the genitourinary tract is close to the rectum, fecal bacteria can ascend the urethra into the bladder. If there is backflow (reflux) of urine from the infected bladder to the ureters, the kidney may become infected. The ascending route from the fecal site is considered to be the major means of transmission of UTI-causing ExPEC to the urinary tract (Sobel, 1985).

Only about 20% of all UTIs in the United States occur in men (Griebling, 2005); 50–60% of women in the United States will have at least one UTI during their lifetime. There is a tendency for UTIs to recur in 25–30% of women after the initial infection, due either to reinfection or recrudescence (Bower et al., 2005; Foxman, 2002; Kucheria et al., 2005). UTIs are the most common bacterial infection in infants <90 days of age, with boys being more susceptible to UTIs than girls; after 3 months of age, UTIs are more prevalent in girls. Uncircumcised boys are about 4 times more likely than circumcised boys to have an infection (Bower et al., 2005; Foxman, 2002; Kucheria et al., 2005). However, Van Howe (2005) has suggested that these differences in UTI incidence are due to sampling and selection bias. The incidence of UTIs in prepubertal girls is 3 to 4 times higher than in boys (Foxman, 2002; Larcombe, 1999).

In the United States, ExPEC cause 85–95% of cases of uncomplicated cystitis in premenopausal women, with 6 to 8 million cases annually (Russo and Johnson, 2003). More than 90% of the approximately 250,000 annual cases of uncomplicated pyelonephritis in premenopausal women are caused by ExPEC, with at least 100,000 cases requiring hospitalization (Russo and Johnson, 2003).

UTIs are the most common bacterial infection seen during pregnancy. Asymptomatic bacteriuria is found in 4–10% of pregnant women. First-time cystitis is seen in 1–4% of pregnant women, and 1–2% of pregnant women are affected with acute pyelonephritis (Foxman, 2002). Women with a history of UTI have an increased risk for UTI during pregnancy. Pyelonephritis during the third trimester may lead to fetal death or infants born with mental retardation, developmental delays, or cerebral palsy (Foxman, 2002).

Approximately 11–25% of elderly noninstitutionalized patients not undergoing catheterization acquire asymptomatic bacteriuria, and about 10% develop symptomatic bacterial UTIs

(Foxman, 2002). Institutionalized and hospitalized elderly persons, especially those undergoing urinary catheterization, have a higher incidence of UTI. In a study of bacteremic UTI (bacteria present in both blood and urine) in 191 elderly patients (average age 83.6 ± 5.9 years), Tal et al. (2005) found that 52.9% of the patients were women, indicating that elderly men, unlike younger men, are as susceptible to UTI as are women. *E. coli* was the causative agent in 89 (46.1%) of the elderly patients (Tal et al., 2005).

Catheter-associated UTI is a common nosocomial infection and accounts for >1 million cases annually in United States hospitals and nursing homes (Foxman, 2002); ExPEC accounts for 25–35% of those cases (Russo and Johnson, 2003). The incidence of bacteriuria during catheterization is 3–10%, and the risk of UTI increases with increasing duration of catheterization (Foxman, 2002).

Community outbreaks of *E. coli*-induced UTIs have been documented in Denmark, Spain, and the United Kingdom (Ramchandani et al., 2005). Manges et al. (2001) described an widespread outbreak of UTIs in women in which a trimethoprim-sulfamethoxazole resistant strain of *E. coli* belonging to a single clonal group (group A) was responsible for roughly half of the community acquired UTIs in women in three states—California, Michigan, and Minnesota. Both Manges et al. (2001) and Ramchandani et al. (2005) suggested that some community outbreaks of UTI are due to the ingestion of contaminated food or water. Johnson et al. (2003a, 2005a, 2005b) have isolated ExPEC, including those strains that cause UTIs, from food products, indicating that the idea of foodborne UTI is a feasible one. However, actual UTI outbreaks due to organisms present in food products have not been demonstrated.

A number of antimicrobials are used to treat *E. coli*-induced UTIs, including β-lactams, quinolones, trimethoprim in combination with sulfamethoxazole, and nitrofuranes (Wagenlehner et al., 2005). Clinicians have noted that there is an increasing trend in the resistance of *E. coli* to commonly prescribed antimicrobial agents used in the treatment of UTIs. Fritzsche et al. (2005) indicated that the resistance to ampicillin in UTI-inducing *E. coli* isolates from Swiss children increased from 33.0% during 1980–1991 to 51.7% during 2001-2003. Similarly, the resistance to trimethoprim plus sulfmethoxazole increased from 15.8% during 1980–1991 to 25.2% for the period 2001–2003 (Fritzsche et al., 2005). In the Czech Republic, the resistance of uropathogenic *E. coli* to ciprofloxacin (a quinolone) increased from 1.6% (21/1320) in 1997 to 9.5% (157/1652) in 2002 (Urbánek et al., 2005). The data from isolates from patients show that UTI-inducing *E. coli* have a high degree of resistance to the β-lactams such as ampicillin and amoxicillin, with a mean resistance of 51.5%. However, resistance was reduced to 12.4% if clavulanic acid, a β-lactamase inhibitor, was combined with amoxicillin to treat UTI (Table 4). *E. coli* strains inducing UTIs were less resistant to trimethoprim plus sulfamethoxazoles than to ampicillin or amoxicillin, but resistance was still substantial. The mean resistance to the quinolones and to first- and second-generation cephalosporins was 15.4%, 16.5%, and 5.0%, respectively. The resistance to third-generation cephalosporins was considerably less (mean resistance 2.6%) than to first- and second-generation cephalosporins. Low resistance to nitrofurantoin was demonstrated in UTI-inducing *E. coli* strains, with mean resistance of 3.9%. These data, while limited, indicate that resistance to ampicillin/amoxicillin (except in the presence of a β-lactamase), trimethoprim plus sulfmethoxazole, first-generation cephalosporins, and to various quinolones was more than 15% and will probably increase if current trends continue. Resistance to second- and third-generation cephalosporins and nitrofurantoin was low, but resistance to these antimicrobials will probably escalate with increased use.

Katouli et al. (2005) indicated that 55/85 (64.7%) of *E. coli* strains causing acute cystitis in young adults in Iran were resistant to >2 antibiotics and 11/85 (12.9%) strains were resistant to 10 antibiotics commonly used to treat UTIs. In a study of 1858 fluoroquinolone-resistant *E. coli* strains isolated from outpatients with UTIs, Karlowsky et al. (2006) found that 27.3%, 54.1%, 7.4%, and 0.4% of the strains were also resistant to 1, 2, 3, or 4 other oral antimicrobials, respectively.

TABLE 4. ANTIMICROBIAL RESISTANCE OF *E. COLI* STRAINS ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS

Reference	*Ampicillin or amoxicillin*[a]	*Trimethoprim + sulfamethoxazole*[b]	*Amoxicillin + clavulanic acid*[c]	*Quinolones*	*First-generation cephalosporin*[a]	*Second-generation cephalosporin*	*Third generation cephalosporin*	*Nitrofurantoin*[d]
Fritzsche et al., 2005, data from Switzerland 2001–2003 (N = 151)	78 (51.7%)[e]	38 (25.2%)	17 (11.3%)	—	Cephalothin 25 (16.6%)	Cefuroxime 2 (1.3%)	Cefriaxone 0 (0.0%)	—
Lorente Garin et al., 2005, data from Spain 2001	785/1315 (59.7%)	381/1315 (29.0%)	129/1315 (9.8%)	Ciprofloxacin 418/1314 (24.3%) Norfloxacin 416/1311 (31.7%)	—	Cefuroxime 52/1289 (4.0%)	—	52/1289 (4.0%)
Andreu et al., 2005, data from Spain 2002 (N = 1989)	1168 (58.7%)	674 (33.9%)	183 (9.2%)	Ciprofloxacin 453 (22.8%)	—	Cefuroxime 185 (9.3%)	Cefixime 89 (4.5%)	113 (5.7%)
Kurutepe et al., 2005, data from Turkey 2003 (N = 159)	78 (49.1%)	66 (41.5%)	44 (27.7%)	Ciprofloxacin 39 (24.5%)	Cefazolin 37 (23.3%)	Cefuroxime 12 (7.5%)	—	14 (8.8%)
Basišić et al., 2005, data from Croatia 2004	123/296 (41.6%)	34/156 (21.8%)	—	Ciprofloxacin 4/52 (7.7%)	—	—	—	—
Ladhani and Gransden, 2003, data from United Kingdom 1996–2000 (N = 1774)	907 (51.1%)	—	64 (3.6%)	Ciprofloxacin 106 (0.6%)	—	Cefuroxime 160 (0.9%)	—	104 (5.9%)
Junquera et al., 2005, data from Spain 2001 (N = 1666)	1003 (60.2%)	563 (33.8%)	52 (3.1%)	Nalidixic acid 561 (33.7%) Norfloxacin 556 (33.4%)	Cefazolin 68 (4.1%)	Cefuroxime 55 (3.3%)	Cefotaxime 25 (1.5%)	42 (2.5%)
Hummers-Pradier et al., 2005, data from Germany 2000–2001 (N = 191)	74 (38.7%)	53 (28.8%)	63 (33.0%)	Ciprofloxacin 17 (8.9%)	Cefazolin 64 (33.5%)	—	Cefixime 5 (2.6%)	4 (2.1%)
Urbánek et al., 2005, data from Czech Republic 2002	—	—	—	Ciprofloxacin 157 (9.5%) Ofloxacin 157 (9.5%)	—	—	—	—

(*continued*)

TABLE 4. ANTIMICROBIAL RESISTANCE OF *E. COLI* STRAINS ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS (*CONTINUED*)

Reference	*Ampicillin or amoxicillin*[a]	*Trimethoprim + sulfa-methoxazole*[b]	*Amoxicillin + clavulanic acid*[c]	*Quinolones*	*First-generation cephalosporin*[a]	*Second-generation cephalosporin*	*Third generation cephalosporin*	*Nitro-furantoin*[d]
(*N* = 1652)								
Fadda et al., 2005, data from Italy 2004 (*N* = 512)	192 (37.5%)	153 (29.9%)	64 (12.5%)	Ciprofloxacin 84 (16.4%) Levofloxacin 87 (17.0%) Prulifloxacin 77 (15.0%)	—	Cefuroxime 55 (10.7%)	—	17 (3.3%)
Drews et al., 2005, data from Canada 2003–2004 (*N* = 767)	—	135 (17.6%)	46 (6.0%)	Ciprofloxacin 91 (11.9%) Nalidixic acid 124 (16.2%)	Cefazolin 40 (5.2%)	Cefprozil 50 (6.5%)	Ceftriaxone 5 (0.7%)	15 (2.0%)
Zhanel et al., 2005, data from Canada and United States 2003–2004 (*N* = 1142)	431 (37.7%)	243 (21.3%)	—	Ciprofloxacin 63 (5.5$) Levofloxacin 58 (5.1%)	—	—	—	13 (1.1%)
Karaca et al., 2005, data from Turkey 2003)	—	633/1644 (38.5%)	—	Ciprofloxacin 317/1277 (24.8%) Ofloxacin 201/1565 (12.8%)	—	—	—	—
Chulain et al., 2005, data from Ireland 2002–2003 (*N* = 723)	377 (52.1%)	—	57 (7.9%)	Naladixic acid 43 (5.9%) Ciprofloxacin 18 (2.5%)	—	Cefoxitin 10 (1.4%)	Cefpodoxime 15 (2.1%)	23 (3.2%)
Karlowsky et al., 2006, data from United States and Canada 2004–2005 (*N* = 1858)	1483 (79.8%)	1236 (66.5%)	—	—	—	—	Cefdinir 167 (9.0%)(4.0%)	74
Mean percent resistance	51.5%	32.3%	12.4%	15.4%	16.5%	5.0%	2.6%	3.9%

[a]Ampicillin, amoxicillin, and the cephalosporins are β-lactams.
[b]Trimethoprim is a pyrimethamine and sulfamethoxazole is a solfonamide.
[c]Clavulanic acid is a β-lactamase inhibitor.
[d]Nitroflurantoin is a nitrofurane.
[e]Number of resistant isolates/total number of isolates (percent resistant isolates).

The phylogenetic grouping of UTI-inducing *E. coli* isolates from a number of studies is presented in Table 5. The majority of strains belonged to phylogenetic group B_2. Johnson et al. (2005c) found that 59/65 (90.8%) of *E. coli* urine isolates from men with febrile UTI belonged to group B_2, whereas only 23/67 (34.3%) of rectal isolates from the same men belonged to the B_2 group. In addition, virulence factors were more prevalent in the urine isolates compared to the rectal *E. coli* isolates.

Genes potentially associated with virulence of UTI-inducing *E. coli* include aer (aerobactin), *kpsMT* (KII capsule), *capIII* (KIII capsule), *cnf1*, *drb* (Dr-binding adhesins), *hly*, *ompT*, *papGI* (P-pili fimbriae class I), *papGII* (P-pili fimbriae class II), *papGIII*, *sfa*, and *fim* (Marrs et al., 2002). Marrs et al. (2005) provide an extensive table listing the virulence factors that may be involved in induction of UTIs by *E. coli*. Almost all cystitis-inducing strains are *fim* type 1 piliated (Abraham et al., 2001), whereas PapGII *E. coli* are commonly associated with the development of pyelonephritis (Larsson et al., 2003).

Many UTI virulence gene clusters are located on PAIs (Table 6). The core element of the *Yersinia* species high pathogenicity island (HPI) is present on PAI IV_{536} (Dobrindt et al., 2002). The HPIs of virulent yersiniae encode the biosynthesis of the siderophore yersiniabactin (Schubert et al., 1999). In *E. coli*, *Yersinia* HPIs are found mainly in phylogenetic groups B_2 and D (Schubert et al., 2002). There is evidence that quinolone resistance in UTI-causing *E. coli* leads to the loss of virulence (Soto et al., 2006; Vila et al., 2002). Horcajada et al. (2005) reported that nalidixic-acid–resistant UTI-inducing *E. coli* from group B_2 showed a decreased presence of certain virulence factors, including *sfa/foc* (S and F1C fimbriae), *hlyD*, and *cnf1*. Since *hly*, *cnf*, and *sfa* are located on PAIs, deletion and transposition of DNA regions during the development of nalidixic acid resistance may have led to a loss of PAIs (Horcajada et al., 2005). When ExPEC strains isolated from UTIs were incubated with subinhibitory concentrations of ciprofloxacin, there was a total or partial loss of the PAIs containing *hly* and *cnf1* (Soto et al., 2006). Kuntaman et al. (2005) noted a high prevalence of fluoroquinolone-resistant *E. coli* among Indonesian patients who had been hospitalized >5 days; the fluoroquinolone-resistant ExPEC isolated from the fecal swabs of these patients showed a decreased presence of virulence factors compared to fluoroquinolone-sensitive ExPEC. Drews et al. (2005) noted a decreased prevalence of virulence factors among uropathogenic *E. coli* that were resistant to ciprofloxacin or nalidixic acid and they demonstrated a decreased incidence of β-hemolytic activity.

There is some indication that *E. coli* strains isolated from patients with different types of UTI vary in virulence. Blanco et al. (1997) found that *pap* or *sfa* operons were more prevalent in uropathogenic *E. coli* strains isolated from pa-

TABLE 5. PHYLOGENETIC GROUPING OF URINARY TRACT INFECTION (UTI)-INDUCING EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM PATIENTS

	Number of patients (%)				
Phylogenetic group	*Bonacorsi et al., 2005* (n = 79)[a]	*Bidet et al., 2005* (n = 75)[b]	*Bingen-Bidois et al., 2002* (n = 100)[c]	*Johnson et al., 2005c* (n = 65)[d]	*Zhang et al., 2002* (n = 93)[e]
A	7 (8.9%)	3 (4.0%)	11 (11.0%)	2 (3.1%)	7 (7.5%)
B_1	1 (1.3%)	1 (1.3%)	1 (1.0%)	3 (4.6%)	3 (3.2%)
B_2	55 (69.6%)	51 (68.0%)	61 (61.0%)	59 (90.8%)	64 (68.8%)
D	16 (20.3%)	20 (26.7%)	27 (27.0%)	1 (1.4%)	19 (20.4%)

[a]French infants <90 days old (mean age 42 days; range, 6–88 days) with UTI; 66 of the infants were uncircumcised males.
[b]French children aged 3 to 120 months with UTI.
[c]French adults (median age, 66 years; range, 19–99 years) with urosepsis. Eighty-one patients were women.
[d]Swedish men with febrile UTI.
[e]United States (Michigan) women aged 18 to 39 years with UTI.

Table 6. Pathogenicity Islands Associated with Uropathogenic *E. coli*

Pathogenicity island	*Virulence factors present*
PAI I_{536}[a]	α-hemolyxin
PAI II_{536}	aP-related fimbriae α-hemolysin
PAI II_{536}[a]	S fimbrial adhesin (*sfa*) Siderophore system (*iro*)
PAI IV_{536}[a]	Yersiniabactin siderophore (HPI)
PAI II_{J96}[b]	Hemolysin (*hly*) Cytotoxic necrotizing factor 1 (*cnf1*) Heat-resistant agglutinin (*hra*)
PAI I_{CFT073}[c]	Hemolysin (*hlyA*) Class III PapG adhesion (*papGIII*)
PAI II_{CFT073}[c]	PapGII adhesin (*papGII*)

[a]Dobrindt et al., 2002.
[b]Bidet et al., 2005; Bingen-Bidois et al., 2002.
[c]Guyer et al., 1998; Rasko et al., 2001.

tients with acute pyelonephritis (44/47; 93.6%); however, *pap* or *sfa* operons were less frequently present in *E. coli* isolated from patients with asymptomatic bacteriuria (24/42; 57.1%), cystitis (78/116; 67.2%), or possible pyelonephritis (26/38; 68.4%). Only 39/149 (26.2%) of *E. coli* isolated from feces of healthy individuals contained the *pap* or *sfa* operons (Blanco et al., 1997).

Virulence genes were more common in pyelonephritis-inducing strains of *E. coli* isolated from children <2 years of age compared to strains isolated from women 18 to 39 years of age with cystitis (Marrs et al., 2002). The genes *aer*, *kpsMT*, *ompT*, *fim*, and *papGAD* II were present in 45/153 (29.4%) of the pyelonephritis-inducing *E. coli* strains, compared to only 33/393 (8.4%) of cystitis-inducing strains (Marrs et al., 2002). In comparing ExPEC strains isolated from women with uncomplicated pyelonephritis (urine isolates) to women with uncomplicated cystitis (urine isolates) or with non-UTI symptoms (fecal isolates), Johnson et al. (2005d) found that pyelonephritis isolates demonstrated a higher prevalence of group B_2 virulence-associated O antigens and virulence factors compared to cystitis and fecal isolates. Guyer et al. (1998) studied strains of UTI-inducing *E. coli* that hybridized with 11 DNA probes targeting the length of PAI $_{ICFT073}$. They found that 78.9% of 67 strains of *E. coli* caused pyelonephritis, 82.3% of 38 strains induced cystitis, 57.6% of 49 strains caused catheter-associated bacteriuria, and 21.5% of 27 fecal samples isolated from healthy women reacted with the probes. The PAI sequences were found significantly more often in clinical strains causing UTIs than in strains causing catheter-associated bacteriuria or in fecal strains (Guyer et al., 1998).

The prevalence of *hly*, *papGIII*, and *cnf1* genes in *E. coli* causing prostatitis in men was significantly higher than in strains causing pyelonephritis or cystitis in women (Ruiz et al., 2002). PAI $_{IICFT073}$ loci were more prevalent in prostatitis-inducing strains of *E. coli* than in cystitis- or pyelonephritis-inducing strains (Parham et al., 2005). The data obtained by Ruiz et al. (2002) and Parham et al. (2005) suggest that clinical isolates of prostatitis-inducing *E. coli* are more virulent than those isolates from cystitis or pyelonephritis cases.

The majority of UTI cases are associated with only a few *E. coli* O-serogroups, including O1, O2, O6, O18, and O75 (Schaeffer, 1983). Johnson et al. (2001e) demonstrated the commonality of virulence genes present in *E. coli* O18:K1:H7 strains isolated from 15 urine samples from women with acute cystitis and 4 cerebrospinal fluid samples isolated from patients with neonatal bacterial meningitis. While the sample size was small, the commonality of virulence factors between UTI and neonatal meningitis-inducing *E. coli* strains suggests that babies of mothers with UTI could be infected during or soon after birth by UTI strains that could induce neonatal meningitis.

Pathogenic events leading to UTIs include bacterial adherence, colonization, avoidance of host defenses, and damage to host tissues. Type 1 fimbriae are one of the most studied elements of uropathogens and have been clearly shown to function as a virulence factor during UTI (Connell et al., 1996; Keith et al., 1986). This adhesin appears to plays a critical role in the initial stages of an infection (Gunther et al., 2002). In contrast, the virulence potential of another adhesin, P-fimbriae, has not been as clearly defined. There are mutant studies both supporting and arguing against inclusion of P-fimbriae in a list of uropathogen virulence factors (Mob-

ley et al., 1993; Roberts et al., 1994; Wullt et al., 2000). However, P-fimbriae have a long history of association with UTI and are, therefore, included in this listing of putative virulence factors. A more recently described adhesin in *E. coli* is the iron-regulated gene homologue adhesin, encoded by the *iha* gene. The exact role that this adhesin plays in UTIs is not understood; however, its deletion from a uropathogenic strain led to the mutant being outcompeted by the parent strain in a murine model of ascending UTI (Johnson et al., 2005e).

The initial step of adherence is followed by colonization of host tissue by ExPEC. To survive and grow within the host, bacteria need to scavenge iron from the surrounding tissue. For this task, bacteria employ siderophores to collect and deliver the iron the pathogen requires. Several genes associated with siderophore activity have been implicated as virulence factors. The *iroN* gene encodes the receptor of the siderophore enterobactin. The presence of this gene contributes to the uropathogen's ability to colonize the mouse bladder, kidneys, and urine during experimental infections (Russo et al., 2002). In a similar manner, the siderophore aerobactin and, more specifically, the gene *iucD* play a role in the colonization of the urinary tract by ExPEC (Torres et al., 2001). Another gene known to contribute to bacterial iron acquisition and virulence is *tonB* (Torres et al., 2001). This gene encodes a cytoplasmic protein that is believed to contribute energy to membrane receptors responsible for bringing iron into the bacteria.

Several of the genes classified as virulence factors in uropathogens play roles in regulatory networks affecting the transcription and expression of many other genes. For example, the *phoU* gene is a negative regulator of the pho regulon, which is known to affect phosphate transport. A mutant strain lacking a functional *phoU* gene was outcompeted by its parent strain in competitive colonization experiments in the mouse UTI model (Buckles et al., 2006). Likewise, mutants of the transcription regulatory genes *degS* and *rfaH* are attenuated for virulence in the urinary tract (Nagy et al., 2002; Redford et al., 2003). The *degS* gene, which encodes a protease, controls the transcription of other genes by acting on the alternative sigma factor σ^E. The product of the *rfaH* gene affects the expression of a diverse group of bacterial components such as lipopolysaccharide, capsule, and hemolysin production, and iron acquisition. Most of these components likely play a role in the pathogenesis in the urinary tract.

The ability to move within the host environment is integral to successful colonization. It is not surprising to find that the *fliC* gene, which encodes flagellin, the structural subunit of flagella, contributes positively to infection of the urinary tract by *E. coli* (Lane et al., 2005; Wright et al., 2005). During colonization of host tissue, a successful pathogen must also be able to withstand or avoid the defensive responses of the host. One area of investigation into the ability of *E. coli* to avoid host defenses has centered on the ability of the uropathogen to be internalized by and propagate within host cells. A gene has been identified, *surA*, which is important for the bacteria's ability to fully invade and propagate in large numbers in host cells (Justice et al., 2006). Without the *surA* gene, the mutant was unable to persist within the urinary tract compared to the wild-type strain.

Another host defense mechanism that uropathogens must overcome is oxidant stress. The *oxyR* gene helps to protect the bacteria against the detrimental effects of oxidative stress. A mutant lacking a functional copy of the *oxyR* gene was outcompeted by its parent strain within the mouse model, implicating this gene as a virulence factor (Johnson et al., 2006). In addition to oxygen stresses, uropathogens are also challenged by a range of different osmotic conditions. The bacteria employ osmoprotectants for stabilization in the face of rapid osmotic changes. The *proP* gene expresses an osmoregulatory transporter. The function of this gene is crucial to *E. coli* during infection of the bladder, since attenuated mutants were recovered in significantly fewer numbers from the murine model of UTI compared to the parent strain (Culham et al., 1998).

Finally, pathogenic organisms secrete toxins to damage the host environment in an attempt to make it more hospitable to their survival. Three toxins present in uropathogenic strains and the genes that encode them, *cnf1*, *hly*, and *sat*, have been identified as virulence factors (Guyer et al., 2002; Nagy et al., 2006;O'Hanley

et al., 1991; Rippere-Lampe et al., 2001). The *cnf1* gene encodes a cytotoxic necrotizing factor that increases the bacteria's resistance to killing by neutrophils. The *hly* genes code for a hemolysin that forms pores in host cells, leading to their destruction, while the *sat* gene encodes for a cytotoxin that forms vacuoles in host cells, most prominently in human kidney cell lines.

Sepsis

Bacteremia is the presence of microorganisms in the circulatory system. If the microorganisms begin to multiply, the bacteremia progresses to septicemia. Sepsis (also known as SIRS, the systemic inflammatory response syndrome) is a grave medical condition induced by an overwhelming infection of the bloodstream. There is widespread activation of inflammation and coagulation pathways with dysfunction of the circulatory system leading to failure of various organs; the mortality rate is high (Andreoli et al., 1997; Annane et al., 2005). In cases of severe sepsis, there is organ dysfunction, decreased perfusion, and hypotension. Septic shock is a subset of severe sepsis and is characterized by the failure of hypotension to respond to fluid resuscitation. Sepsis can be caused by a microbial infection that originates from the kidneys (UTI), bowel (peritonitis), skin (cellulitis), or lungs (pneumonia), as well as other bodily sites. Neonates, the elderly, and immunocompromised individuals are particularly at risk for bacterial sepsis (Martin et al., 2003; Stoll et al., 2005).

Sepsis during the first week of life is known as early onset neonatal sepsis. The early onset syndrome may be due to infection of the fetus from ascending spread of the microorganisms from the lower genital tract of the mother or transplacentally through maternal bacteremia (Schrag and Schuchat, 2005). Late onset neonatal sepsis can be due to bacterial infection during passage through the birth canal or from the hospital or home environment (Schrag and Schuchat, 2005). Worldwide, 4 to 5 million infants die during the first 4 weeks of life; 98% of these deaths occur in less developed countries (Vergnano et al., 2005; Zupan, 2005). According to Vergnano et al. (2005), deaths from neonatal sepsis and meningitis account for most of the neonatal deaths in developing countries.

Early onset neonatal sepsis is a serious problem in neonates, especially in very low birth weight preterm infants, and places infants at risk for death or chronic sequelae including hearing loss, seizure, and neurodevelopmental defects (Jones et al., 2004; Moore et al., 2003). Hyde et al. (2002), using data from selected counties in California and Georgia for 1998–2000, identified 408 cases of early onset sepsis. Group B *Streptococcus* (GBS) accounted for most of the cases (40.7%) and *E. coli* accounted for 70 cases (17.2%). They noted an increase in resistance of *E. coli* to ampicillin during the reporting period; mortality was higher with ampicillin-resistant strains than with ampicillin-sensitive strains of *E. coli*. Among 28,659 deliveries in a Florida teaching hospital during the period 1998–2002, 102 cases of early onset neonatal sepsis were identified: *E. coli* was the cause in 41 cases (40.2%) (Mayor-Lynn et al., 2005). Neonates with *E. coli*-induced sepsis had a lower birth weight, required an approximately 4-fold longer stay in intensive care, often required mechanical ventilation, and had an almost 3 times higher mortality compared to those neonates with septicemia caused by GBS (Mayor-Lynn et al., 2005).

A massive effort has been underway to reduce GBS infection in neonates by the use of intrapartum antimicrobial prophylaxis. Moore et al. (2003) reported that GBS early onset neonatal sepsis declined substantially in developed countries with the introduction of intrapartum antibiotic therapy. In the United States, the rate (per 1000 live births) for GBS-induced sepsis was 5.9 for 1991–1993, 1.7 for 1998–2000, and 1.8 for 2000–2003 (Stoll et al., 2005). The ratefor *E. coli*-induced sepsis was 3.2 for 1991–1993, 6.8 for 1998–2000, and 7.0 for 2002–2003 (Stoll et al., 2005). The overall rate of early onset neonatal sepsis for very low weight preterm infants has remained stable from 1991–2003. However, early onset sepsis due to GBS decreased by approximately two-thirds, whereas *E. coli*-induced sepsis doubled between 1991 and 2003 (Stoll et al., 2005). It is not clear why the incidence of neonatal sepsis due to *E. coli* has increased (Stoll et al., 2005).

The annual number of adult sepsis cases in the United States increased from 164,072 in 1979 to 659,935 in 2000 (Martin et al., 2003). The mean age of the patients increased from 54.7 years for 1979–1984 to 60.8 years for the period 1995–2000. Approximately 48% of the patients were men. In the United States, the mortality rate for septicemia in elderly populations for 1986–1997 was 22.6/100,000 for individuals aged 65–74 years, 60.0/100,000 for ages 75–84, and 177.6/100,000 for patients >85 years old (McBean and Rajamani, 2001). The population shift to a larger number of elderly people indicates that there will be a steady increase in the morbidity and mortality due to sepsis.

Individuals at the extremes of age are the most susceptible to bacterial-induced, community acquired septicemia; *E. coli* was found to be the most frequent cause of septicemia in infants <1 year of age and in the elderly >65 years of age (Diekema et al., 2002). For the first six months of 1998, 4579 cases of bacterial septicemia were identified in hospitals in Canada, Latin America, and the United States. The most common cause for septicemia was coagulase-positive *Staphylococcus aureus* (22.5%), followed by *E. coli* (18.9%) (Diekema et al., 2000). Gram-negative bacteria were responsible for 4267 cases of nosocomial and community acquired septicemia in selected centers in Canada, Latin America, and the United States in 1997; *E. coli* was responsible for 41.0% of those cases (Diekema et al., 1999). In 1997–1998, *E. coli* was isolated in 20.9% of nosocomial cases of septicemia in European hospitals and was the most frequently isolated bacterium in septicemia cases (Fluit et al., 2001).

The most commonly reported pathogen leading to hospitalization for septicemia in patients >65 years of age in the United States was *E. coli*; the most frequent comorbidity was diabetes (McBean and Rajamani, 2001). Jackson et al. (2005) conducted a population-based cohort study of 46,238 noninstitutionalized elderly (>65 years) members of a health cooperative in Washington state to determine the incidence of *E. coli* bacteremia. In a three-year time period, 1998–2001, 184 cases of community-associated bacteremia were due to an infection by *E. coli*. The overall rate of *E. coli*-induced bacteremia (per 100,000 person-years) was 150 cases; the range (per 100,000 person-years) was 97 for people aged 65–69 to 452 for those aged >85 (Jackson et al., 2005). Urinary incontinence was a major factor contributing to bacteremia in the elderly, suggesting a urinary source for *E. coli* bacteremia (Jackson et al., 2005). Similar to the findings of McBean and Rajamani (2001), Jackson et al. (2005) found that diabetes was commonly associated with *E. coli* bacteremia.

Johnson et al. (2001d) determined the phylogenetic grouping of 181 *E. coli* isolates from bacteremic patients. Most of the strains (65.7%) belonged to phylogenetic group B_2, while 11.6%, 10.5%, and 12.2% belonged to groups A, B_1, and D, respectively. More than half (97/181, 53.6%) of the bacteremic-inducing *E. coli* strains were of urinary or pulmonary tract origin; 78/97 (80.4%) of those strains belonged to group B_2 (Johnson et al., 2002b). Sannes et al. (2004), studying 63 *E. coli* isolates from bacteremic veterans with a mean age of 71.6 years, found that 11.1% belonged to group A, 3.2% to group B_1, 66.7% to group B_2, and 19.0% to group D. Virulence factors were concentrated in the B_2 and D groups. The virulence gene ompT in the *E. coli* isolates was strongly predictive for bacteremia (Sannes et al., 2004). The data obtained by Johnson et al. (2001d, 2002b) and Sannes et al. (2004) indicate that bacteremia-inducing *E. coli* strains are typical ExPEC pathogens.

By using the suppression subtractive hybridization technique, Mokady et al. (2005) subtracted the genome of *E. coli* K12 from septicemia-inducing strains of *E. coli* serogroups O2 and O78 to determine potential virulence factors that may be involved in septicemia. Putative virulence factors for the serotype O2 and O78 strains include iron uptake systems (aerobactin, yersiniabactin, and IroN receptor), serum resistance, and adhesins (type 1 pili, curli, and P pili). Non-fimbrial adhesins were present only in serotype O78, and the K-1 capsule was present only in O2 (Mokady et al., 2005). A new type III secretion system has been reported to be involved in the virulence of septicemic ExPEC (Ideses et al., 2005). The new type III secretion system was designated *E. coli* type III secretion system 2 (ETT2) to differentiate it from the locus of enterocyte effacement–encoded type III system (ETT1). Using a septicemic O78 *E. coli* strain, Ideses et al. (2005)

demonstrated the presence of a degenerate (nonsecretion competent) ETT2 gene cluster; nonetheless, the degenerate cluster contributed toward virulence in a chick model for septicemia.

Russo and Johnson (2003) estimated that *E. coli* is the cause of 17% of the cases of severe sepsis (dysfunction of at least one organ system) in the United States. They estimated there were 127,500 cases of *E. coli*-induced severe sepsis, with 40,000 deaths, in 2001; the mortality rate was approximately 30%. The annual health care costs were estimated at $1.1 to $2.8 billion dollars (Russo and Johnson, 2003). However, Weycker et al. (2003) estimated that the total charges for severe sepsis patients (18 to 85 years of age) admitted to hospitals were about $45,835/patient (in 2001 dollars).

Pneumonia and surgical site infections

The risk of developing pneumonia increases with age, and it is the most common cause of death in nursing homes. Hospitalization rates for pneumonia in those aged >65 increased approximately 29% between 1988–1990 and 2000–2002. For the period 2000–2002, the hospitalization rate (per 1000 population) for pneumonia in individuals aged >85 was 51, compared to 26 for those aged 75–84, and 12 for individuals aged 65–74 (Fry et al., 2005). Gram-negative bacteria (excluding *Haemophilus pneumoniae*) are the most frequent cause of pneumonia in long-term care facilities (Muder, 1998). *E. coli* was the cause of 356/8,891 (4%) of nosocomial cases of pneumonia in United States hospitals during 1990–1992 (Emori and Gaynes, 1993) and was the cause of 147/1,854 (7.9%) of nosocomial pneumonia cases in European hospitals in 1997–1998 (Fluit et al., 2001).

Russo et al. (2000, 2005) used a rat pulmonary infection model to study ExPEC-induced pneumonia. The ExPEC strain used was CP9 (O4/K54/H5), possessing a group 3 capsule (K54), O4-specific LPS antigen, a-hemolysin (Hly), CNF, class I and III PapG adhesins, and type 1 pilus. The strain was also complement-resistant. Using isogenic mutants of strain CP9 lacking the K54 antigen, O4 antigen, and Hly, the researchers found that the presence of the O antigen decreased pulmonary neutrophil influx, whereas the K54 antigen increased it. These results suggest that K54 capsular polysaccharide is proinflammatory and stimulates the host defense. The O4 antigen attenuates neutrophil influx and downregulates host defense mechanisms. In addition, the O mutant was cleared from the lung more rapidly than the wild-type strain. *hly* contributed to ExPEC virulence by increasing neutrophil death (Russo et al., 2000, 2005). Lung injury in the rat was significantly greater with the Hly-positive wild-type strain as compared to the Hly-minus mutant (Russo and Johnson, 2000, 2005). Therefore, the rat pneumonia model indicates that the O antigen and *hly* are important virulence factors for ExPEC strains that induce pneumonia.

For the period 1990–2002, postoperative infections increased approximately 80% in people aged 65 and older (Larkin, 2006). ExPEC strains account for 8% of the surgical site infections, resulting in 24,000–64,000 cases annually, with an estimated cost of $94–252 million (Russo and Johnson, 2003).

FUTURE DIRECTIONS IN CONTROL AND TREATMENT OF ExPEC-INDUCED DISEASES

The ubiquity of ExPEC and other *E. coli* strains in physical and biological environments precludes their eradication from those environments. However, a heightened state of hygiene, including thorough handwashing, cleanliness in food handling, thorough cooking of foods, and proper disposal of human and animal waste can greatly contribute to limiting human exposure to ExPEC. The prevention of ExPEC infections is of pressing concern from both the public health and economic perspectives. Clinicians are increasingly aware that antimicrobial resistance is on the rise in ExPEC strains. UTI-inducing ExPEC strains are resistant to commonly used antimicrobials, and it is probable that ExPEC strains that induce other extraintestinal diseases have increased resistance to antimicrobials. Therefore, there is a need for new antimicrobial agents to combat ExPEC-induced syndromes.

The need to suppress ExPEC-induced diseases is particularly important because of the increase in the number of immunocompromised individuals. Approximately 20% of the population of the United States suffers from some degree of immune compromise, making them more susceptible to infectious diseases. These include those >65 years of age, pregnant women, cancer patients, organ transplant patients, residents in nursing and other care facilities, and HIV-positive patients. In particular, the elderly are very susceptible to ExPEC-induced diseases, including UTIs, pneumonia, and septicemia (Angus et al., 2001; Jackson et al., 2005; McBean and Rajamani, 2001; Muder, 1998; Tal et al., 2005). The elderly made up 18.4% of the population in 2003 and are expected to make up 25.2% of the population by 2020. As the elderly population increases, there is the likelihood that ExPEC-induced infections will increase, as well. The increase in antimicrobial resistance of ExPEC combined with increasing numbers of ExPEC-induced infections will make it more difficult and costly to manage these infections in the near future (Russo et al., 2003).

The approach to UTI treatment and prevention remains dependent on antimicrobial therapy. However, due to the increase in antimicrobial resistance seen with UTI-inducing *E. coli* strains, there have been attempts to find alternative methods for treatment or prevention of UTIs in women. A number of clinical trials indicate that cranberry juice can prevent UTIs (Raz et al., 2004; Stapleton, 2003). Studies indicate that cranberry juice does not exert its protective effect by the acidification of urine, but through compounds in the juice that inhibit the adhesion of *E. coli* to uroepithelial cells. While the clinical trials do indicate some effectiveness in preventing UTIs by the ingestion of cranberry juice, the evidence is not completely clear due to the small number of trials, short trial periods, small numbers of participants (and high drop out rates), trials that were not randomized or blinded, and the failure to report the concentrations of cranberry juice used (Raz et al., 2004; Stapleton, 2003). Obviously, a high degree of standardization needs to be introduced before clinicians can be completely certain that ingestion of cranberry juice can prevent UTIs. Studies on the treatment of UTIs with cranberry juice during an infection have not been reported.

The predominant microbial flora of the normal human vagina consist of lactobacilli, which exert a protective effect against bacterial infections (Antonio et al., 1999; Stapleton, 2003). There is evidence that the instillation of probiotics (selected lactobacilli) into the vagina (Reid, 2001; Reid et al., 1995) or the ingestion of an oral probiotic containing live lactic acid bacteria (Reid et al., 2001) may reduce the incidence of recurrent UTIs in women. However, little work has been done to understand the role of probiotics in the control of UTIs. In order to be effective, the *Lactobacillus* strains selected as probiotics for UTI infection prevention should have the ability to colonize the uroepithelium, inhibit pathogen binding and growth, produce hydrogen peroxide, and resist killing by spermicides (Reid and Bruce, 2001).

Uehling et al. (2001) inserted vaginal suppositories containing a killed suspension of 10 uropathogenic bacterial strains, including 6 strains of *E. coli*, to induce mucosal immunity in women who had recurrent UTIs. The reinfection interval for women treated with the killed bacterial suspension was significantly delayed in comparison with control patients who received a placebo.

A few studies have been conducted to determine the feasibility of using pili as vaccines to prevent UTI. Adhesive pili mediate the colonization of the uroepithelium by uropathogenic *E. coli*. Type 1 piliated *E. coli* have been associated with cystitis (Mulvey et al., 2001) and bind to the luminal surface of both human and mouse bladder epithelium *in vivo* (Langermann et al., 1997). Langermann et al. (1997) prepared antibodies against FimH (the tip of the pilus that binds to host cell mannosides) by injecting mice with a complex of FimC (the chaperone that is involved in assembling the pilus) and FimH or with a mannose-binding FimH truncate. Both antigens elicited a strong response against FimH; however, whole type 1 pili elicited a poor anti-FimH response. Antibodies to FimH inhibited the binding of type 1 pili-positive *E. coli* to J82 cells (human bladder epithelial cells) (Langermann et al., 1997). C3H mice immunized with the FimH antigens

demonstrated a 100- to 1000-fold decrease in the number of bacteria recovered from the bladder when challenged with uropathogenic *E. coli* as compared to nonimmunized control animals (Langermann and Ballou, 2001; Langermann et al., 1997).

There is a similarity in bladder, renal, and ureteral physiology in monkeys and humans, and both are subject to cystitis, pyelonephritis, and ureteral reflux leading to ascending UTI (Langermann and Ballou, 2001). Cynomolgus monkeys, vaccinated with the FimC-FimH complex, developed long-lasting serum IgG antibodies to FimH. The antibodies prevented cystitis and inflammation in ~75% of the monkeys challenged with type 1–piliated *E. coli*; cystitis and bladder inflammation developed in unvaccinated control monkeys (Langermann and Ballou, 2001; Langermann et al., 1997). The IgG antibodies induced by FimH did not have an appreciable effect on *E. coli* populations of the gut.

While mice and monkeys are protected against cystitis by FimH vaccination, the efficacy of FimH vaccines in preventing cystitis in humans has not been demonstrated. Langermann and Ballou (2003) describe potential procedures that could be used for developing and testing of a FimC-FimH vaccine in human UTI patients.

P-fimbriated *E. coli* strains associated with pyelonephritis bind to epithelial cells via Gala1-4Galβ oligosaccharide sequences (globoseries) of kidney cell surface glycosphingolipids (GSLs). Svensson et al. (2001) suggested carbohydrate receptor depletion as a means of preventing UTIs. Preincubation of A498 (kidney tubular epithelial) cells with the GSL synthesis inhibitor, N-butyldeoxuynojirimycin (NB-DNJ), led to a reduction in GSL expression. Challenge of the NB-DNJ–treated cells with P-fimbriated *E. coli* resulted in a decrease in attachment of bacteria to the kidney cell line (Svensson et al., 2001). Mice fed NB-DNJ demonstrated a striking decrease in kidney GSL expression, and reduced numbers of challenge *E. coli* were isolated from the kidneys and bladders of the treated mice compared to control mice. In addition, mice fed NB-DNJ and challenged with P-fimbriated *E. coli* showed a reduction in the number of urine neutrophils and a decrease in bacterial-induced mucosal inflammation. NB-DNJ was well tolerated by the mice, with little adverse effects (Svensson et al., 2001). NB-DNJ has been used to treat type I Gaucher disease in humans, a genetic disorder of lipid metabolism resulting in accumulation of abnormal GSLs in mononuclear phagocytes, and it may prove to be useful in preventing UTIs in humans (Cox et al., 2003).

Subcutaneous immunization of mice with the purified denatured siderophore receptor IroN led to production of IgG antibodies against IroN protein; however, IgA was not produced (Russo et al., 2003). IroN immunization protected mice challenged by UTI-inducing *E. coli* against renal infection (pyelonephritis) but not against bladder infection (cystitis). While animal models indicate that vaccines show promise in preventing UTIs (and possibly other ExPEC-induced diseases), the diversity of virulence factors associated with ExPEC-induced UTI and other diseases will make it difficult to convince vaccine developers to spend time and money generating vaccines that may be effective against ExPEC-induced diseases in humans.

Stapleton (2003) suggested the use of oligosaccharide-based competitive inhibitors to prevent UTIs through interference with bacterial adherence and colonization. The binding of class II PapG adhesin from a UTI-causing *E. coli* strain to sugar fragments from the globoseries of glycolipids was demonstrated *in vitro* by Larsson et al. (2003). Similarly, Bouckaert et al. (2005) determined that type 1 pili would bind to various mannosides. These studies may lead to the development of saccharide competitive inhibitors that can prevent pyelonephritis and cystitis by UTI-inducing *E. coli*.

Patients with spinal cord injury (SCI) are prone to UTI, and approximately 40% of patients with SCI die of renal-related problems (Foxman, 2002). Antimicrobial treatment of UTI in SCI patients is ineffective. Darouiche et al. (2001) instilled the bladders of 44 adult SCI patients with a nonpathogenic *E. coli* strain (strain 83972, isolated from a case of asymptomatic bacteriuria); successful bladder colonization was found in 30 patients. The mean rate of symptomatic UTI in the colonized SCI patients

was 0.06 UTI episodes per patient-year, compared to 1.8 episodes per patient-year in the 14 noncolonized CSI patients. Darouiche et al. (2001) called the protective effect induced by the nonpathogenic *E. coli* active bacterial interference. While the study was limited to a small population, active bacterial interference showed a definite protective effect against UTIs in SCI patients.

Using a mouse model of UTI, Roos et al. (2006) instilled 109 CFU of a uropathogenic strain of *E. coli* or a 1:1 mixture of the pathogen and *E. coli* strain 83972 directly into mouse bladders. At 24 h, the urine from both groups of mice contained approximately the same number of bacteria, but in the mice receiving both the pathogen and strain 83972, the pathogenic *E. coli* made up only 1.3% of the population, indicating that strain 83972 could outcompete the pathogen. Thus, strain 83972 may be useful in eliminating uropathogenic *E. coli* from the bladders of infected individuals.

While most of the studies on prevention of ExPEC-induced diseases have been limited to UTIs, some of the alternative measures to antimicrobial treatment may be applicable to other diseases caused by ExPEC. These treatments could include development of vaccines and/or saccharide competitive inhibitors that interfere with ExPEC adhesins.

CONCLUSION

Reid et al. (2004) pointed out that there is little appreciation of the threats posed by ExPEC on the part of individuals working in the various health sectors, in spite of the fact that the estimated annual number of cases due to ExPEC-induced UTIs, pneumonia, surgical site infections, and sepsis range from 6.7 to 8.6 million, at an annual cost of $1.5–2.3 billion dollars, and these estimates do not include all the cases of illness caused by ExPEC (Russo and Johnson, 2003). In contrast, the annual number of cases induced by enterohemorrhagic STEC O157 has been estimated by Frenzen et al. (2005) at 73,500, with 61–65 deaths, and an annual cost of $405 million. While Russo and Johnson (2003) did not estimate the number of deaths due to ExPEC-induced infections, the number of deaths is probably much higher than deaths due to enterohemorrhagic *E. coli* O157. Thus, on the basis of economic and morbidity estimates (as well as potential mortality), ExPEC is an important pathogen and should be taken seriously.

There is a commonality of ExPEC-associated virulence factors among the various extraintestinal syndromes, and animal ExPEC strains share virulence factors with human ExPEC strains. The commonality seen in ExPEC strains suggests that they are specific neither for disease syndromes nor for hosts. In immunocompetent hosts, more severe diseases induced by ExPEC such as pyelonephritis, bacteremia, or meningitis are caused by group B_2 strains with a greater number of virulence factors, as compared to strains that cause milder diseases such as cystitis. The requirement for ExPEC virulence factors is considerably reduced when the host is immunocompromised due to age, immune status, or underlying disease. For example, group A strains caused neonatal meningitis only in those neonates with an underlying immune or medical condition (Bingen et al., 1998). Bonacorsi et al. (2003) suggested that detection of a group A ExPEC isolate in a neonatal meningitis case indicates immune deficiency or other underlying medical problems.

Russo and Johnson (2003) suggested that the neglect of ExPEC-induced diseases is due to the fact that, in the past, these organisms were highly susceptible to antimicrobial treatment. However, recent data indicate an increased resistance of ExPEC to antimicrobials: many compounds, useful in the past, are less effective as therapeutic agents. At present, alternatives to antimicrobials in the treatment of ExPEC-induced diseases are lacking. The increasing resistance of ExPEC strains to antimicrobials led Russo and Johnson (2003) to observe that there was "no relief in sight."

Considering the microbial resistance of ExPEC strains now seen in clinical practice, Russo and Johnson (2006) suggest that it is time to consider the potential of immunization strategies as a means of preventing ExPEC-induced extraintestinal diseases. They suggest that a polyvalent subunit vaccine combining adhesins, outer membrane proteins, and detoxified lipid A/core saccharides of lipopolysac-

carides from ExPEC strains may lead to a useful strategy to prevent ExPEC-induced diseases. They also suggest that a whole-cell vaccine containing multiple strains of wild-type ExPEC engineered so that factors (capsule, O antigen) that impede optimal host responses are inactivated and in which critical antigenic determinants are overexpressed could be an another approach. While vaccine development will be difficult, research in this area is necessary in order to combat ExPEC-induced diseases.

The recent studies of Johnson et al. (2003a, 2005a, 2005b) demonstrating the presence of ExPEC in food products suggest that these represent a new class of foodborne pathogens. However, the studies establishing the presence of ExPEC strains in foods were limited to one area in Minnesota. There is a need to expand such studies to other areas of the United States to obtain a better picture of the extent of ExPEC contamination in foods and any potential or direct link to foodborne diseases.

A number of studies have indicated the commonality of virulence factors in human and animal strains of ExPEC, suggesting that ExPEC strains are zoonotic pathogens, as well. To determine if ExPEC are zoonotic agents, experimental inoculation of animals with human strains of ExPEC could be performed. Such experiments have not been reported in the literature.

DISCLAIMER

Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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