Food reservoir for *Escherichia coli* causing community-acquired urinary tract infections

by

Caroline Vincent

Department of Microbiology and Immunology

McGill University, Montréal

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ABSTRACT

Closely related strains of *Escherichia coli* have been shown to cause extraintestinal infections in unrelated persons. This study tests whether a food reservoir may exist for these *E. coli*. Isolates from three sources collected over the same time period and geographic area were compared. The sources comprised *E. coli* isolates from women with urinary tract infection (UTI) (n = 353); retail meat (n = 417); and restaurant/ready-to-eat foods (n = 74). *E. coli* were evaluated for antimicrobial susceptibility and O:H serotype and compared by using six different genotyping methods. We identified 17 clonal groups that contained *E. coli* isolates (n = 72) from more than one source. *E. coli* from retail chicken (O25:H4-ST131 and O114:H4-ST117) and honeydew melon (O2:H7-ST95) were indistinguishable from or closely related to *E. coli* from human UTIs. This study provides strong support for the role of food reservoirs in the dissemination of *E. coli* causing community-acquired UTIs.

RÉSUMÉ

Il a été démontré que des souches de *Escherichia coli* étroitement reliées causaient des infections extraintestinales chez des personnes non-reliées. Cette étude teste l'hypothèse selon laquelle il existerait un réservoir alimentaire pour ces souches d'*E. coli*. Des isolats provenant de trois sources différentes et récoltés durant les mêmes périodes et régions géographiques ont été comparés. Les sources incluaient des isolats d'*E. coli* provenant de femmes soufrant d'infection urinaire (IU) (n=353); de viande vendue au détail (n = 417); et d'aliments de restauration/prêts-à-manger (n =74). Les *E. coli* ont été évalués pour leur susceptibilité aux agents antimicrobiens et leur sérotype O:H, et ont été comparés par l'intermédiaire de six différentes méthodes de génotypage. Nous avons identifié 17 groupes clonaux contenant des isolats d'*E. coli* (n = 72) provenant de plus d'une source. Des *E. coli* provenant de viande de poulet (O25:H4-ST131 et O114:H4-ST117) et de melon au miel (O2:H7-ST95) étaient indistinguables ou étroitement reliés à des *E. coli* provenant d'IUs. Cette étude supporte fortement le rôle des réservoirs alimentaires dans la dissémination du *E. coli* causant des IUs acquises dans la communauté.

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- Caroline Vincent, M.Sc. candidate, McGill University
  Conducted most of the laboratory experiments, collected and interpreted the data, prepared and presented the poster at the ASM meeting, and wrote the manuscript.

- Patrick Boerlin, Co-investigator, University of Guelph
  Contributed to the conception and design of the study, assisted with data interpretation, reviewed the manuscript and provided insight.

- Gabhan Chalmers, Research technician, University of Guelph
  Performed antimicrobial resistance genotyping.

- Danielle Daignault, Microbiologist, Public Health Agency of Canada
  Performed antimicrobial susceptibility testing.

- Charles M. Dozois, Investigator, INRS-Institut Armand-Frappier
  Contributed to the conception and design of the study.

- Lucie Dutil, Veterinary epidemiologist, Public Health Agency of Canada
  Assembled the retail meat isolate collection, contributed to the conception and design of the study and provided insight.

- Chrissi Galanakis, Honor student, McGill University
  Contributed to data collection.

- Philippe Garneau, Research agent, Université de Montréal
  Performed microarray experiments.

- Josée Harel, Co-investigator, Université de Montréal
  Assisted with data interpretation and provided insight.

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- Kim Ziebell, Investigator, Public Health Agency of Canada
  Performed the serotyping.

- Amee R. Manges, Principal investigator, McGill University
  Conceived and designed the study, supervised the M.Sc. candidate, provided epidemiological expertise and edited the manuscript.

Other manuscripts that I have co-authored during the course of my program but that are not included as part of this thesis are as follows:

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STATEMENT OF ORIGINALITY

The results reported in this thesis provide the strongest molecular-epidemiological evidence available to date for the role of food reservoirs or foodborne transmission in the dissemination of *E. coli* causing common community-acquired urinary tract infections (UTIs). These findings are supported by pulsed-field gel electrophoresis, a highly discriminating genotyping method, which identified genetically indistinguishable or closely related *E. coli* strains from food and human UTI sources.

LIST OF ABBREVIATIONS

ASB	asymptomatic bacteriuria
ATCC	American Type Culture Collection
APEC	avian pathogenic *E. coli*
cfu	colony-forming unit
CgA	clonal group A
CI	confidence interval
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CLB	Cell Lysis Buffer
CLSI	Clinical and Laboratory Standards Institute
CSB	Cell Suspension Buffer
dNTP	deoxynucleoside triphosphate
EDTA	ethylenediaminetetraacetic acid
ERIC2	enterobacterial repetitive intergenic consensus sequence 2
ESBL	extended-spectrum β-lactamase
ExPEC	extraintestinal pathogenic *E. coli*
LB	Luria-Bertani
MIC	minimum inhibitory concentration
MLST	multilocus sequence typing
MLVA	multilocus variable number tandem repeat analysis
MNEC	meningitis-associated *E. coli*
MSHS	McGill Student Health Service

NARMS	National Antimicrobial Resistance Monitoring System
PAI	pathogenicity island
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
RUTI	recurrent urinary tract infection
ST	sequence type
TBE	Tris borate EDTA
TE	Tris EDTA
TMP-SMX	trimethoprim-sulfamethoxazole
UPEC	uropathogenic *E. coli*
UTI	urinary tract infection
UV	ultraviolet

1. LITERATURE REVIEW

1.1 Urinary tract and other extraintestinal infections

1.1.1 Generalities

Urinary tract infections (UTIs) are considered to be one of the most common bacterial infections (1). The population at highest risk for community-acquired UTIs are sexually active women of childbearing age that are otherwise healthy. The majority of these infections are uncomplicated in nature, that is, they occur in people without underlying anatomic or functional abnormalities of the urinary tract (e.g., catheterization, neurogenic bladder) or comorbidities such as diabetes (2). Complicated UTIs have a more diverse etiology than uncomplicated UTIs and can be polymicrobial (3). The rest of this thesis will focus on uncomplicated, community-acquired UTIs in young women.

1.1.2 Etiology, incidence and associated costs

The majority (80-90%) of uncomplicated UTIs are caused by *E. coli*, or extraintestinal pathogenic *E. coli* (ExPEC) and, to a lesser extent, by *Staphylococcus saprophyticus* (5-15%). *Klebsiella*, *Enterobacter* and *Proteus* species are also responsible for a small portion of these infections (3-5).

ExPEC are responsible for an enormous burden of illness, death, and associated medical and indirect costs. Every year, an estimated 6-

8 million cases of uncomplicated UTI occur in the United States, and 130-175 million cases occur globally (1;6). Other extraintestinal infections, such as pyelonephritis, meningitis, and sepsis contribute to another million cases annually in the United States. The urinary tract is the most common source for *E. coli* causing bloodstream infections, which cause 40,000 deaths from sepsis each year in the United States (1;6). It is estimated that by age 24, one woman out of three will have at least one physician-diagnosed UTI requiring antimicrobial therapy, and up to 60% of all women will experience at least one UTI during their lifetime (7). Community-acquired UTIs are responsible for over $1 billion of direct health care costs in the United States annually (6;7). The raising prevalence of antimicrobial resistance among uropathogens is further adding to the cost of treating these infections, since drug-resistant infections often require more complicated treatment regimens and result in more treatment failures. In outpatient settings, a recent study from the United Kingdom estimated that at a minimum the total cost for the treatment and re-consultation for an antimicrobial-resistant UTI episode was 25% higher than for a UTI episode caused by a pan-susceptible *E. coli* isolate (8).

1.1.3 Pathogenesis

The primary reservoir for ExPEC is the host's own intestinal tract. This has been confirmed by the identification of the same UTI-causing

E. coli strain in the fecal flora of individual patients (9;10). Identical ExPEC strains can also be shared among sexual partners and household members, suggesting both sexual and person-to-person transmission (11-13). UTI occur when the bacteria leave the rectum, colonize the vagina and the periurethral opening, and ascend the urethra to infect the bladder (Figure 1). The bacteria can also migrate further up into the kidney and cause pyelonephritis (kidney infection). A critical step of the infection process involves the attachment of the bacteria to the luminal surface of the bladder. ExPEC possess filamentous adhesive organelles, such as type 1 fimbriae, that allow them to tightly bind the uroepithelium, therefore facilitating the colonization of host tissues and allowing the bacteria to withstand the bulk flow of urine. Recent research has shown the ability of ExPEC to invade the superficial cells of the bladder and persist in an intracellular reservoir that may serve as a source for recurrent UTIs (RUTI) (14;15).

1.1.4 Risk factors

Because their urethra is shorter and closer to the anus, women have a significantly higher risk than men to experience a UTI. Other subpopulations that are more susceptible to UTI include children, pregnant women, the elderly, catheterized or immunocompromised patients, and patients with spinal cord injuries, diabetes, multiple sclerosis, or underlying urologic abnormalities (1). Individual factors that were found to increase

the risk of uncomplicated UTI or RUTI in women include recent or frequent sexual intercourse, use of diaphragm or spermicide-based contraceptives, which is associated with *E. coli* vaginal colonization, a history of RUTIs, and nonsecretion of blood group antigens, which is associated with the presence of *E. coli*-binding glycolipids on the surface of uroepithelial cells (1;4;16).

1.1.5 Syndromes

UTI refers to the presence of bacterial pathogens in the urinary tract and can be classified into three different syndromes: cystitis, pyelonephritis and asymptomatic bacteriuria (ASB).

Cystitis, also known as lower UTI, is an acute infection of the bladder or urethra. Typical clinical manifestations include dysuria, frequent need or urgency of urination, suprapubic pain, and low back pain in some cases (16).

Pyelonephritis, or upper UTI, refers to an infection of the kidney and renal pelvis. Characteristic symptoms include fever, flank pain and tenderness, dysuria, nausea, vomiting, abdominal pain and, in severe cases, sepsis or septic shock (16).

ASB is defined as the presence of a significant concentration of bacteria in the urine (typically $\geq 10^5$ colony-forming units [cfu] per ml of urine), but without accompanying symptoms. This condition is frequent among patients with indwelling catheters and the institutionalized elderly

(16). However, due to its lack of clinical significance, treatment of ASB is not recommended, except in two specific cases: before urologic surgery (to avoid post-operative complications) and during pregnancy (due to an increased risk of pyelonephritis) (4;16).

1.1.6 Recurrent UTIs (RUTIs)

RUTIs are a frequent clinical problem in young women. Twenty-seven to 44% of women will experience at least one recurrence within six months after a primary UTI (17;18). RUTIs can be divided into two main categories: relapse and reinfection. A relapse usually occurs within two weeks after treatment cessation, whereas a reinfection occurs more than two weeks after the completion of therapy (4). The subsequent episode of cystitis can often be attributable to the same strain as the one implicated in the original infection. Persistence of the bacteria within the host's bladder or colonic flora, or reintroduction from an external reservoir, for example the sexual partner, may explain these same-strain recurrences (16).

1.1.7 Other extraintestinal infections

UTI is the most frequent infection caused by ExPEC (6). Other types of extraintestinal infection include neonatal meningitis, intraabdominal infection, nosocomial pneumonia, osteomyelitis, cellulitis,

and wound infections. All of these infections can give rise to bacteremia and sepsis, which can be fatal (6;19;20).

1.1.8 Prevention

A well-studied method for the prevention of UTI consists of regularly consuming cranberry products. Proanthocyanidins, which are the active compound of cranberries, are thought to inhibit the attachment of *E. coli* to the uroepithelium, thus preventing subsequent colonization and infection. A recent Cochrane review on the efficacy of cranberries in preventing UTI concluded that cranberry juice may help in reducing the incidence of UTI in women who tend to have recurrences (21). However, convincing clinical trials are still missing and the use of cranberries as a prophylactic agent remains controversial (22).

For women at risk for RUTIs, the discontinuation of diaphragm or spermicide use is the first recommendation. When behavioral changes are not sufficient, an effective strategy to prevent RUTIs consists of using low doses of antimicrobial prophylaxis, either on a regular basis or after sexual intercourse. Patient-initiated treatment can also be used at the onset of symptoms (16;23). Immunoactive prophylaxis, which consists of consuming bacterial extracts in order to stimulate the innate immune system, is another option (2). In a meta-analysis on the safety and efficacy of bacterial lysates in the prevention of RUTI, a product called OM-89 (Uro-Vaxom) was shown to reduce the number of UTI recurrences by

39%, and appears to be a potential alternative to antimicrobial prophylaxis (24).

1.1.9 Treatment

Lower UTIs are usually treated empirically with oral antimicrobials. Indeed, due to time and cost constraints, urine cultures and microbiological diagnosis are not systematically performed. The actual first-line treatment consists of a three-day course of trimethoprim-sulfamethoxazole (TMP-SMX). However, the use of this drug is not recommended in geographic areas where resistance prevalence in uropathogens reaches 20% (16;25). Recent studies from Canada have shown that we are approaching this threshold, with resistance rates of 15-19% among *E. coli* isolated from urine samples (26-28). Fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin) are an effective but more expensive alternative to TMP-SMX (4;25;29;30). Emerging resistance to this class of antimicrobials actually raises concern, since it may limit their use in the treatment of more severe infections such as pyelonephritis (16;25). Other treatment options include a five- to seven-day regimen with nitrofurantoin, and a single three-gram oral dose of fosfomycin. However, these two antibiotics are thought to exhibit more adverse effects and result in lower cure rates compared to TMP-SMX and fluoroquinolones (25;29;30). β-lactams agents, which include penicillins, cephalosporins, monobactams and carbapenems, are sometimes used for UTI therapy but are less

effective than the aforementioned drugs due to frequent bacterial resistance (25;29;30).

1.2 Extraintestinal pathogenic *E. coli* (ExPEC)

1.2.1 Intestinal reservoir

ExPEC differ from diarrheagenic strains of *E. coli*, such as *E. coli* O157:H7, in that they do not cause gastrointestinal diseases. Similarly, diarrheagenic *E. coli* strains are largely unable to cause disease outside of the intestinal tract (19;20). ExPEC can, along with commensal strains, asymptomatically colonize their host's intestinal tract. They may even, at any given time point, constitute the predominant fecal *E. coli* strain in approximately 20% of healthy individuals (31). Therefore, the intestinal acquisition of ExPEC is not sufficient for infection to occur. The pathogen needs to leave the intestine and colonize a normally sterile body site in order to initiate an extraintestinal infection. In the case of UTI, this can be prompted by sexual intercourse or the placement of an indwelling catheter, for example.

1.2.2 Virulence factors

Most commensal strains of *E. coli* that inhabit the gastrointestinal tract belong to phylogenetic lineages A and B1, and tend to lack virulence genes (19;20). This is in contrast to ExPEC, which derives predominantly

from phylogroups B2 and D, and possess specific extraintestinal virulence factors such as adhesins (P and type 1 fimbriae), iron-acquisition systems (aerobactin), host defense-subverting mechanisms (capsule, O antigens), and toxins (hemolysin, cytotoxic necrotizing factor 1) (6;19;20). Many of these virulence factors are found on pathogenicity islands (PAIs), which are large genomic regions that are thought to be acquired by horizontal transfer. PAIs are typically absent from the genomes of commensal *E. coli* strains (32;33).

1.2.3 Pathotypes

Human ExPEC can be further categorized into uropathogenic *E. coli* (UPEC), meningitis-associated *E. coli* (MNEC) and septicemia-associated *E. coli* (SEPEC). ExPEC can also cause illness in animals; avian pathogenic *E. coli* (APEC) causes extraintestinal infections such as colibacillosis and septicemia in poultry, resulting in significant economic losses (34). Each of these pathotypes is typically defined by the clinical syndrome produced or alternatively by the presence of a particular set of virulence factors (35-37). However, the virulence genes associated with each pathotype are not mutually exclusive, and a single virulence factor can be pathologically relevant to several clinical syndromes (19;20). The traditional characterization of *E. coli* pathotypes relies on the identification of somatic (O), capsular (K) and flagellar (H) antigens, which are determined by serotyping. Specific antigen types have been associated

with certain pathotypes. For example, expression of the K1 capsular antigen has been shown to contribute to the pathogenesis of MNEC (38). On the other hand, *E. coli* of serotypes O2 and O78 are responsible for about 80% of avian septicemia cases (39), and a limited number of O-serotypes (O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75) account for up to 81% of UPEC-associated infections (31;40).

1.3 Outbreaks of community-acquired extraintestinal infections caused by *E. coli*

1.3.1 Introduction

Community-acquired extraintestinal infections are generally considered to be sporadic infections that are caused by a diverse pool of *E. coli* strains. Unlike enteric diseases such as salmonellosis or gastroenteritis, extraintestinal infections are not usually associated with outbreaks. However, certain ExPEC lineages have exhibited epidemic behavior, and there is mounting evidence that they are responsible for community-wide outbreaks.

In this thesis, a clonal group or cluster will be defined as a group of isolates that exhibits identical phenotypic and genotypic traits, as determined by a strain typing method such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) or serotyping. This definition is based on the fact that a clonal group (or a clone) arises from the asexual reproduction of a common precursor strain.

1.3.2 *E. coli* O15:K52:H1

The first outbreak of extraintestinal *E. coli* infections ever reported occurred in South East London in the winter of 1986-1987. There, a highly virulent and multidrug-resistant clonal group of *E. coli* O15:K52:H1 was found to be responsible for 15% of all UTI cases and caused three deaths from septicemia (41). *E. coli* O15:K52:H1 was later recognized as an endemic cause of UTI and bacteremia in Denmark and Spain (42-44). It was also identified beyond Europe, in the United States and in Canada, which suggests its broad dissemination (45;46).

1.3.3 *E. coli* O78:H10

In 1991, a cluster of multidrug-resistant *E. coli* O78:H10 was identified in Copenhagen, Denmark. The outbreak strain was responsible for 18 cases of community- and hospital-associated UTI over an eight-month period (47). Between 1956 and 1990, only 30 strains of serotype O78:H10 were identified among the thousands received from all around the world by the WHO *Escherichia* Centre at the Statens Serum Institut in Copenhagen (47).

1.3.4 *E. coli* clonal group A

In 2001, Manges and colleagues reported the discovery of *E. coli* clonal group A (CgA). This clonal group, characterized by serotype

O11/O77/O17/O73:K52:H18, caused 11% of all UTIs, and 49% of TMP-SMX-resistant UTIs in a single California community over a four-month period (48). It also caused antimicrobial-resistant UTIs in Michigan, Minnesota, and Colorado (49), as well as pyelonephritis in several states (50). CgA isolates were highly homogenous with respect to enterobacterial repetitive intergenic consensus sequence 2 (ERIC2) polymerase chain reaction (PCR) fingerprinting patterns, O:H serotype, PFGE profiles, virulence-factor profiles and antibiotic resistance patterns. This high degree of genetic homogeneity, combined with the presence of an unusual serotype (the somatic antigens O11, O77, O17 and O73 are not frequently encountered among UTI-causing *E. coli*), suggested that CgA was a newly emerging pathogenic clonal group (48). One year after the possible CgA outbreak, the proportion of drug-resistant UTIs caused by this clonal group had dropped by 38% ($p<0.001$) (51). This temporal decline in the prevalence of CgA indicated that the number of cases followed an epidemic pattern (rapid increase followed by a sharp decline), which provides further evidence that CgA was responsible for a community-wide epidemic of UTI.

1.3.5 CTX-M extended-spectrum β-lactamase-producing *E. coli* (O25:H4-ST131)

Extended-spectrum β-lactamases (ESBLs) are plasmid-borne bacterial enzymes that mediate resistance to aztreonam, penicillins, and

third and fourth generation cephalosporins such as ceftazidime, cefotaxime, and cefepime. Resistance to aminoglycosides and TMP-SMX is often co-transferred on the same plasmid (52).

ESBLs can be divided into distinct families or types. ESBLs belonging to the CTX-M family were first described in 1986, in a strain of *E. coli* (53). Unlike the traditional TEM and SHV-derived ESBLs, which have been mostly restricted to nosocomial klebsiellae, CTX-M enzymes are mainly detected in community-associated *E. coli* infections, and have recently emerged as the dominant ESBL type worldwide (54-57). CTX-M-producing *E. coli* are not only resistant to penicillins and cephalosporins, but also carry resistance to multiple other medically significant antimicrobial drugs, including fluoroquinolones. As these ESBL-producing *E. coli* are frequently found in human feces, it has been suggested that they could be spreading via the food chain (57). Once in the gut, these multidrug-resistant *E. coli* may serve as a source for urinary tract and bloodstream infections. The ability of CTX-M-producing *E. coli* to rapidly disseminate in the community and cause severe infections with limited treatment options raises concern; some authors have even referred to a 'CTX-M β-lactamase pandemic' (58;59).

In 2000-2001, clonally related strains of ESBL-producing *E. coli* were found to be responsible for a community-onset outbreak of UTI in the Calgary Health Region. This was the first ever reported clonal outbreak of ESBL-producing organisms occurring outside of a hospital setting (60). The PFGE-defined clonal group contained 59 isolates (recovered from 59

patients); these isolates were resistant to ciprofloxacin and produced CTX-M-14 β-lactamase. The distribution of UTI cases caused by the epidemic strain followed an epidemic pattern: from a single case detected in June 2000, the outbreak reached its highest point between October and December 2000, with 17 cases reported. The number of subsequent cases gradually went down to 5 in December 2001, and the epidemic strain seemingly disappeared from the population thereafter (60). The temporal and geographical clustering of the cases, combined with the fact that they were caused by closely related *E. coli* strains, suggests that an outbreak of UTI occurred in this Calgary community.

Between 2004 and 2006, Mendonça *et al.* investigated the prevalence of CTX-M enzymes among clinical isolates of *E. coli* in Portugal (61). A cluster of 91 CTX-M-15-producing isolates was identified based on PFGE. These isolates exhibited high levels of resistance to quinolones and aminoglycosides, and the majority were recovered from out- and inpatient urine samples (61). The authors suggested that the country-wide dissemination of CTX-M-producing *E. coli* in Portugal might be due to the introduction and spread of a major clone between the hospitals and the community.

In 2003-2004, the Health Protection Agency's Antibiotic Resistance Monitoring and Reference Laboratory analyzed a set of 287 CTX-M-producing *E. coli* forwarded from 42 centres throughout the United Kingdom (62). A single CTX-M-producing strain, designated epidemic strain A, was found to account for a cluster of 110 PFGE-related isolates

retrieved from six centres. A sizeable fraction of these isolates were recovered from community patients (23%; the remainder being from hospitalized patients), and the majority were from urine and blood specimens (62). The epidemic strain belonged to serotype O25:H4 and sequence type (ST) 131 (as determined by MLST), and was resistant to ciprofloxacin and trimethoprim (62;63).

One year later, Lau *et al.* also identified an important genetic lineage of *E. coli* belonging to ST131 in the Northwest of England. This lineage was responsible for 59% of cephalosporin-resistant UTIs and bacteremia cases among community and hospital patients (64). The presence of CTX-M enzymes was not evaluated, but further investigation into ST131 isolates revealed that they belonged to serotype O25, and were linked to the United Kingdom's epidemic strain A (63;64).

E. coli O25:H4-ST131 has been identified in several other countries, including Canada (46;65;66), Spain (67;68), Italy (67), Turkey (69), Croatia (70), Japan (71), Norway (72), Korea (73), France (74), Indonesia (75), and the United States (76). This recently emerged clonal group has rapidly disseminated around the globe (77) and actually contributes to a considerable fraction of community-onset extraintestinal infections caused by CTX-M-producing *E. coli*. O25:H4-ST131 belongs to the highly virulent phylogenetic group B2 and harbors plasmids that encode resistance to multiple antimicrobial agents (78;79). Therefore, this clonal group represents a serious threat to public health. In the near

future, carbapenems are likely to become the only choice available for the empirical treatment of community-acquired extraintestinal infections (78).

1.3.6 Summary

These outbreak reports suggest that community-onset epidemics of extraintestinal infection caused by *E. coli* may be more common than initially expected. The increasing prevalence of drug-resistant UTIs in certain communities may be attributable to the introduction of a single multidrug-resistant clonal group of ExPEC, rather than changes in the frequency of antibiotic use (51). Since urine cultures are not automatically performed for most UTI cases, there is no doubt that other large-scale outbreaks of extraintestinal *E. coli* infections have occurred, or may actually be ongoing, without being detected. Unlike with foodborne enteric diseases, there is no surveillance system actually in place to monitor and identify clonal outbreaks of extraintestinal infections. These outbreaks are more difficult to detect because there is a time lag ranging from several weeks to months between the acquisition of ExPEC by the gut and the development of an infection. This is in contrast to exposure to diarrheagenic *E. coli*, which usually results in immediate disease manifestation.

The source(s) of these epidemics was never identified. However, several authors have suggested that a point source, such as contaminated food products, may play a role in the local dissemination of clonally related

E. coli strains (47;48;51;54;55;57;60;78). Indeed, the number of cases implicated in these outbreaks, their temporal clustering, and the absence of obvious epidemiological connections between affected individuals argues against household and sexual contact as the primary modes of transmission.

1.4 Evidence for a food animal reservoir of ExPEC

1.4.1 Introduction

It is unclear how ExPEC are acquired by the human gut, but it has been suggested that foods might be a source of antimicrobial-resistant ExPEC in humans. Indeed, the food supply is an established reservoir for several pathogenic bacteria, such as *E. coli* O157:H7, *Salmonella enterica*, *Campylobacter jejuni*, and *Listeria monocytogenes* (80). The use of antimicrobial agents for clinical therapy, prophylaxis and growth promotion in animal husbandry ultimately favors the selection of antimicrobial-resistant microorganisms. Pathogenic and antimicrobial-resistant bacteria that are found in the fecal flora of food animals can contaminate meat carcasses during the slaughtering process and be transferred to humans via the food supply (81-85). Indeed, pathogenic bacteria found on retail meat items can easily be transmitted to food handlers and consumers via cross-contamination between raw and cooked items, consumption of undercooked meat, and poor hygiene practices in the kitchen (86;87).

1.4.2 Similarities between avian and human ExPEC

Avian and human ExPEC typically cause disease in different hosts. However, since they both have the ability to cause extraintestinal infections, the host specificitiy of APEC and human-associated pathotypes is being challenged. Indeed, several studies have shown that APEC and human ExPEC have overlapping virulence traits, serotypes, and phylogenetic groups, and also share the ability to cause disease in chicks (88-90). Since no distinctive host-associated virulence characteristics appears to exist between avian and human ExPEC, it has been suggested that APEC may be able to cause disease in human hosts (39;88-93). The recent sequencing of an O1:K1:H7 APEC strain, designated APEC O1, revealed extensive similarities between this strain and the genome of three UPEC reference strains (94), suggesting that there is no obvious genetic evidence for a host- or syndrome-exclusive pathotype between avian and human ExPEC (39;94). Therefore, these findings support the possibility that some APEC may behave as zoonotic pathogens.

In a comparative genotyping study of 59 O1:K1:H7/NM ExPEC isolates, Mora and co-authors identified 39 strains from various pathotypes that belonged to phylogroup B2 and ST95. By PFGE, some of these isolates exhibited similar profiles. Six clusters including APEC and human ExPEC strains were identified, suggesting recent divergence from a common ancestor (95). It must be noted, however, that the isolates included in the study were not epidemiologically linked (they were

collected from four countries over a time-period of 15 years). Nonetheless, these results suggest that APEC may be related to human ExPEC.

The similarities observed between human and avian ExPEC suggest that their associated pathotypes are not host-specific. Therefore, APEC may possibly be transmitted to humans via contaminated poultry meat, establish themselves in the human gut, and, under certain circumstances, go on to cause disease at an extraintestinal site. However, convincing epidemiological evidence showing that foodborne APEC are a cause of disease in humans is still lacking.

1.4.3 Antimicrobial-resistant *E. coli* and ExPEC in retail foods

Several studies have reported the presence of antimicrobial-resistant *E. coli* in raw retail meats from groceries stores (96-98). The selection for antimicrobial-resistant organims is likely the result of antimicrobial use in food animal production. Between 1999 and 2003, Johnson and colleagues examined the presence of antimicrobial-resistant ExPEC within large sets of retail food items from community markets in Minnesota (99;100). They observed that poultry samples exhibited the highest level of *E. coli* contamination (92%) compared to beef or pork (69%) and other foods items (9%) (99). Among *E. coli*-positive samples, retail poultry exhibited a significantly higher prevalence of antimicrobial resistance (86-94%) and ExPEC-associated virulence traits (40-46%) compared to other meat and food sources. These findings suggest that

retail meats purchased in supermarkets, particularly poultry products, are often extensively contaminated with antimicrobial-resistant *E. coli*, and some of them carry typical ExPEC virulence features.

1.4.4 Similarities between antimicrobial-resistant ExPEC of human and poultry origin

To address whether poultry products may be a source of antimicrobial-resistant ExPEC in humans, researchers have examined the similarities between drug-resistant and drug-susceptible *E. coli* isolates of human fecal and poultry origin with regards to their phylogenetic groups, ExPEC-associated virulence factors and O-antigens (101;102). Drug-susceptible human isolates were found to differ considerably from other human and poultry isolates. On the other hand, drug-resistant human isolates closely resembled poultry isolates, and drug-susceptible and drug-resistant poultry isolates were largely indistinguishable (101;102). These findings suggest that (i) drug-resistant poultry-source *E. coli* isolates likely arise *de novo* from susceptible poultry-source precursors, which is consistent with on-farm emergence of resistance due to the selective pressure from antimicrobial drug use in animal husbandry, and (ii) drug-resistant human fecal *E. coli* isolates may originate from a poultry reservoir, which is consistent with the hypothesis of foodborne transmission of pathogenic *E. coli* from poultry to humans.

In a molecular-epidemiological study of fluoroquinolone-resistant *E. coli* isolates from humans and chickens, Johnson *et al.* (102) found one instance of a close PFGE match between a retail chicken and a human fecal *E. coli* isolate. Both isolates were ciprofloxacin-resistant, O76 antigen positive and contained the following virulence determinants: *fimH* (type 1 fimbriae adhesin), *iutA* (aerobactin receptor), *traT* (serum resistance-associated), and *ompT* (outer membrane protease). In another study, eight food-associated ExPEC isolates were found to closely resemble *E. coli* isolates recovered from human extraintestinal infections according to O antigen and random amplified polymorphic DNA profiles (RAPD) (99;100).

These observations suggest that retail chicken may be a source for the acquisition of potentially pathogenic antimicrobial-resistant *E. coli* by humans, and raise concerns regarding the human health impact of antimicrobial drug use in food animal production.

1.4.5 Epidemiologic association between retail meat consumption and UTIs caused by antimicrobial-resistant *E. coli*

In 2003-2004, Manges *et al.* conducted a case-control study on the dietary habits of women with UTI caused by antimicrobial-resistant *E. coli*. Their results showed that women infected with multidrug-resistant *E. coli* were significantly more likely to report frequent (≥4-6 times per week) chicken consumption, and women with UTI caused by ampicillin- or

cephalosporin-resistant *E. coli* were more likely to report frequent pork consumption (103). This study provided epidemiologic evidence that antimicrobial-resistant, UTI-causing *E. coli* may have a food reservoir, possibly in retail chicken or pork.

1.4.6 Summary

Retail meat, particularly poultry, may represent an important but unrecognized vehicle for the community-wide dissemination of antimicrobial-resistant ExPEC. Upon consumption, ExPEC will colonize the intestinal tract and become available to cause a UTI when risk factors (e.g., frequent sexual intercourse) are present. If there is a food animal reservoir for ExPEC, then the use of antimicrobials in food animal production may select for antimicrobial-resistant forms of ExPEC (104), and the public health implications for animal husbandry, food safety and clinical management of human infections would be significant.

2. INTRODUCTION

Evidence showing that food can be a reservoir for ExPEC includes (i) the observation of community-based outbreaks of extraintestinal infections caused by epidemic strains of *E. coli* causing uncomplicated UTI (41;47;48;60-62;64) and other severe infections (41;50;62;64); (ii) the determination that these epidemic strains share antimicrobial resistance patterns, virulence-factor profiles, serotypes, and genotypes with isolates recovered from retail meat (99;100;102); and (iii) the epidemiologic association between retail meat consumption and the intestinal acquisition of antimicrobial-resistant *E. coli* causing UTI (103).

2.1 Objective and hypothesis

In this thesis, I report the results of a study designed to address whether a food reservoir exists, possibly in retail chicken meat and other food products, for *E. coli* causing human extraintestinal infections. The objective was to compare the genotypes of systematically selected *E. coli* isolates recovered from (i) human cases of community-acquired UTI, (ii) retail meats, and (iii) restaurant/ready-to-eat foods, in order to determine whether UTI-causing *E. coli* are genetically related to *E. coli* from food sources (retail meat or restaurant/ready-to-eat foods). Isolates from all three sources were collected over the same time period (2005-2007) and geographic sampling area (Montréal and nearby regions).

Our *a priori* hypothesis, based on previous research, was that *E. coli* from human UTIs would most closely resemble the *E. coli* from retail chicken meat. We did examine *E. coli* recovered from beef and pork meats, but we focused on isolates recovered from retail chicken products. We also looked at isolates recovered from restaurant/ready-to-eat foods.

3. METHODS

3.1 Study design

This study is based on an ecologic design. *E. coli* isolates recovered from three sources (human UTI, retail meat and restaurant/ready-to-eat foods) were systematically sampled over the same time period (2005-2007) and geographic area. Human UTI and restaurant/ready-to-eat isolates were from Montréal, Québec. Retail meat isolates from Québec and parts of Ontario were included because women with infections were primarily from these regions. We hoped to maximize the probability that matching genotypes between *E. coli* from these three sources could be identified. *E. coli* isolates from each source were cultured and processed separately in different laboratory rooms to prevent cross-contamination. All organisms were stored in Luria-Bertani (LB) broth (Difco) with 15% (vol/vol) glycerol at -80°C until use. The systematic sampling schemes and details for each source are outlined below.

3.2 Sampling of *E. coli* causing human UTIs

Urine cultures were obtained in collaboration with two clinics from the Montréal area: the McGill Student Health Services (MSHS) and the Centre Local de Service Communautaire (CLSC) Métro Guy. *E. coli* isolates were recovered from urine samples of women aged 18-45 years presenting with UTI to one of these two clinics from June 2005 to May

2007. UTI was clinically defined as the presence of two or more relevant symptoms, including dysuria, increased urinary frequency or urgency, pyuria, and hematuria, and by the presence of more than 10^2 cfu of *E. coli* per ml of clean-catch urine (105). Exactly 1,395 consecutive urine specimens were collected and cultured. The study protocol was approved by the McGill University Institutional Review Board (A01-M04-05A).

Details about specimen culture and bacterial identification of *E. coli* have been previously described (66). Briefly, urine samples were immediately cultured on Uricult (Orion Diagnostica) MacConkey/cysteine lactose electrolyte-deficient agar dipslides. One arbitrarily selected colony (or multiple if morphologically different colonies were present) was selected from the MacConkey side. Lactose- and indole- positive colonies were presumptively identified as *E. coli* (106). Those isolates that were either lactose or indole negative were cultured on CHROMagar orientation plates (Becton Dickinson and Company) and subjected to lysine decarboxylase testing (Moeller Decarboxylase, PML Microbiologicals). The reference strains used for decarboxylase testing included *Klebsiella pneumoniae* (ATCC 13883) and *Enterobacter cloacae* (ATCC 13047). Isolates with a typical *E. coli* appearance on the CHROMagar plate and positive for lysine decarboxylase were considered to be *E. coli*. One *E. coli* isolate from each urine culture was arbitrarily selected for further analysis. If a woman had RUTIs during the study period, only the isolate from the first episode was included. A total of 599 *E. coli* isolates were available for this study.

The final study sample (n = 353) of *E. coli* isolates was systematically sampled from the entire eligible human clinical isolate collection in the following manner. All cephalothin-resistant *E. coli* (n = 19) were included, as cephalosporin resistance phenotype has been associated with UTI outbreaks and is a common characteristic of poultry-derived *E. coli* isolates (60-62;64;96;99). Isolates that were known to be part of a clonal group (n = 46), meaning that in earlier studies these *E. coli* were found to be genetically related to other *E. coli* isolates causing UTI in unrelated women, were included (66). We hypothesized that these *E. coli* would be more likely to be associated with food sources as they caused UTIs in multiple, unrelated women. A random sample of *E. coli* isolates resistant to one or more antimicrobial agents was assembled (n = 172). We chose to oversample resistant *E. coli*, as antimicrobial resistance has been associated with possible outbreaks of extraintestinal *E. coli* infections (41;47;48;60-62;64). A random sample of fully susceptible *E. coli* isolates (n = 116) was selected. An *E. coli* isolate could occur only once in the final study sample.

3.3 Sampling of *E. coli* from retail meats

A sample of 417 *E. coli* isolates recovered from fresh, raw retail chicken, beef, and pork meat was systematically selected from the collection of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). This program led by the Public Health

Agency of Canada monitors temporal and regional trends in antimicrobial resistance in selected bacterial species obtained from chain, independent and butcher stores in several Canadian provinces (107). Protocols for sample collection, *E. coli* isolation, and antimicrobial susceptibility testing are detailed in CIPARS's annual reports (107-109). Briefly, CIPARS' retail meat sampling protocol involves a yearly collection of 100 isolates each from poultry (chicken legs or wings [skin on]), pork (shoulder chops) and beef (ground beef) items from randomly selected regions of each participating province (107).

E. coli isolates collected by the CIPARS in Montréal, areas of Québec outside Montréal, and parts of Ontario from January 2005 to July 2007 were included in the study as follows. All CIPARS isolates from Montréal were included because all UTIs occurred in Montréal (n = 197). All CIPARS nalidixic acid–resistant *E. coli* from all regions of Canada were included (n = 24); these isolates have been associated with reduced susceptibility to fluoroquinolones in both animal and human-related isolates (110). Randomly selected susceptible and resistant isolates from outside Montréal, including regions of Québec and Ontario, were selected to better represent the possible sources of retail meat exposure for the UTI cases. The overall sampling fraction for retail chicken meat isolates was approximately 60%, given that our primary hypothesis focused on poultry products. The sampling fraction for retail beef and pork was 20% each. There has been a strong association between ExPEC clonal groups and antimicrobial resistance (41;47;48;60-62;64). Our targeted sampling

fraction for antimicrobial resistance was 60% for each retail meat category; however, only 25% of retail beef isolates were resistant to one or more antimicrobials.

3.4 Sampling of *E. coli* from restaurant/ready-to-eat foods

We included all 74 *E. coli* isolates from restaurant/ready-to-eat food sources collected in Montréal between February 2005 and October 2007 by the Division de l'Inspection des Aliments, Ville de Montréal (111;112). These isolates were recovered from a range of prepared and ready-to-eat foods, including meats, fruits, vegetables and other items. They were collected as part of routine surveillance activities and from complaint-related inspections of restaurants and establishments offering ready-to-eat foods.

3.5 Antimicrobial susceptibility testing

Human clinical and restaurant/ready-to-eat food isolates had been previously screened for resistance to ampicillin, cephalothin, chloramphenicol, ciprofloxacin, nitrofurantoin, streptomycin, tetracycline, and TMP-SMX by the disk diffusion method as part of another study (66).

Extended antimicrobial susceptibility testing for both human UTI and restaurant/ready-to-eat food isolates was performed by the Public Health Agency of Canada, Laboratory for Foodborne Zoonoses. Retail

meat isolates had been tested prior to this study using the same procedure outlined below. Minimum inhibitory concentration (MIC) values were determined for 15 antimicrobial agents (amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and TMP-SMX) by the broth microdilution method (113). Testing was performed by using the Sensititre Automated Microbiology System (Trek Diagnostic Systems Ltd) and National Antimicrobial Resistance Monitoring System (NARMS) susceptibility panel CMV1AGNF. Isolates were defined as resistant, intermediate, or susceptible to each antimicrobial agent according to the most current breakpoints of the Clinical and Laboratory Standards Institute (CLSI) (114). Since no CLSI *Enterobacteriaceae* interpretive criteria is available for streptomycin, breakpoints were based on the MIC distribution and harmonized with NARMS data (107). Isolates exhibiting intermediate resistance were classified as susceptible. Multidrug resistance was defined as resistance to three or more antimicrobial classes, including aminoglycosides (amikacin, gentamicin, kanamycin, and streptomycin); penicillins (amoxicillin-clavulanic acid and ampicillin); cephalosporins (cefoxitin, ceftiofur, ceftriaxone, and cephalothin); quinolones (ciprofloxacin and nalidixic acid); sulfonamides (sulfisoxazole and trimethoprim-sulfamethoxazole); tetracycline; nitrofurantoin; and chloramphenicol.

3.6 DNA isolation

E. coli isolates were plated on LB agar and single colonies were picked and inoculated into 3 ml LB broth for 16 h with agitation at 37°C. A 1-ml suspension of bacteria was centrifuged and the pellet was subjected to total DNA extraction and purification with the DNeasy blood and tissue kit (QIAGEN) according to the manufacturer's protocol. DNA samples were run on a 0.8% agarose gel to ensure their quality and quantified by using the NanoDrop 1000 Spectrophotometer (Thermo Scientific). DNA dilutions of 10ng/µl were used for multilocus variable number tandem repeat analysis (MLVA) and MLST.

3.7 Multilocus variable number tandem repeat analysis (MLVA)

MLVA was performed on all *E. coli* isolates by the McGill University and Génome Québec Innovation Centre. The method was as previously described by Manges *et al.* (115). Essentially, eight genomic loci containing variable numbers of tandem repeats were amplified in separate PCR reactions using fluorescent primers. The PCR reaction mixture included PCR Buffer (1×), $MgCl_2$ (0.5 mM), deoxynucleoside triphosphate (dNTP; 0.2 mM each), forward and reverse primers (0.2 µM each), Qiagen HotStart *Taq* (0.04 U/µl), and bacterial DNA (20 ng). The thermal cycling was as follows: initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s, and a final extension step at 72°C for 7 min.

Fragments were resolved by capillary electrophoresis, using a 3730 DNA analyzer (Applied Biosystems). Raw fragment lengths for each locus were binned manually using a minimum threshold of ±3-bp to distinguish between different alleles. *E. coli* CFT073, K12, and O157:H7 were used as positive controls in every amplification and genotyping run. The set of 8 alleles for each isolate was defined as the MLVA profile.

3.8 Enterobacterial repetitive intergenic consensus sequence 2 (ERIC2) PCR fingerprinting

E. coli isolates exhibiting identical MLVA profiles were grouped and compared by ERIC2 PCR fingerprinting (116-118) to confirm isolates' relatedness within each MLVA cluster. ERIC2 is a repetitive element-based PCR technique; it uses a single primer that matches target sequences which are present in multiple locations in the bacterial genome. DNA fragments of varying sizes are amplified and separated using gel electrophoresis, resulting in a unique DNA fingerprint for each isolate.

Boiled lysates were used as a source of template DNA. Briefly, bacteria were harvested from 1 ml of an overnight culture, resuspended in 100 µl of PCR-grade water and boiled for 10 min. The DNA supernatant was obtained by centrifugation and used in the PCR reaction. Amplification was performed in a 25 µl reaction volume containing PCR Buffer (1×), $MgCl_2$ (5.0 mM), dNTPs (0.4 mM each; Invitrogen), primer (1.0 µM; Sigma-Genosys), *Taq* DNA polymerase (0.08 U/µl; Invitrogen),

and 2.0 µl of template DNA. The cycling routine included a preliminary denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 4.5 min, and a final extension at 72°C for 1 min. PCR products were electrophoresed in 1% agarose gel, stained with ethidium bromide and visualized using an ultraviolet (UV) transilluminator (ChemiGenius2, Syngene). The human prototype CgA strain (ATCC BAA-457) was used as a positive control for each ERIC2 PCR run. Isolates with fingerprints that were indistinguishable on visual inspection were grouped and selected for further typing.

3.9 Clonal group definition

A clonal group was defined as two or more *E. coli* isolates exhibiting indistinguishable MLVA and ERIC2 PCR profiles. We focused only on clonal groups that contained isolates from more than one source. Groups containing isolates from retail meat and restaurant/ready-to-eat sources were included to determine whether *E. coli* strains from retail meat could be identified in prepared foods. Each clonal group was given a designation that includes the serotype and the ST, as in serotype O25:H4 and ST131 (O25:H4-ST131).

All clonal group isolates were subjected to antimicrobial resistance gene typing, and selected isolates from each clonal group were evaluated by PFGE, serotyping, MLST, and phylogenetic typing to confirm the

identities of these clonal groups and to define their within-group variability. Finally, selected isolates from the most homogenous clonal groups were evaluated for the presence or absence of several virulence genes with a DNA microarray.

3.10 Antimicrobial resistance gene detection

All clonal group members were subjected to antimicrobial resistance genotyping. Template DNA was obtained by the boiling method of Kozak *et al.* (119). Multiplex PCRs were used to detect β-lactamase-encoding genes (bla_{SHV}, bla_{OXA-1}, and bla_{CMY-2}), as well as the major genes for resistance to streptomycin (*strA*/*strB* and *aadA*), kanamycin (*aphA1*, *aphA2* and *aadB*), gentamicin [*aac*(3)IV], sulfonamides (*sul1*, *sul2*, and *sul3*), and tetracycline [*tet*(A), *tet*(B), and *tet*(C)] (119;120). Single PCRs were used to amplify the β-lactamase-encoding gene bla_{TEM} (119), the integrase gene from class I integron *intI1* (121), and several genes for resistance to trimethoprim (*dhfrI*, *dhfrIb*, *dhfrV*, *dhfrIX*, *dhfrXII*, *dhfrXIII*, *dhfrXIV*, *dhfrXVI*, *dhfrXVII*). Primers and PCR conditions are described in Table 1. Multiplex PCRs were performed with a Qiagen multiplex PCR kit in 25 µl reaction volumes containing 1× Qiagen multiplex PCR master mixture, 1× Q-solution, and 1× primer mixture according to the manufacturer's instructions. *dhfrIb* and *dhfrXIV* were amplified with the same primers and the amplicon, if present, was sequenced to identify the corresponding gene variant. Primers for the amplification of all *dhfr* genes

were designed from conserved regions based on sequence alignment of each gene and its variants available from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

3.11 Pulsed-field gel electrophoresis

Selected isolates from each clonal group were evaluated by *Xba*I PFGE. The standardized protocol for molecular subtyping of *E. coli* O157:H7 by PFGE, as established by the PulseNet network of the Centers for Disease Control and Prevention, was used (122). Briefly, 1 ml of bacterial culture was centrifuged and the pellet was resuspended in Cell Suspension Buffer (CSB; 100 mM Tris, 100 mM ethylenediaminetetraacetic acid (EDTA) [pH 8.0]). Bacterial suspensions were further diluted with CSB to an optical density of 0.7 at 610 nm and mixed in a 1:1 volume ratio with an agarose solution consisting of 1% Pulse Field Certified Agarose (Bio-Rad Laboratories) and 1% sodium dodecyl sulfate (SDS) in Tris EDTA (TE) buffer (10 mM Tris, 1 mM EDTA [pH 8.0]). The mixture was dispensed into the wells of disposable plug molds (Bio-Rad Laboratories) and, once solidified, the plugs were lysed overnight at 54°C in Cell Lysis Buffer (50 mM Tris, 50 mM EDTA [pH 8.0], 1% Sarcosyl, 0.5 mg/ml proteinase K). Plugs were then washed two times with double distilled water for 15 min and six times with TE buffer for 10 min. All washing steps were performed at 50°C in a shaking water bath. The plugs were subsequently restricted overnight at 37°C with *Xba*I

endonuclease (60 U/plug, Invitrogen), washed with 0.5× Tris borate EDTA (TBE) buffer (54 g/l of Tris, 27.5 g/l of boric acid, and 6.45 g/l of EDTA), and loaded directly onto a 1% Pulsed Field Certified Agarose gel (Bio-Rad Laboratories). DNA fragments were resolved with the CHEF Mapper electrophoresis appartus (Bio-Rad Laboratories) in 0.5× TBE buffer at 14°C. Electrophoresis was performed for 23 h at a gradient of 6 V/cm, with initial and final switch times of 2.2 and 54.2 s, respectively. The gels were then stained in 0.1% ethidium bromide solution and visualized using a UV transilluminator (ChemiGenius2, Syngene). On each gel, the human prototype CgA strain ATCC BAA-457 and Lambda Ladder PFG Marker (New England BioLabs) were used as positive control and molecular weight marker, respectively.

Restriction-fragment patterns were visually compared. According to the criteria of Tenover *et al.*, isolates exhibiting identical patterns were considered genetically indistinguishable, those exhibiting 1-3 band differences were considered closely related and those exhibiting 4-6 band differences in their PFGE patterns were considered to be possibly related (123). The genetic relatedness of isolates exhibiting related *Xba*I PFGE patterns was confirmed by using a second restriction enzyme, *Not*I.

3.12 Serotyping

O- and H-serotyping was performed by the Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, using established protocols.

Isolates that did not react with O antiserum were classified as nontypeable (ONT), and those that were nonmotile were denoted NM.

3.13 Multilocus sequence typing (MLST)

MLST was performed to assess the degree of evolutionary relatedness between isolates (124). Gene amplification of seven housekeeping genes (*adk* [adenylate kinase], *fumC* [fumarate hydratase], *gyrB* [DNA gyrase], *icd* [isocitrate/isopropylmalate dehydrogenase], *mdh* [malate dehydrogenase], *purA* [adenylosuccinate dehydrogenase], and *recA* [ATP/GTP binding motif]) was carried out by using the primers and conditions specified by the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Amplification was performed in a total volume of 50 µl containing PCR Buffer (1×), $MgCl_2$ (1.25 mM), dNTPs (0.2 mM each; Invitrogen), forward and reverse primers (2.0 µM; Integrated DNA Technologies), *Taq* DNA polymerase (0.04 U/µl; Invitrogen), and DNA (25 ng). The cycling parameters consisted of an initial denaturation step of 2 min at 95°C, followed by 30 cycles of denaturation for 1 min at 95°C, annealing for 1 min at specific temperature (http://mlst.ucc.ie/mlst/dbs/Ecoli), and extension for 2 min at 72°C, and a final extension step of 5 min at 72°C. Amplicons were sequenced with the forward PCR primers at the McGill University and Génome Québec Innovation Centre, using a 3730xl DNA analyzer (Applied Biosystems). Allele designations, STs, and ST

complexes (defined as a group of at least three STs sharing six alleles in pair-wise comparisons) were assigned to each isolate based on query results from the electronic database of the *E. coli* MLST web site.

3.14 Phylogenetic typing

Phylogenetic group determination was done according to the method of Clermont *et al.* (125). Isolates were assigned to one of four major phylogenetic groups (A, B1, B2, or D) based on the presence of two genes (*chuA* and *yjaA*) and a DNA fragment (TSPE4.C2), as determined by triplex PCR. The TspE4C2 primers previously described (125) were replaced by TspE4C2II'F (5'-AGTAATGTCGGGGCATTCAG-3') and TspE4C2II'R (5'-TCGCGCCAACAAAGTATTACG-3') (J.R. Johnson, personal communication). Bacterial DNA was obtained by the boiling method described above for ERIC2 PCR, except that cells were resuspended in 200 µl PCR-grade water instead of 100 µl. Amplification was carried out in a 20 µl mixture containing PCR Buffer (1×), $MgCl_2$ (4 mM), dNTPs (0.8 mM each; Invitrogen), forward and reverse primers (1.0 µM each; Integrated DNA Technologies), *Taq* DNA polymerase (0.125 U/µl; Invitrogen), and 3.0 µl of template DNA. The reaction mixture was subjected to a two-step PCR including an initial denaturation for 12 min at 94°C, 30 cycles of 5 s at 94°C and 10 s at 59°C, and a final extension step of 5 min at 72°C. PCR products were electrophoresed in

2.0% agarose gels, stained with ethidium bromide and visualized using a UV transilluminator (ChemiGenius2, Syngene).

3.15 *E. coli* virulence microarray

Representative isolates from highly homogenous clonal groups were evaluated by using an oligonucleotide-based DNA microarray. The microarray used in this study is an updated version of a previously validated microarray (126) targeting 325 *E. coli* virulence genes or markers, as well as their variants. The microarray was designed to detect a large spectrum of virulence genes representative of all *E. coli* pathotypes and includes virulence factors such as adhesins, the locus of enterocyte effacement, colicins and microcins, toxins, iron acquisition and transport systems, capsular and somatic antigens, hemolysins and hemagglutinins, as well as newly recognized or putative *E. coli* virulence genes.

Bacterial DNA extraction and labeling (Bioprime DNA labeling system, Invitrogen) were performed as previously described (127). Briefly, 4 µl of template DNA was added to a final reaction volume of 50 µl containing 20 µl of random-primer solution, 1 µl of high-concentration DNA polymerase (Klenow fragment; 40 U/µl), 5 µl of dNTPs (1.2 mM dATP, 1.2 mM dGTP, 1.2 mM dTTP, and 0.6 mM dCTP in TE buffer), and 2 µl of 1 mM Cy5-labelled dCTP. Labeling reactions were performed in the dark at 37°C for 3.5 h and stopped by adding 5 µl of 0.5 M EDTA (pH 8.0). The

labeled samples were purified with a PureLink PCR purification kit (Invitrogen) according to the manufacturer's instructions.

Pre-hybridization and hybridization of labeled DNA was performed as described elsewhere (128). Microarray slides were scanned at a resolution of 10 µm at 85% laser power with a ScanArray Lite fluorescent microarray analysis system (Perkin-Elmer). Acquisition and quantification of fluorescent spots intensities was performed using the ScanArray Express software version 2.1 (Perkin-Elmer) (127). The local background was subtracted from the recorded spot intensities and the median value of each set of triplicate spotted oligonucleotides was then compared to the median value of the negative control spots present on the array. Oligonucleotides with a signal-to-noise fluorescence ratio greater than 2.0 were considered positive.

Customized algorithms executed in MS-Excel were used to assign a specific pathotype to each *E. coli* isolate according to its set of virulence genes or markers. Isolates were designated diarrheagenic *E. coli* pathotypes based on the presence of particular virulence markers such as the locus of enterocyte effacement genes and heat-stable, heat-labile, or Shiga-like toxin-encoding genes (126;127). Based on the criteria of Johnson *et al.* (129), isolates were classified as ExPEC according to the possession of two or more of the following virulence genes: P or P-related fimbriae; S, F1C, F17, or F165 fimbriae; Dr binding adhesins; aerobactin siderophore system; and *kpsM* II (group 2 capsule synthesis). Isolates were assigned to a particular ExPEC subgroup based on the presence of

the following genes or gene groups: for UPEC, K1 capsule (*neuA* or *kpsM* II), iron acquisition system (aerobactin or yersiniabactin), P or P-related fimbriae, and S, F1C, F17, or F165 fimbriae; for SEPEC, aerobactin siderophore system, K1 capsule (*neuA* or *kpsM* II), *traT* (serum resistance-associated), *iss* (increased serum survival), *cdtB* (cytolethal distending toxin), *gafD* (F17 fimbriae adhesin), *F17A* (F17 fimbriae structural subunit), and *f165(1)A* (F165(1) fimbriae); and for MNEC, iron acquisition system (aerobactin or yersiniabactin), *ibeA* (invasion of brain endothelium), and *neuA* and *neuC* (K1 capsule synthesis). Isolates were classified as potentially APEC based on the presence of at least four of the five following genes or gene groups: *iss*; *tsh* (temperature-sensitive hemagglutinin); P, F, or S fimbriae; iron acquisition system (aerobactin or yersiniabactin); and K1 capsule (*neuA* or *kpsM* II) (130). It must be noted, however, that no consensus APEC genotype currently exists. Isolates lacking one or more of the genes defining a given pathotype were considered nonpathogenic. The virulence score was the number of different virulence genes detected, adjusted for multiple detection of operon-associated genes.

3.16 Extended-spectrum β-lactamase testing

Members of clonal group O25:H4-ST131 were tested for ESBL phenotype. ESBL-production was assessed by a disk diffusion method, as described by the CLSI (114). Inhibition zones obtained with ceftazidime

and cefotaxime disks were compared to those obtained with ceftazidime-clavulanic acid and cefotaxime-clavulanic acid disks, respectively (Sensi-Disc, Becton Dickinson and Company). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains.

3.17 Statistical analyses

Proportions and 95% confidence intervals (CI) for proportions were estimated. Differences in proportions and virulence scores were assessed by Chi-square and Mann-Whitney U test, respectively. Statistical significance was defined as a p value <0.05. All analyses were conducted using Stata version 9.0 (StataCorp LP).

4. RESULTS

4.1 Description of study sample

We collected and analyzed a total of 844 *E. coli* isolates from human UTIs (n = 353), retail meats (n = 417), and restaurant/ready-to-eat foods (n = 74). Details regarding the year of isolation, geographic location, and specific meat or food source are summarized in Table 2.

4.2 Clonal group identification

MLVA and ERIC2 PCR identified 17 clonal groups containing isolates from more than one source. Eleven clonal groups contained isolates from human UTI and retail meat sources; five clonal groups contained isolates from retail meat and restaurant/ready-to-eat sources; and one clonal group contained isolates from human UTI and restaurant/ready-to-eat sources. The 17 clonal groups accounted for a total of 72 isolates (range 2-14 isolates per group), including 25 isolates (35%) from human UTI, 39 isolates (54%) from retail meat and 8 isolates (11%) from restaurant/ready-to-eat food. Among retail meat isolates, 32 (82%) were from retail chicken and the remainder were from retail beef or pork.

4.3 Antimicrobial susceptibility

The frequencies of resistance to specific antimicrobial agents among clonal group members are presented in Table 3. Thirty-four isolates (47%) were resistant to at least one antimicrobial agent, although resistant isolates were originally oversampled. Of these, 13 (38%) were from human UTI, 20 (59%) were from retail meat and only one (3%) was from a restaurant/ready-to-eat source. The prevalence of resistance to all antimicrobial agents was consistently lower among restaurant/ready-to-eat food isolates than among human UTI or retail meat isolates. Overall, resistance rates were highest for tetracycline (35%), sulfisoxazole (31%) and ampicillin (28%). Resistance to amoxicillin-clavulanic acid, cefoxitin and ceftiofur was not observed in human UTI isolates, while resistance to chloramphenicol and ciprofloxacin was not detected in retail meat isolates. Restaurant-ready-to-eat food isolates only exhibited resistance to tetracycline. One isolate recovered from retail chicken (EC01DT05-0408-01) exhibited resistance to eight antimicrobial agents (amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, streptomycin, sulfisoxazole, tetracycline, and TMP-SMX). Multidrug resistance (defined as resistance to three or more antimicrobial classes) was observed in 32% (95% CI 0.15-0.54) of human UTI isolates, in 33% (95% CI 0.19-0.50) of retail meat isolates, and in none of restaurant/ready-to-eat food isolates.

4.4 Resistance genotypes

The distribution of resistance determinants among the 72 clonal group members are reported in Table 4. Altogether, resistance genes were detected in 34 (47%) isolates, in 94% (33 of 35) of resistant or intermediate isolates, and in 3% (1 of 37) of pan-susceptible isolates. Among human UTI isolates, the most common resistance genes included bla_{TEM}, *tet*(B) and *strA*/*strB*, whereas among retail meat isolates the most frequent resistance genes were *tet*(A), *sul1*, and *aadA*. One isolate from a human UTI (MSHS 825A) possessed seven distinct resistance determinants (*sul2*, *tet*(B), bla_{OXA-1}, bla_{TEM}, *strA*/*strB*, *aadA*, and *dhfrXIV*) as well as the *intI1* gene of the class I integron. The integrase gene (*intI1*) from class I integron was also detected in a considerable fraction (47%) of resistant isolates. The integron-associated genes *aadA* and *sul1* were present (either alone or in combination) in 81% of *intI1*-positive isolates. The resistance genes *sul3*, *aadB*, *acc*(3)IV, *dhfrIb*, *dhfrIX*, *dhfrXIII* and *dhfrXVI* were not identified in any isolates.

4.5 Correlation between resistance phenotypes and genotypes

The genotypes did not always correlate with the resistance phenotype of each isolate. One isolate from retail meat (EC01DT05-0224-01) carried the β-lactamase resistance gene bla_{CMY-2} but was phenotypically susceptible to all antimicrobial agents tested. On the other

hand, one isolate (MSHS 161) from human UTI source displayed resistance to cephalothin but harbored no identified resistance gene.

Based on MICs, 27 isolates were classified as intermediate or resistant to tetracycline. Of these, 16 (59%) had *tet*(A), 8 (30%) had *tet*(B), 2 (7%) had *tet*(C), and 1 (4%) had no corresponding tetracycline resistance gene. Among ampicillin-resistant isolates (n=20), 1 (5%) had bla_{CMY-2}, 2 (10%) had bla_{SHV}, 11 (55%) had bla_{TEM}, 1 (5%) had bla_{CMY-2} and bla_{TEM} together, 1 (5%) had bla_{OXA-1} and bla_{TEM} together, and 4 (20%) had none of the β-lactamase genes investigated.

Of the 10 streptomycin-resistant isolates, 6 (60%) had *strA*/*strB*, and 4 (40%) had *strA/strB* and *aadA* together. Sixty-two isolates were phenotypically susceptible to streptomycin. The majority of these isolates (52 isolates [84%]) carried neither *aadA* nor *strA/strB*. However, 7 isolates (11%) carried *aadA*, 2 isolates (3%) carried *strA/strB*, and 1 isolate (2%) carried both *aadA* and *strA/strB*. None of the gentamicin-resistant isolates (n=8), carried the associated *aac*(3)IV gene.

4.6 Clonal group characterization

Among the 72 clonal group members, 57 representative isolates were selected for evaluation by PFGE, MLST, serotyping, and phylotyping (Table 5). Based on PFGE patterns, we identified two clonal groups (Group 1 and 2) that contained genetically indistinguishable isolates and

one clonal group (Group 3) that contained closely related isolates from food and human UTI sources.

4.6.1 Clonal group 1 - O25:H4-ST131

Clonal group 1 contained three *E. coli* isolates from phylogenetic group B2 that were characterized as O25:H4-ST131. One isolate was from retail chicken meat and the two others were from cases of human UTI; all isolates were recovered from the Montréal area (Table 5). The *Xba*I PFGE patterns of the first human isolate (MSHS 161) and the retail chicken isolate (EC01DT06-1737-01) were indistinguishable, and the second human isolate (MSHS 1134A) differed by one band from the other two patterns (Figure 2A). The *Not*I PFGE patterns of the two human isolates (which were indistinguishable) differed from the retail chicken isolate by a single band (Figure 2B). The retail meat isolate from this clonal group was susceptible to all antimicrobial agents tested and had no resistance gene detected. One of the two isolates from human UTI (MSHS 161) was resistant to cephalothin but did not have an associated resistance gene; the other isolate (MSHS 1134A) was resistant to ampicillin, streptomycin, sulfisoxazole, and tetracycline, and contained bla_{TEM}, *strA*/*strB*, *sul2*, and *tet*(A) genes. None of the isolates from this clonal group had an ESBL phenotype. Virulence microarray testing assigned all O25:H4-ST131 isolates into the MNEC pathotype (Table 6).

4.6.2 Clonal group 2 - O2:H7-ST95

Clonal group 2 contained nine *E. coli* isolates from phylogroup B2 that were characterized as O2:H7-ST95. One isolate was from a restaurant/ready-to-eat food source (a honeydew melon) and the remaining were from cases of human UTI. All O2:H7-ST95 *E. coli* isolates were identified on the Island of Montréal (Table 5). The *Xba*I PFGE patterns were indistinguishable for three of the human UTI isolates (MSHS 100, 186, and 811) and the restaurant/ready-to-eat food isolate (68616.01); the other five O2:H7-ST95 isolates differed by one band (MSHS 1229), two bands (MSHS 95 and MSHS 1062), and four bands (MSHS 782 and MSHS 819) from the food source isolate, respectively (Figure 2A). The *Not*I PFGE patterns for MSHS 100 and MSHS 186 were indistinguishable from the restaurant/ready-to-eat food isolate, and the other human infection isolates differed by one to seven bands from the honeydew melon isolate (Figure 2B). The food source *E. coli* isolate was fully susceptible and did not carry any resistance gene. Most isolates from human UTI source were also susceptible, except for two: one isolate (MSHS 811) was resistant to ampicillin and carried bla_{TEM}; the other isolate (MSHS 1229) was resistant to ampicillin, sulfisoxazole, and TMP-SMX, and possessed bla_{TEM}, *sul2*, *strA*/*strB*, *dhfrV* and *intI1*. All O2:H7-ST95 isolates that were tested on microarray were classified as ExPEC, and shared virulence traits of both UPEC and APEC pathotypes (Table 6).

4.6.3 Clonal group 3 - O114:H4-ST117

Clonal group 3 contained 14 *E. coli* isolates from phylogroup D and ST117. Two isolates were from human UTI and 12 isolates were from retail chicken meat; 9 of these isolates are shown in Table 5. Two isolates, characterized as O114:H4-ST117, were related by PFGE. The first isolate (EC01DT05-0789-01) was recovered from a retail chicken sample in Toronto, Ontario, and the second (MSHS 1014A) was recovered from a human UTI case that occurred in Montréal. Their *Xba*I PFGE patterns differed by five bands, while their *Not*I PFGE patterns differed by more than six bands (Figure 3). Both isolates were fully susceptible and did not carry any resistance determinant. Virulence microarray testing did not assign any of these two PFGE-related isolates to a specific pathotype, and they were thus considered nonpathogenic (Table 6).

Three clonal groups (Groups 4-6, Table 5) exhibited shared phylotypes, MLSTs, and serotypes, but the PFGE patterns were not related.

4.6.4 Clonal group 4 - O4:H5-ST493

Clonal group 4 contained two *E. coli* isolates from phylogenetic group B2 that were characterized as O4:H5-ST493. One isolate (EC01DT05-1012-01) was recovered from a sample of retail pork in Lambton County, Ontario, and the other (MSHS 769) was from a case of

human UTI in Montréal (Table 5). The retail meat isolate was resistant to kanamycin and harboured *aphA2*. The human clinical isolate was resistant to ampicillin, sulfisoxazole, tetracycline, and TMP-SMX, and carried bla_{SHV}, *sul1*, *aadA*, *dhfrI*, and *intI1*. Both O4:H5-ST493 isolates were classified as ExPEC; the human UTI isolate also exhibited some of the virulence characteristics of UPEC and APEC (Table 6).

4.6.5 Clonal group 5 - O36:NM-ST401

Clonal group 5 contained three *E. coli* isolates from phylogroup A that were characterized as O36:NM-ST401. One isolate (EC01DT06-1265-01) was from retail beef and the two others (76083.08 and 76083.10) were from restaurant/ready-to-eat food source (both from chicken dishes); two of these isolates are shown in Table 5. All 3 isolates were identified in Montréal, were fully susceptible and had no resistance gene detected. Two O36:NM-ST401 isolates were tested on virulence microarray and were shown to be nonpathogenic (Table 6).

4.6.6 Clonal group 6 - O172:H16-ST295

Clonal group 6 contained two *E. coli* isolates from phylogroup B1 that were characterized as O172:H16-ST295. One isolate (EC01DT06-0274-01) was recovered from a sample of retail chicken in Roussillon, Québec, and the other (79287) was identified in a chicken dish prepared

by a restaurant/ready-to-eat food establishment in Montréal (Table 5). Both isolates were susceptible to all antimicrobial agents tested, carried none of the resistance genes screened and fell into the nonpathogenic virulence subgroup (Table 6).

4.6.7 Other clonal groups

The serotypes and PFGE patterns of the other clonal groups (Groups 7-17, Table 5) were variable. Among these, five clonal groups (Groups 7-11, Table 5) were homogenous with respect to phylotypes and MLSTs. One of these five clonal groups contained an *E. coli* isolate (EC01DT06-0649-01) characterized as O17/O73/O77:H18-ST69 [also known as CgA (48)], which was identified in a sample of retail meat pork in Montréal (Group 7, Table 5). This isolate exhibited some of the typical features of CgA. Notably, it showed resistance to TMP-SMX, and carried several virulence genes, including *iutA* (aerobactin receptor), *kpsM* II (group 2 capsule synthesis), *traT* (serum resistance-associated), and *ompT* (outer membrane protease), but lacked the F16 *papA* (P fimbriae structural subunit) allele (48;131). Five clonal groups (Groups 12-16, Table 5) only shared phylotypes and one clonal group (Group 17, Table 5) exhibited different MLSTs, serotypes, phylotypes, and PFGE patterns.

4.6.8 Phylogenetic typing

The clonal group isolates were equally distributed among the 4 major phylogenetic groups: half of the strains (49%) belonged to the virulence-associated groups B2 and D, and the remaining half (51%) belonged to the less virulent, commensal-associated groups A and B1. The majority of the strains belonging to phylogenetic group B2 were from Clonal groups 1 and 2 (12/14; 86%), whereas the majority of strains belonging to phylogenetic group D were from Clonal group 3 (9/14; 64%).

4.6.9 Virulence profiles

Extended virulence profiles were determined for 21 isolates from 7 highly related clonal groups (Groups 1-7) to assess their degree of within-group diversity and their virulence potential (Table 6). Of the 325 virulence genes included on the microarray, 85 (26%) were detected in at least one isolate. The following virulence genes were present in all tested isolates: *csgA* (curli structural subunit), *csgE* (curli assembly), *fimH* (type I fimbriae adhesin), *gad* (glutamate decarboxylase A), *ibeB* (invasion of brain endothelium), *ompA* (outer membrane protein), *artJ* (L-arginine periplasmic binding protein), *mviM*, and *mviN* (putative virulence factors). The aggregate virulence scores ranged from 12 to 38 (median 31). As a comparison, CFT073, a pyelonephritogenic *E. coli* isolate that was shown to be highly virulent in a mouse model of ascending UTI (132), had a virulence score of 35, whereas the nonpathogenic laboratory reference

strain *E. coli* K12 had a virulence score of 12 (126). The virulence scores did not differ significantly (P = 0.11) between resistant and susceptible isolates. Overall, virulence profiles were very similar within each clonal group, ranging from 77.4% to 98.8%. *E. coli* isolates classified as nonpathogenic belonged to phylogenetic groups A and B1, whereas pathotype-associated isolates belonged to phylogenetic groups B2 or D. The only exception was Clonal group 3: although isolates from this clonal group belonged to phylogenetic group D, they were classified as nonpathogenic.

5. DISCUSSION

The aim of this work was to investigate whether a food reservoir exists for *E. coli* causing UTIs. To this end, we used several genotyping methods to compare a large set of temporally and geographically matched *E. coli* strains obtained from food and human clinical sources. We identified *E. coli* isolates recovered from retail chicken and other food sources that were indistinguishable from or closely related to isolates recovered from human UTIs. Our *a priori* hypothesis, based on previous results from molecular epidemiologic studies, suggested that retail meat, specifically chicken meat, could be the main reservoir for *E. coli* causing human extraintestinal infections. This study is the first to report convincing evidence for this hypothesis, based on the high degree of genetic similarity observed between certain food and human UTI-causing isolates.

5.1 Antimicrobial resistance phenotypes and genotypes

Johnson and collaborators have shown that antimicrobial-resistant *E. coli* from human feces and bloodstream infections tend to be more similar, compared to their antimicrobial-susceptible counterparts, to antimicrobial-resistant and -susceptible *E. coli* from fecal and meat poultry sources (101;102). These findings suggested that the selection of resistant *E. coli* isolates is more likely to occur in the food animal reservoir, and subsequent contamination of poultry products may allow the transfer of

these antimicrobial-resistant strains to humans. In this study, we observed genetically related *E. coli* from food sources and human infections that were susceptible, implying that both resistant and susceptible extraintestinal infection-causing *E. coli* may be transmitted to humans via the food supply.

Twenty-nine percent of the 72 clonal group-associated isolates exhibited multidrug resistance. This phenotype may result from the acquisition of resistance gene cassettes through mobile genetic elements such as plasmids, transposons, and integrons (133;134). In fact, 62% of multidrug-resistant isolates possessed the class I integron gene *intI1*. The presence of such genetic elements may explain the occurrence of both susceptible and highly resistant *E. coli* in a same clonal group. Exposure to antimicrobial selection pressure in the human and animal reservoirs is likely to contribute to the acquisition or loss of resistance determinants, since they are easily exchanged between strains. This is in contrast to virulence genes, which are relatively stable over time. Further investigations into the presence or absence of other mobile genetic elements such as antimicrobial resistance plasmids among some of our *E. coli* strains are planned.

The accumulation of resistance genes on transferable DNA elements is a major obstacle for antimicrobial resistance control efforts. The horizontal transfer of mobile DNA elements in natural environments and in the intestinal flora of humans and food animals contributes to the

rapid dissemination of antimicrobial resistance genes in bacterial populations (135;136). Moreover, the physical linkage of multiple resistance genes on a single DNA element means that the use of a given antimicrobial agent may not only select for resistance to that agent, but also to a variety of others. Therefore, if the use of a certain antimicrobial agent is being restricted as part of a measure to limit the emergence of resistance, its associated resistance gene may still be co-selected and perpetuated via the use of other antimicrobial agents. This phenomenon explains the continuous presence of chloramphenicol resistance in food-producing animals 25 years after its ban as a growth promoter in Canada (137-140).

We noticed that all eight streptomycin-susceptible isolates that carried the *aadA* gene were also positive for *intI1*. Lanz *et al.* suggested that the *aadA* gene cassette may not be inserted in the integron structure of these isolates. The presence of such silent gene cassettes in susceptible isolates may eventually contribute to the emergence of new resistance phenotypes (141).

The molecular mechanisms underlying antimicrobial resistance are various and complex, and the presence or absence of a specific gene does not always accurately predict the resistance phenotype (133). In this study, the apparent absence of a resistance gene in a phenotypically resistant isolate may be explained by the presence of a resistance gene that was not assessed. These genes may include, but are not limited to

tet(Q) and *tet*(M) for resistance to tetracycline; *aacC2* for resistance to gentamicin; and *ampC* for resistance to ampicillin. Another explanation is that certain resistance phenotypes are caused by point mutations or resistance mechanisms such as efflux pump overexpression, rather than being caused by gene acquisition. In these cases, no associated resistance gene is to be expected with a resistant phenotype (142). The presence of a resistance gene in a correspondingly susceptible isolate may be the result of silent gene effects. Alternatively, it is possible that the breakpoints used for certain antimicrobials are not accurate, resulting in the misclassification of susceptible and resistant isolates. This might be the case for streptomycin, since previous studies have shown that streptomycin resistance genes are often detected in phenotypically susceptible isolates, which suggests that the breakpoint of ≥64 µg/µl used for this antimicrobial is too high for epidemiological purposes (141;143).

5.2 Identification of common genotypes between *E. coli* from human UTI and food sources

We identified members of the *E. coli* O25:H4-ST131 clonal group, which has recently been associated with ESBL production and fluoroquinolone resistance in several locations around world (67;77). Strains from this clonal group exhibited a robust and highly homogenous virulence gene profile, suggesting considerable extraintestinal pathogenic potential. ESBL production was not detected. Nonetheless, this clone

undoubtfully has the potential to acquire ESBL-encoding plasmids. *E. coli* belonging to clone O25:H4-ST131 but without CTX-M β-lactamase were also identified in the stools of healthy volunteers in Paris, France, and among fluoroquinolone-resistant UTI-causing isolates in Canada and Europe (46;67;144). These findings suggest that O25:H4-ST131 was already an established clone before it acquired the ability to produce CTX-M enzymes. To confirm this hypothesis, it would be interesting to compare our O25:H4-ST131 isolates to the European CTX-M-producing clone (77), in order to assess their degree of genetic relatedness.

The food source *E. coli* O25:H4-ST131 was recovered from retail chicken, suggesting that meat products may be an important reservoir implicated in the transmission and dissemination of this clonal group. Support to this hypothesis is provided by the recent findings of Cortés *et al.*, who identified genetically related *E. coli* O25:H4-ST131 producing CTX-M-9 from poultry farms and human extraintestinal infections (145). If the use of antimicrobials in food animal production promotes the selection of ESBL-producing *E. coli*, there may be subsequent amplification and transmission of these highly resistant organisms to consumers via the food chain (146).

Some of the virulence genes that have been previously associated with clonal group O25:H4-ST131 (73;77) were also detected in this study. These include *fimH* (type I fimbriae adhesin), *fyuA* (yersiniabactin receptor), *usp* (uropathogenic specific protein), *malX* (pathogenicity island marker), *kpsM* II (group 2 capsule synthesis), *ompT* (outer membrane

protease), and *traT* (serum resistance-associated). Several additional virulence genes were also detected.

E. coli strains of serotype O2, ST95 and phylogenetic group B2 have been previously associated with extraintestinal disease in both humans and avian hosts (92;95). In this study, we identified *E. coli* O2:H7-ST95 (from phylogroup B2) in food and human UTI samples; these isolates were genetically indistinguishable (or closely related) according to PFGE. Isolates from this clonal group also exhibited a wide range of virulence traits, many of which are frequently identified in both APEC and UPEC isolates, namely P (*papC* and *PapG* allele II) and type 1 (*fimH*) fimbriae, yersiniabactin (*fyuA*, *irp1* and *irp2*) and sit (*sitA* and *sitD*) operons, outer membrane protease (*ompT*), and serum resistance (*traT*) (88;92;95). Interestingly, the O2 serogroup is one of the most commonly occurring somatic antigens in both APEC and UPEC (34;40;88). Several studies have suggested, based on the similarities between APEC and human ExPEC, that APEC may be able to cross host boundaries and cause disease in humans (39;88-93). Our results provide additional support for this hypothesis.

The O2:H7-ST95 food source isolate identified in this study was from a ready-to-eat honeydew melon. The origin of this *E. coli* contamination could be a food handler with poor hygiene practices. However, given the APEC features of this isolate, cross-contamination in the kitchen with chicken products may be a more likely hypothesis.

5.3 Transfer of ExPEC from retail meat to prepared foods

The observation of several clonal groups containing isolates recovered from retail meats and meat-containing dishes (restaurant/ready-to-eat foods) confirms that prepared foods can be contaminated with *E. coli* originating from raw meats. Cross-contamination between different food items and undercooking of meats may allow the transfer and persistence of *E. coli* in foods and significantly contribute to the burden of foodborne disease.

5.4 Person-to-person transmission of ExPEC

The identification of two clonal groups containing closely related isolates from retail chicken meat and human UTI supports our *a priori* hypothesis. However, we cannot completely exclude the possibility that food source isolates originated from human contamination during food processing or handling, even though this type of contamination seems less likely than a contamination due to the animal's feces during the slaughtering process. Person-to-person transmission of ExPEC may presumably occur via the fecal-oral route, as is the case with bacillary dysentery caused by *Shigella* (147). In this case, food would serve as a vehicle for the transmission of ExPEC between an intestinally colonized food handler and the consumer. Upon ingestion of the contaminated food

item, the consumer will in turn become intestinally colonized with ExPEC and may, when risk factors such as sexual intercourse occur, go on to develop a urinary tract or other extraintestinal infection.

5.5 Virulence potential and phylogenetic background

The abundance of ExPEC-associated virulence genes carried by some of the isolates under study is of concern, because it suggests a high likelihood of virulence and extraintestinal pathogenic potential. *E. coli* from Clonal groups O25:H4-ST131, O2:H7-ST95, O114:H4-ST117, and O4:H5-ST493, as well as the CgA-associated Clonal group 7 exhibited high virulence scores (≥26). Interestingly, all of these groups belonged to phylotypes B2 and D, which corroborates previous findings associating virulent isolates with these phylogenetic groups (148).

There was no significant association between resistance status and virulence score. In fact, a substantial fraction of isolates (9/21; 43%) exhibited extensive virulence profiles, combined with resistance to first-line therapeutic agents. If such isolates are confirmed to originate from a food reservoir, it will represent a serious threat to public health.

5.6 Limitations of the study

This study was based on an ecologic design and samples from three distinct reservoirs were compared across a common geographic area and time-frame. Food-to-human transmission was not directly assessed. An epidemiologic study designed to measure the individual risk of UTI based on food exposure would have been ideal but extremely complex and difficult to perform. Epidemiologic information on the patients with UTI was not available. Information on travel history, antimicrobial use, diet, and other factors would have been useful to describe the study population and assess the significance of other possible transmission routes that might explain our results.

The study also oversampled retail chicken meat. Consequently, isolates from retail beef and pork were underrepresented. It is possible that clonal groups containing closely related isolates from human UTI and beef or pork samples would have been otherwise identified. There is insufficient power in our sampling strategy to exclude the existence of these groups. Despite oversampling isolates from retail chicken meat, we observed that 82% (a considerably greater fraction than the 61% initial sampling fraction) of retail meat isolates belonging to a clonal group were associated with chicken. We recently conducted a retrospective study involving additional sampling of *E. coli* from retail beef and pork which confirmed that these isolates are less clonally related to human UTI isolates than those from retail chicken (Prussing *et al.*, manuscript in

preparation). We also oversampled antimicrobial resistant isolates; however, the majority (53%) of isolates that belonged to a clonal group was fully susceptible.

Gene detection was used to make inferences regarding the virulence potential of the isolates under study. Although the best way to assess the pathogenicity of an isolate remains the use of an animal model, it has been shown that the virulence factor profile of an *E. coli* isolate is a good predictor of its *in vivo* pathogenicity in an animal model of extraintestinal infection (149). Additionally, Picard *et al.*, have suggested that the number of ExPEC virulence genes in an isolate is proportional to its pathogenic potential (150).

5.7 Strengths of the study

Strengths of the study include the substantial sample size, the systematic collection of UTI samples, the analysis of concurrent human and food isolates from the same geographic location, and the use of several different genotyping methods to compare the isolates. The systematic collection of all consecutive urine specimens from women presenting to the participating clinics with a suspected UTI is important in order to have a representative and unbiased sample of UTI. Because empirical therapy is the standard approach for the management of uncomplicated UTIs, the specimens that are normally collected and

transmitted to the clinical laboratory are more likely to be representative of women with recurrent or complicated UTI (151).

5.8 Perspectives

Our next step will be directed towards determining whether the *E. coli* that causes UTI originates from a food animal reservoir, or whether these *E. coli* are transmitted from person-to-person via food. In order to do so, we will collect *E. coli* isolates from abattoir sources. These isolates, in contrast to the *E. coli* recovered from retail meats, are very unlikely to be contaminated by food handlers, due to the strict sanitary measures in place in the slaughterhouses. The isolates will be retrospectively selected from the same time-period and geographical area as our human UTI set of isolates. Both groups will then be compared to assess their genetic similarity and determine which of the two proposed transmission pathways applies to ExPEC.

5.9 Significance and implications

Overall, a small proportion of analyzed isolates (72/844; 8.5%) were found to belong to a clonal group. This suggests that only a limited number of *E. coli* found in foods may have the ability to cause extraintestinal infections in humans. Nonetheless, the mere presence of ExPEC in a food reservoir is worrisome, given that these strains are able to cause mild to

life-threatening extraintestinal infections, especially if they are resistant to antibiotics of importance to human medicine. This underscores the significance of our findings in terms of food safety and public health.

We assembled a set of 844 *E. coli* isolates from different 3 sources in 2 provinces during a 3 year period. Given the ecologic design of the study, the fact that a retail chicken meat isolate and a human UTI isolate were found to be indistinguishable by PFGE is surprising and compelling. The genomic diversity within *E. coli* has been estimated to be extensive enough that the probability to obtain a PFGE match between two isolates from different environments by chance is extremely low (102). Indeed, PFGE is a highly discriminatory genotyping method; it is the gold standard to identify epidemic strains during outbreak investigations (123;152). Even though our total sample size represented only a tiny fraction of the *E. coli* found in foods and human extraintestinal infections, we were able to detect several instances of groups containing closely related isolates from both sources. It is therefore probable that a food reservoir exists and that foodborne transmission of ExPEC is not rare.

6. CONCLUSIONS

In summary, our findings provide compelling evidence that some *E. coli* from retail chicken meat and other food sources are closely related to *E. coli* causing human UTIs. Since a food animal reservoir apparently exists for *E. coli* causing urinary tract and other extraintestinal infections, this further reinforces the need for responsible antimicrobial stewardship in human and veterinary medicine, and particularly in food animal production, in order to limit the emergence and dissemination of therapy-refractory pathogens. Alternative measures to reduce the risk of infection or even eliminate these organisms from the food supply may include the promotion and adoption of more hygienic food-handling practices (86;87;153), and the irradiation of meat products prior to distribution (154).

7. FIGURES AND TABLES

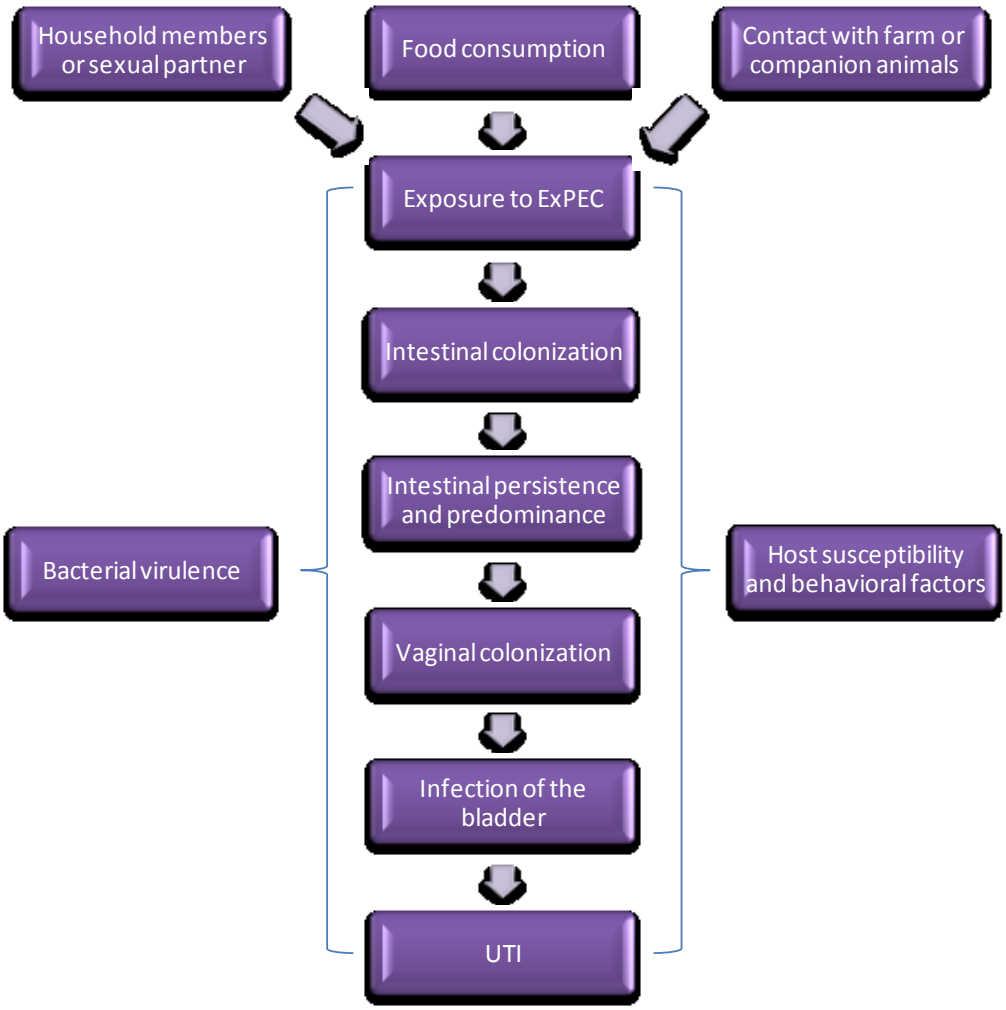

Figure 1. Pathogenesis of uncomplicated UTIs. Various types of exposure can lead to the intestinal acquisition of ExPEC. Once in the intestine, ExPEC will establish itself as part of an asymptomatic reservoir. Those strains that are able to persist, or alternatively predominate in the intestinal flora may, in certain conditions, be released from the intestine and colonize an extraintestinal body site, resulting in infection. In the case of an uncomplicated UTI, the pathogen will transit by the vaginal mucosa before reaching the urethra. Subsequent ascension into the urethra and colonization of the bladder will result in a UTI. In every case, disease development will be affected by behavioral factors and host susceptibility, as well as the degree of virulence of the bacteria.

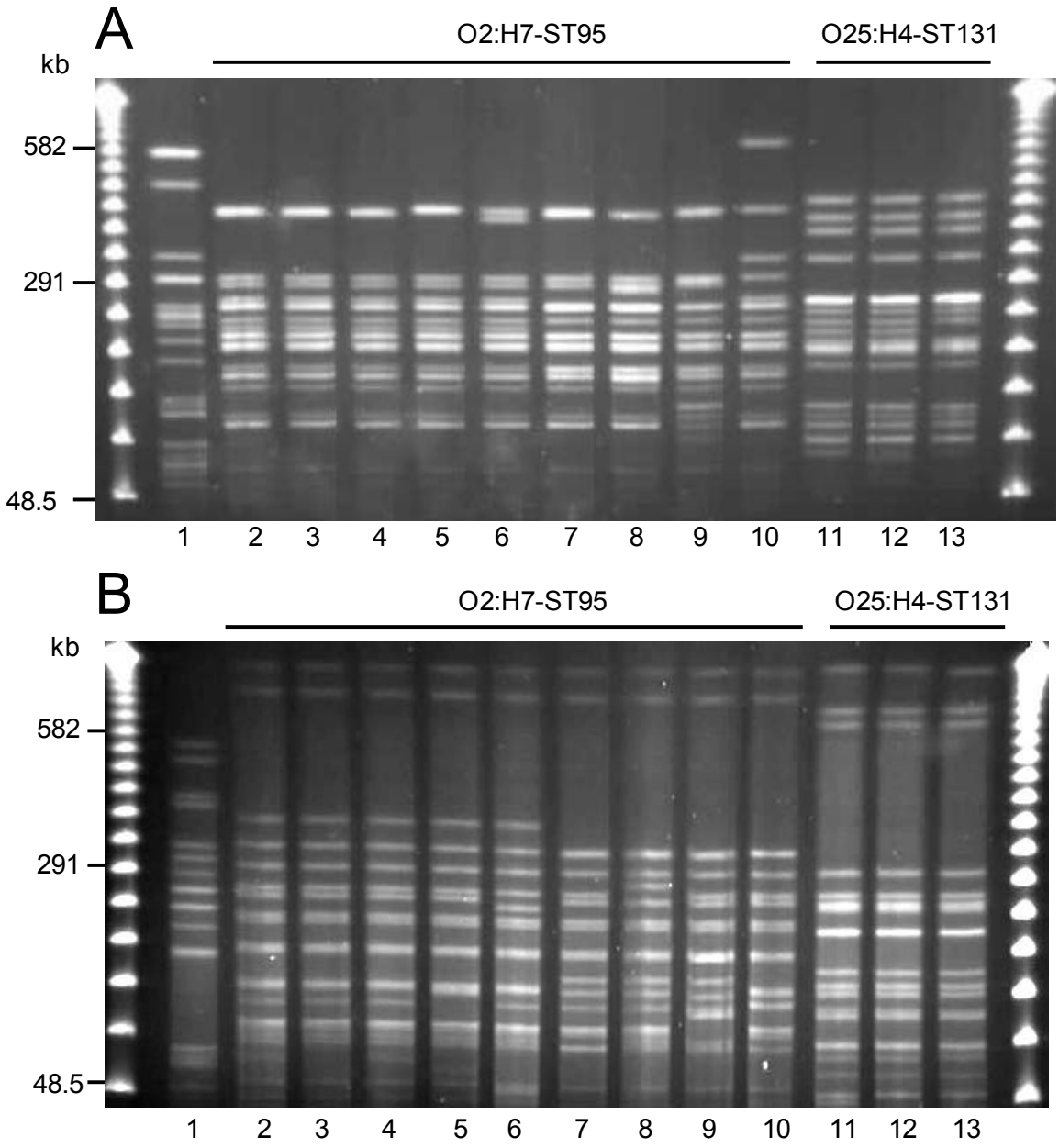

Figure 2. PFGE patterns for *E. coli* O2:H7-ST95 and *E. coli* O25:H4-ST131. A) *Xba*I; B) *Not*I. Lane 1 is the positive control *E. coli* O11:H18-ST69 (ATCC BAA-457); lane 2 is an *E. coli* O2:H7-ST95 isolate from a restaurant sample of honeydew melon (68616.01); lanes 3-10 are *E. coli* isolates from human UTI cases (lane 3, MSHS 100; lane 4, MSHS 186; lane 5, MSHS 811; lane 6, MSHS 1229; lane 7, MSHS 95; lane 8, MSHS 1062; lane 9, MSHS 782; lane 10, MSHS 819); lane 11 is an *E. coli* O25:H4-ST131 isolate from a retail chicken sample (EC01DT06-1737-01); and lanes 12 and 13 are *E. coli* isolates from human UTI cases (lane 12, MSHS 161; lane 13, MSHS 1134A). Outer lanes are pulsed-field molecular weight markers.

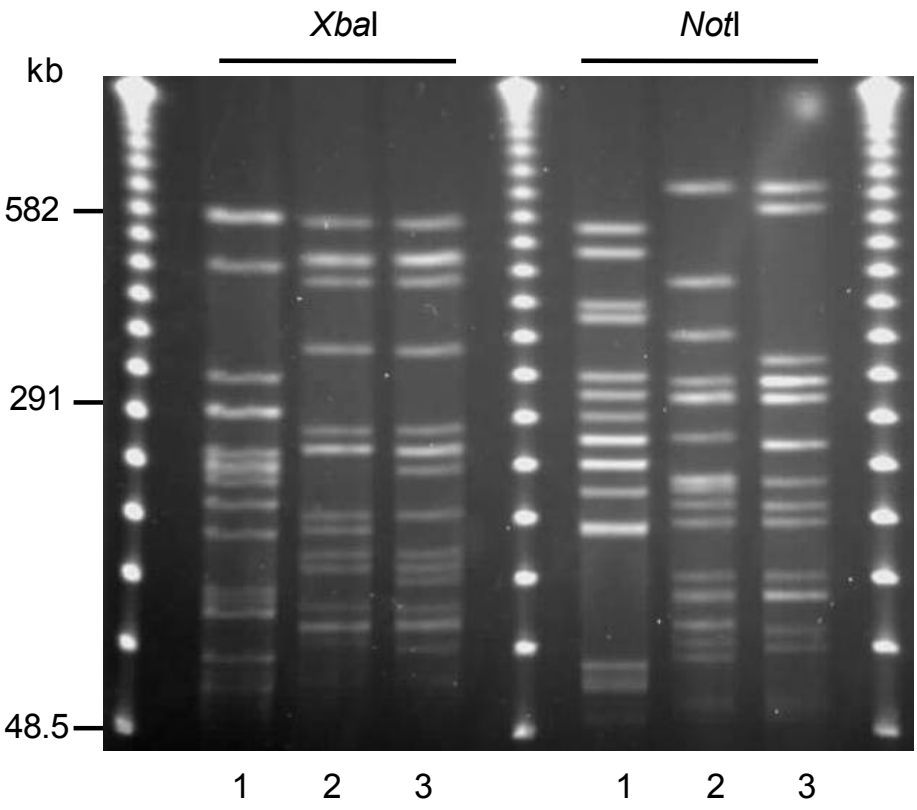

Figure 3. *Xba*I and *Not*I PFGE patterns for *E. coli* O114:H4-ST117 (lanes 2 and 3). Lane 1 is the positive control *E. coli* O11:H18-ST69 (ATCC BAA-457); lane 2 is an *E. coli* O114:H4-ST117 isolate from a retail chicken sample (EC01DT05-0789-01); and lane 3 is an *E. coli* isolate from a human UTI case (MSHS 1014A). Outer and center lanes are pulsed-field molecular weight markers.

Table 1. PCR conditions and control strains used for the detection of antimicrobial resistance genes in *E. coli* isolates

PCR	Gene	Primer sequence (5'-3')		Final primer concentration (µM)	Annealing temperature (°C)	Amplicon size (bp)	Positive control	Reference
		Forward	Reverse					
1	*sul1*	CGGCGTGGGCTACCTGAACG	GCCGATCGCGTGAAGTTCCG	0.2	66	433	AMR130	(119)
1	*sul2*	CGGCATCGTCAACATAACCT	TGTGCGGATGAAGTCAGCTC	0.3	66	721	AMR130	(119)
1	*sul3*	CAACGGAAGTGGGCGTTGTGGA	GCTGCACCAATTCGCTGAACG	0.2	66	244	RL0044	(119)
2	*tet*(A)	GGCGGTCTTCTTCATCATGC	CGGCAGGCAGAGCAAGTAGA	0.1	63	502	R08	(119)
2	*tet*(B)	CGCCCAGTGCTGTTGTTGTC	CGCGTTGAGAAGCTGAGGTG	0.2	63	173	PB#11	(119)
2	*tet*(C)	GCTGTAGGCATAGGCTTGGT	GCCGGAAGCGAGAAGAATCA	0.5	63	888	PB#2	(119)
3	*aadA*	GTGGATGGCGGCCTGAAGCC	AATGCCCAGTCGGCAGCG	0.1	63	525	AMR075	(119)
3	*strA/strB*	ATGGTGGACCCTAAAACTCT	CGTCTAGGATCGAGACAAAG	0.4	63	893	AMR075	(119)
3	*aac*(3)IV	TGCTGGTCCACAGCTCCTTC	CGGATGCAGGAAGATCAA	0.2	63	653	AMR075	(119)
4	*aphA1*	ATGGGCTCGCGATAATGTC	CTCACCGAGGCAGTTCCAT	0.4	55	600	AMR61	(119)
4	*aphA2*	GATTGAACAAGATGGATTGC	CCATGATGGATACTTTCTCG	0.1	55	347	AMR20	(119)
4	*aadB*	GAGGAGTTGGACTATGGATT	CTTCATCGGCATAGTAAAAG	0.2	55	208	TN1409	(119)
5	bla_{CMY-2}	GACAGCCTCTTTCTCCACA	TGGACACGAAGGCTACGTA	0.2	62	1000	R1414	(119)
5	bla_{SHV}	AGGATTGACTGCCTTTTTG	ATTTGCTGATTTCGCTCG	0.4	62	393	SHV4339	(119)
5	bla_{OXA-1}	TATCTACAGCAGCGCCAGTG	CGCATCAAATGCCATAAGTG	0.1	62	199	RI0035	(120)
6	bla_{TEM}	TTAACTGGCGAACTACTTAC	GTCTATTTCGTTCATCCATA	0.2	55	247	TEM4676	(119)
7	*intI1*	CGGAATGGCCGAGCAGATC	CGCAACTGGTCCAGAACCTTG	0.25	68	853	RL079	(121)
8	*dhfrI*	AATGGTAGCTATATCGAAGAATGGA	CCCTTTTGCCAGATTTGGTAACTAT	0.25	60	462	AMR042	This study
9	*dhfrIb*/*dhfrXIV**	TCATTGATRGCTGCGAAAGC	CCCTTTTTCCAAATTTGATAGC	0.25	54	461	RL0139	This study
10	*dhfrV*	GGCTGCAAAAGCGAAAAACG	CCCTTTTGCCAAATTTGATAGC	0.25	58	453	RL0456	This study
11	*dhfrIX*	GGCTTCTCTAAACATGATTGTCG	TCAGTAATGGTCGGGACCTC	0.25	53	462	C600	This study
12	*dhfrXII*	TTTATCTCGTTGCTGCGATG	AGGCTTGCCGATAGACTCAAG	0.25	55	155	AMR588	This study
13	*dhfrXIII*	GAATCGGTCCGCATTTATCTG	GGAGTGCGTGTACGTGATTGT	0.25	54	465	dhfr13	This study
14	*dhfrXVI*	GCCAAGTCGAAGAACGGTAT	TTAACTCTTTTGCCAGATTTGAT	0.25	54	474	RI0164	This study
15	*dhfrXVII*	GAAAATATCATTGATTTCTGCAGTG	TTTTTCCAAATCTGGTATGTATAATTT	0.25	54	474	RI0254	This study

**dhfrIb* and *dhfrXIV* were amplified with the same primer pair and the amplicon, if present, was sequenced to identify the corresponding gene variant.

Table 2. Sources of 844 *E. coli* isolates collected and analyzed, by year and location

Source	Total no. (%)	Year, no. (%) isolates			Location, no. (%) isolates		
		2005	2006	2007	Quebec	Ontario	Other†
Human							
Urinary tract infection	353 (42)	103 (29)	175 (50)	75 (21)	353 (100)	0	0
Retail meat							
All	417 (49)	178 (43)	158 (38)	81 (19)	264 (63)	139 (33)	14 (3)
Chicken	253 (61)	107 (42)	101 (40)	45 (18)	141 (56)	99 (39)	13 (5)
Beef	82 (20)	37 (45)	26 (32)	19 (23)	81 (99)	1 (1)	0
Pork	82 (20)	34 (41)	31 (38)	17 (21)	42 (51)	39 (48)	1 (1)
Restaurant/Ready-to-eat foods							
All	74 (9)	19 (26)	33 (45)	22 (30)	74 (100)	0	0
Chicken	21 (28)	7 (33)	6 (29)	8 (38)	21 (100)	0	0
Beef	13 (18)	3 (23)	6 (46)	4 (31)	13 (100)	0	0
Pork	5 (7)	0	4 (80)	1 (20)	5 (100)	0	0
Fish/seafood	6 (8)	2 (33)	2 (33)	2 (33)	6 (100)	0	0
Other meat‡	9 (12)	1 (11)	7 (78)	1 (11)	9 (100)	0	0
Other food§	20 (27)	6 (30)	8 (40)	6 (30)	20 (100)	0	0
Total	844 (100)	300 (36)	366 (43)	178 (21)	691 (82)	139 (16)	14 (2)

†Other location includes British Columbia (n = 4) and Saskatchewan (n = 10).

‡Other meat includes bison, lamb, duck, and snail.

§Other food includes fruits (honeydew melon), vegetables, cheese, rice, cousous, and pasta.

Table 3. Frequencies of antimicrobial resistance among 72 *E. coli* isolates from clonal groups identified within 3 sources*

Antimicrobial agent†	Source, no. (%) isolates			
	Total (n = 72)	Human UTI (n = 25)	Retail meat (n = 39)‡	RRE (n = 8)
Amoxicillin-clavulanic acid	4 (6)	0	4 (10)	0
Ampicillin	20 (28)	9 (36)	11 (28)	0
Cefoxitin	3 (4)	0	3 (8)	0
Ceftiofur	3 (4)	0	3 (8)	0
Chloramphenicol	2 (3)	2 (8)	0	0
Ciprofloxacin	1 (1)	1 (4)	0	0
Gentamicin	8 (11)	2 (8)	6 (15)	0
Kanamycin	5 (7)	1 (4)	4 (10)	0
Nalidixic acid	8 (11)	4 (16)	4 (10)	0
Streptomycin	10 (14)	4 (16)	6 (15)	0
Sulfisoxazole	22 (31)	8 (32)	14 (36)	0
Tetracycline	25 (35)	10 (40)	14 (36)	1 (13)
Trimethoprim-sulfamethoxazole	10 (14)	6 (24)	4 (10)	0
Resistance to ≥ 1 antimicrobial agent	34 (47)	13 (52)	20 (51)	1 (13)
Multidrug resistance§	21 (29)	8 (32)	13 (33)	0

*UTI, urinary tract infection; RRE, restaurant/ready-to-eat foods.
†Resistance to amikacin or ceftriaxone was not detected in any *E. coli* isolate.
‡Retail meat includes chicken (n=32), beef (n=4), and pork (n=3).
§Defined as resistance to ≥ 3 antimicrobial classes.

Table 4. Frequencies of antimicrobial resistance genes in 72 *E. coli* isolates from clonal groups identified within 3 sources*

Target	Resistance gene†	Source, no. (%) isolates			
		Total (n = 72)	Human UTI (n = 25)	Retail meat (n = 39)‡	RRE (n = 8)
Sulfonamides	*sul1*	13 (18)	4 (16)	9 (23)	ND
	sul2	10 (14)	4 (16)	6 (15)	ND
Tetracycline	*tet*(A)	15 (21)	4 (16)	11 (28)	1 (13)
	tet(B)	8 (11)	5 (20)	3 (8)	ND
	tet(C)	2 (3)	ND	2 (5)	ND
Ampicillin	bla_{CMY-2}	3 (4)	ND	3 (8)	ND
	bla_{SHV}	2 (3)	1 (4)	1 (3)	ND
	bla_{OXA-1}	1 (1)	1 (4)	ND	ND
	bla_{TEM}	13 (18)	8 (32)	5 (13)	ND
Kanamycin	*aphA1*	4 (16)	1 (4)	3 (8)	ND
	aphA2	1 (1)	ND	1 (3)	ND
Streptomycin	*strA*/*strB*	13 (18)	5 (20)	8 (21)	ND
	aadA	12 (17)	3 (12)	9 (23)	ND
Class I integron	*intI1*	16 (22)	5 (20)	11 (28)	ND
Trimethoprim	*dhfrI*	2 (3)	1 (4)	1 (3)	ND
	dhfrV	1 (1)	1 (4)	ND	ND
	dhfrXII	2 (3)	1 (4)	1 (3)	ND
	dhfrXIV	3 (4)	1 (4)	2 (5)	ND
	dhfrXVII	2 (3)	2 (8)	ND	ND
Total no. (%) of isolates positive for any resistance gene		34 (47)	12 (48)	22 (56)	1 (13)

*UTI, urinary tract infection; RRE, restaurant/ready-to-eat foods; ND, not detected.

†The resistance genes *sul3*, *aadB*, *acc*(3)IV, *dhfrIb*, *dhfrIX*, *dhfrXIII* and *dhfrXVI* were not detected in any *E. coli* isolates.

‡Retail meat includes chicken (n=32), beef (n=4), and pork (n=3).

Table 5. Characteristics of *E. coli* clonal groups identified within isolates from 3 sources (human clinical, retail meat and restaurant/ready-to-eat foods)*

Clonal group and strain	Isolate source	Type of sample	Location†	Year	Genotype			MLST		Serotype	Phylo
					MLVA	ERIC2 PCR	*Xba*I PFGE	ST	ST complex		
1											
EC01DT06-1737-01	Retail meat	Chicken	Montréal	2006	1.033	33.01	33A.0	131	None	O25:H4	B2
MSHS 161	Human	UTI	Montréal	2005	1.033	33.01	33A.0	131	None	O25:H4	B2
MSHS 1134A	Human	UTI	Montréal	2007	1.033	33.01	33A.1	131	None	O25:H4	B2
2											
68616.01	RRE	Honeydew	Montréal	2005	1.018	18.01	18A.0	95	95	O2:H7	B2
MSHS 100	Human	UTI	Montréal	2005	1.018	18.01	18A.0	95	95	O2:H7	B2
MSHS 186	Human	UTI	Montréal	2005	1.018	18.01	18A.0	95	95	O2:H7	B2
MSHS 811	Human	UTI	Montréal	2006	1.018	18.01	18A.0	95	95	O2:H7	B2
MSHS 1229	Human	UTI	Montréal	2007	1.018	18.01	18A.1	95	95	O2:H7	B2
MSHS 95	Human	UTI	Montréal	2005	1.018	18.01	18A.2	95	95	O2:H7	B2
MSHS 1062	Human	UTI	Montréal	2007	1.018	18.01	18A.2	95	95	O2:NM	B2
MSHS 782	Human	UTI	Montréal	2006	1.018	18.01	18A.4	95	95	O2:H7	B2
MSHS 819	Human	UTI	Montréal	2006	1.018	18.01	18A.4	95	95	O2:H7	B2
3											
EC01DT05-0789-01	Retail meat	Chicken	Ontario	2005	1.023	23.01	23A.0	117	None	O114:H4	D
MSHS 1014A	Human	UTI	Montréal	2007	1.023	23.01	23A.5	117	None	O114:H4	D
EC01DT05-0224-01	Retail meat	Chicken	Ontario	2005	1.023	23.01	23B	117	None	ONT:NM	D
EC01DT06-1887-01	Retail meat	Chicken	Montréal	2006	1.023	23.01	23C	117	None	O143:H4	D
EC01DT07-0956-01	Retail meat	Chicken	Other	2007	1.023	23.01	23D	117	None	O53:H4	D
EC01DT05-1700-01	Retail meat	Chicken	Quebec	2005	1.023	23.01	NT	117	None	O160:H4	D
EC01DT07-1050-01	Retail meat	Chicken	Ontario	2007	1.023	23.01	NT	117	None	O45:H4	D
EC01DT07-1090-01	Retail meat	Chicken	Montréal	2007	1.023	23.01	NT	117	None	O24:H4	D
MSHS 133	Human	UTI	Montréal	2005	1.023	23.01	NT	117	None	O24:NM	D
4											
EC01DT05-1012-01	Retail meat	Pork	Ontario	2005	1.102	102	102A	493	12	O4:H5	B2
MSHS 769	Human	UTI	Montréal	2006	1.102	102	102B	493	12	O4:H5	B2
5											
EC01DT06-1265-01	Retail meat	Beef	Montréal	2006	2.107	107	107A	401	None	O36:NM	A
76083.08	RRE	Chicken	Montréal	2007	2.107	107	107B	401	None	O36:NM	A
6											
EC01DT06-0274-01	Retail meat	Chicken	Quebec	2006	2.097	97.01	97A	295	None	O172:H16	B1
79287	RRE	Chicken	Montréal	2007	2.097	97.01	97B	295	None	O172:H16	B1
7											
EC01DT06-0649-01	Retail meat	Pork	Montréal	2006	1.116	116	116A	69	69	O17/73/106:H18	D
MSHS 719	Human	UTI	Montréal	2006	1.116	116	116C	69	69	O44:H18	D
MSHS 956	Human	UTI	Montréal	2007	1.116	116	116D	69	69	ONT:H18	D
8											
EC01DT06-0604-01	Retail meat	Chicken	Other	2006	1.037	37.01	NT	648	None	O49:H10	D
CLSC 36	Human	UTI	Montréal	2005	1.037	37.01	37A	648	None	O1:H42	D
9											
EC01DT06-0006-01	Retail meat	Chicken	Quebec	2006	1.002	2.01	2A	746	None	O33:NM	A
MSHS 624	Human	UTI	Montréal	2006	1.002	2.01	2B	746	None	O20:H4	A
10											
EC01DT05-0408-01	Retail meat	Chicken	Montréal	2005	1.003	2.01	2C	10	10	O153:NM	A
EC01DT05-1925-01	Retail meat	Chicken	Quebec	2005	1.003	2.01	2D	10	10	O106:H4	A
EC01DT06-1546-01	Retail meat	Chicken	Montréal	2006	1.003	2.01	2E	UNK	None	O21:H25	A
EC01DT07-0491-01	Retail meat	Chicken	Montréal	2007	1.003	2.01	2F	10	10	ONT:H4	A
EC01DT07-1162-01	Retail meat	Chicken	Ontario	2007	1.003	2.01	2G	10	10	ONT:NM	A
MSHS 233	Human	UTI	Montréal	2005	1.003	2.01	2H	10	10	ONT:H32	A
MSHS 892	Human	UTI	Montréal	2006	1.003	2.01	2I	10	10	O101:NM	A
11											
EC01DT05-0420-01	Retail meat	Beef	Montréal	2005	2.061	2.01	2J	10	10	ONT:H27	A
77392	RRE	Beef	Montréal	2007	2.061	2.01	2K	10	10	O71:H32	A
12											
EC01DT06-0907-01	Retail meat	Beef	Montréal	2006	1.015	15.01	15A	278	278	O178:H7	B1
MSHS 1118	Human	UTI	Montréal	2007	1.015	15.01	15B	196	None	O8:H7	B1
13											
EC01DT06-1854-01	Retail meat	Chicken	Ontario	2006	1.106	106	106A	446	446	O153:H8	B1
CLSC 95	Human	UTI	Montréal	2006	1.106	106	106B	58	155	O36:H25	B1
14											
EC01DT05-1261-01	Retail meat	Chicken	Montréal	2005	1.106	113	113A	101	101	O29:H12	B1
EC01DT05-1455-01	Retail meat	Chicken	Montréal	2005	1.106	113	113B	UNK	None	O157:H10	B1
EC01DT06-0760-01	Retail meat	Chicken	Montréal	2006	1.113	113	113C	602	446	O102:H21	B1
MSHS 472	Human	UTI	Montréal	2006	1.113	113	113D	101	101	O82:NM	B1
15											
EC01DT05-2243-01	Retail meat	Chicken	Ontario	2005	2.024	24.01	24A	641	86	O30:H25	B1
53573.29	RRE	Chicken	Montréal	2005	2.024	24.01	24B	711	None	O120:H10	B1
16											
EC01DT05-0925-01	Retail meat	Beef	Montréal	2005	2.112	112	112A	UNK	None	O107:H7	B1
73073	RRE	Snail	Montréal	2006	2.112	112	112B	58	155	O154:H25	B1
17											
EC01DT05-0469-01	Retail meat	Pork	Ontario	2005	1.047	15.01	15C	642	278	O105:H4	B1
MSHS 689	Human	UTI	Montréal	2006	1.047	15.01	15D	UNK	None	O174:H7	A

*MLVA, multilocus variable number tandem repeat analysis; ERIC2, enterobacterial intergenic consensus sequence 2; PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; ST, sequence type; RRE, restaurant/ready-to-eat foods; UTI, urinary tract infection; Phylo, phylotype; NT, nontypeable; UNK, unknown; NM, non-motile; ONT, serogroup nontypeable.

†Montréal = city of Montréal; Quebec = province of Quebec, outside Montréal; Ontario = province of Ontario; Other = Saskatchewan and Britsh Columbia.

Table 6. Pathotypes and virulence scores nd genotypes of *E. coli* clonal groups identified w ithin isolates from 3 sources (human clinical, retail meat and restaurant/ready-to-eat foods)*†

Virulence Genotype§ — Adhesins

Clonal group and strain	Pathotype‡	Virulence score	*csgA*	*csgE*	*fimA*	*fimH*	*f165(1)A*	*iha*	*lpfA* (O113)	*pixA*	*papA* (F9)	*papA* (F10)	*papA* (F11)	*papA* (F13)	*papA* (F16)	*papC*	*papG* I	*papG* II	*papG* III	*papG* IV	*focA*	*focG*	*sfaD*	*pilL*
1																								
EC01DT06-1737-01	ExPEC; MNEC	32	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MSHS 161	ExPEC; MNEC	32	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MSHS 1134A	ExPEC; MNEC	31	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2																								
68616.01	ExPEC; UPEC; APEC	31	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	+
MSHS 100	ExPEC; UPEC; APEC	32	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-
MSHS 186	ExPEC; UPEC; APEC	30	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	+
MSHS 811	ExPEC; UPEC; APEC	32	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-
MSHS 1229	ExPEC; UPEC; APEC	30	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	+
MSHS 95	ExPEC; UPEC; APEC	31	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-
MSHS 782	ExPEC; UPEC; APEC	28	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-
3																								
EC01DT05-0789-01	nonpathogenic	31	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MSHS 1014A	nonpathogenic	35	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4																								
EC01DT05-1012-01	ExPEC; UPEC; APEC	35	+	+	+	+	-	-	-	-	-	+	-	-	-	+	+	-	+	-	-	-	+	-
MSHS 769	ExPEC	38	+	+	+	+	-	-	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+	-
5																								
EC01DT06-1265-01	nonpathogenic	14	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76083.08	nonpathogenic	12	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6																								
EC01DT06-0274-01	nonpathogenic	17	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79287	nonpathogenic	17	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7																								
EC01DT06-0649-01	ExPEC	26	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MSHS 719	ExPEC; APEC	28	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
MSHS 956	ExPEC; APEC	33	+	+	+	+	-	+	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-

Virulence Genotype§ — Colicins and microcins; Toxins; Iron acquisition and transport systems

Clonal group and strain	*cia*	*cvaC*	*mchB*	*mcbA*	*cka*	*ce1a*	*astA*	*LT-IIbB*	*sat*	*cnf1*	*chuA*	*fepC*	*iroN*	*fyuA*	*irp1*	*irp2*	*iucD*	*iutA*	*sitA*	*sitD*
1																				
EC01DT06-1737-01	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-	-	+	+
MSHS 161	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-	-	+	+
MSHS 1134A	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+
2																				
68616.01	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
MSHS 100	-	-	+	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
MSHS 186	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
MSHS 811	-	-	-	+	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
MSHS 1229	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
MSHS 95	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
MSHS 782	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+
3																				
EC01DT05-0789-01	-	-	+	-	-	-	-	+	-	-	+	+	-	+	+	+	+	+	+	+
MSHS 1014A	-	+	+	-	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+
4																				
EC01DT05-1012-01	-	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+
MSHS 769	-	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+
5																				
EC01DT06-1265-01	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76083.08	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6																				
EC01DT06-0274-01	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79287	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7																				
EC01DT06-0649-01	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+
MSHS 719	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+
MSHS 956	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+

Virulence Genotype§ — Capsular and somatic antigens; Hemolysins and hemagglutinins

Clonal group and strain	*kpsM* II	*kpsM* III	*neuA*	*neuC*	K5 *kfiB*	*rfc*	*wzy* (O114)	*hlyA*	*hlyE*	*hra*	*tsh*	*vat*
1												
EC01DT06-1737-01	+	-	+	+	-	-	-	-	+	-	-	-
MSHS 161	+	-	+	+	-	-	-	-	+	-	-	-
MSHS 1134A	+	-	+	+	-	-	-	-	+	-	-	-
2												
68616.01	+	-	+	+	-	-	-	-	+	-	-	+
MSHS 100	+	-	+	+	-	-	-	-	+	-	-	+
MSHS 186	+	-	+	+	-	-	-	-	+	-	-	+
MSHS 811	+	-	+	+	-	-	-	-	+	-	-	+
MSHS 1229	+	-	+	+	-	-	-	-	+	-	-	+
MSHS 95	+	-	+	+	-	-	-	-	+	-	-	+
MSHS 782	+	-	+	+	-	-	-	-	+	-	-	+
3												
EC01DT05-0789-01	-	-	-	-	-	-	+	-	+	-	-	+
MSHS 1014A	-	-	-	-	-	-	+	-	+	-	+	+
4												
EC01DT05-1012-01	+	-	-	-	-	-	-	+	+	+	-	+
MSHS 769	-	+	-	-	-	+	-	+	+	+	-	+
5												
EC01DT06-1265-01	-	-	-	-	-	-	-	-	+	-	-	-
76083.08	-	-	-	-	-	-	-	-	+	-	-	-
6												
EC01DT06-0274-01	-	-	-	-	-	-	-	-	+	-	-	-
79287	-	-	-	-	-	-	-	-	+	-	-	-
7												
EC01DT06-0649-01	+	-	-	-	-	-	-	-	+	-	-	-
MSHS 719	+	-	-	-	+	-	-	-	-	-	-	-
MSHS 956	+	-	-	-	-	-	-	-	+	-	-	-

Virulence Genotype§ — Various functions

Clonal group and strain	*agn43*	*ccdB*	*deoK*	*fliC*	H7 *fliC*	*flmA54*	*gad*	*ibeA*	*ibeB*	*iss*	*malX*	*ompA*	*ompT*	*senB*	*pic*	*traT*
1																
EC01DT06-1737-01	-	-	+	+	-	-	+	+	+	+	+	+	+	-	-	+
MSHS 161	-	-	+	+	-	-	+	+	+	+	+	+	+	-	-	+
MSHS 1134A	-	-	+	+	-	-	+	+	+	+	+	+	+	-	-	+
2																
68616.01	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
MSHS 100	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
MSHS 186	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
MSHS 811	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
MSHS 1229	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
MSHS 95	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
MSHS 782	-	-	+	+	+	-	+	-	+	+	+	+	+	+	-	+
3																
EC01DT05-0789-01	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+
MSHS 1014A	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+
4																
EC01DT05-1012-01	+	-	-	+	-	-	+	-	+	+	+	+	+	-	-	-
MSHS 769	+	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+
5																
EC01DT06-1265-01	-	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-
76083.08	-	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-
6																
EC01DT06-0274-01	-	-	-	-	-	+	+	-	+	+	-	+	+	-	-	-
79287	-	+	-	-	-	+	+	-	+	+	-	+	+	-	-	+
7																
EC01DT06-0649-01	-	-	-	+	-	-	+	-	+	+	-	+	+	-	-	+
MSHS 719	+	+	-	+	-	-	+	-	+	+	-	+	+	+	-	+
MSHS 956	+	+	-	+	-	-	+	-	+	+	-	+	+	+	-	+

Virulence Genotype§ — New ly recognized or putative *E. coli* virulence genes; ETT2 elements

Clonal group and strain	*artJ*	*b1121*	*b1432*	*gimB*	*iol*	*mviM*	*mviN*	*rtx*	*ureD*	*usp*	*eivG*	*eprJ*	*spaS* (*eprS*)	*z4184*
1														
EC01DT06-1737-01	+	+	+	-	-	+	+	-	+	+	-	-	-	-
MSHS 161	+	+	+	-	-	+	+	-	+	+	-	-	-	-
MSHS 1134A	+	+	+	-	-	+	+	-	+	+	-	-	-	-
2														
68616.01	+	+	-	+	-	+	+	-	+	+	-	-	-	-
MSHS 100	+	+	-	+	+	+	+	-	+	+	-	-	-	-
MSHS 186	+	+	-	+	-	+	+	-	-	+	-	-	-	-
MSHS 811	+	+	-	+	+	+	+	-	+	+	-	-	-	-
MSHS 1229	+	+	-	+	-	+	+	-	-	+	-	-	-	-
MSHS 95	+	+	-	+	+	+	+	-	+	+	-	-	-	-
MSHS 782	+	+	-	+	-	+	+	-	-	+	-	-	-	-
3														
EC01DT05-0789-01	+	+	-	-	+	+	+	-	+	-	-	-	-	-
MSHS 1014A	+	+	-	-	+	+	+	-	+	-	-	-	-	-
4														
EC01DT05-1012-01	+	+	+	+	+	+	+	-	+	+	-	-	-	-
MSHS 769	+	+	+	+	+	+	+	-	+	+	-	-	-	-
5														
EC01DT06-1265-01	+	-	-	-	+	+	+	-	+	-	-	+	+	+
76083.08	+	-	-	-	-	+	+	-	-	-	-	+	+	+
6														
EC01DT06-0274-01	+	+	-	-	-	+	+	-	-	-	-	+	-	-
79287	+	+	-	-	+	+	+	-	-	-	-	+	+	+
7														
EC01DT06-0649-01	+	+	-	-	+	+	+	+	+	-	-	-	-	-
MSHS 719	+	+	-	-	+	+	+	-	-	-	+	+	+	+
MSHS 956	+	+	-	-	+	+	+	+	+	-	+	+	+	+

*ST, sequence type; ETT2, *E. coli* type III secretion system 2.

†All isolates in Clonal group 1 w ere characterized as O25:H4-ST131, phylotype B2; all isolates in Clonal group 2 w ere characterized as O2:H7-ST95, phylotype B2; all isolates in Clonal group 3 w ere characterized as O114:H4-ST117, phylotype D; all isolates in Clonal group 4 w ere characterized as O4:H5-ST493, phylotype B2; all isolates in Clonal group 5 w ere characterized as O36:NM-ST401, phylotype A; all isolates in Clonal group 6 w ere characterized as O172:H16-ST295, phylotype B1; isolate EC01DT06-0649-01 in Clonal group 7 w as characterized as O17/73/106:H18-ST69 phylotype D; and isolates MSHS 719 and MSHS 956 in Clonal group 7 w ere characterized as O44/ONT:H18-ST69, phylotype D.

‡Pathotypes w ere attributed to each *E. coli* isolate according to its set of virulence genes or markers (see methods section). ExPEC, extraintestinal pathogenic *E. coli* ; MNEC, meningitis-associated *E. coli* ; UPEC, uropathogenic *E. coli* ; APEC, avian pathogenic *E. coli* .

§Only those virulence genes that w ere positive for at least one isolate are show n. *csgA* , curli structural subunit; *csgE* , curli assembly; *fimA* , type 1 fimbriae structural subunit; *fimH* , type 1 fimbriae adhesin; *f165(1)A* , F165(1) fimbriae structural subunit; *iha* , adhesin-siderophore; *lpfA* (O113), LPF fimbriae structural subunit variant; *pixA* , Pix pili structural subunit; *papA* , P fimbriae structural subunit, w ith its F9, F10, F11, F13, and F16 alleles; *papC* , P fimbriae assembly; *papG* alleles I, II, III, and IV, P fimbriae adhesin variants; *focA* , F1C fimbriae major structural subunit; *focG* , F1C fimbriae minor structural subunit; *sfaD* , S fimbriae assembly; *pilL* , type IV pilus lipoprotein; *cia* , colicin Ia; *cvaC* , microcin V; *mchB* , microcin H47; *mcbA* , microcin B17; *cka* , colicin K; *ce1a* , colicin E1; *astA* , enteroaggregative *E. coli* heat-stable toxin; *LT-IIbB* , heat-labile enterotoxin type IIb, subunit B; *sat* , secreted autotransporter toxin; *cnf1* , cytotoxic necrotizing factor 1; *chuA* , heme receptor; *fepC* , enterobactin transport; *iroN* , salmochelin receptor; *fyuA* , yersiniabactin receptor; *irp1* and *irp2* , yersiniabactin biosynthetic proteins; *iucD* , aerobactin biosynthesis; *iutA* , aerobactin receptor; *sitA* ar *sitD* , iron and manganese ABC transporters; *kpsM* II, group 2 capsule synthesis; *kpsM* III, group 3 capsule synthesis; *neuA* and *neuC* , K1 capsule biosynthesis; K5 *kfiB* , K5 group 2 capsule variant; *rfc* , O4 lipopolysaccharide; *wzy* (O114), O114 lipopolysaccharide; *hlyA* , alpha hemolysin; *hlyE* , silent hemolysin; *hra* , heat-resistant agglutinin; *tsh* , temperature-sensitive hemagglutinin; *vat* , vacuolating autotransporter toxin; *agn43* , antigen 43 precursor; *ccdB* , F-plasmid-encoded cytotoxic protein; *deoK* , deoxyribokinase; *fliC* , flagellin structural subunit; H7 *fliC* , flagellin variant; *flmA54* , flagellin variant; *gad* , glutamate decarboxylase A; *ibeA* and *ibeB* , invasion of brain endothelium; *iss* , increased serum survival; *malX* , pathogenicity island marker; *ompA* , outer membrane protein; *ompT* , outer membrane protease; *senB* , enteroinvasive *E. coli* enterotoxin production; *pic* , autotransporter protease; *traT* , serum resistance-associated; *artJ* , L-arginine periplasmic binding protein; *b1121* and *b1432* , putative virulence factors; *gimB* , genetic island; *iol* , myo-inositol uptake; *mviM* and *mviN* , putative virulence factors; *rtx* , putative RTX family exoprotein; *ureD* , urease-associat *usp* , uropathogenic specific protein; *eivG* , *eprJ* and *spaS* , *E. coli* type III secretion system 2 proteins; *z4184* , *E. coli* type III secretion system 2 transcriptional regulator.

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