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Retail Meat Consumption and the Acquisition of Antimicrobial Resistant *Escherichia coli* Causing Urinary Tract Infections: A Case–Control Study

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Abstract

Background. The increasing incidence of community-acquired urinary tract infections (UTIs) caused by antimicrobial resistant *Escherichia coli*, and observations of potential outbreaks of UTI-causing *E. coli*, suggest that food may be an important source of *E. coli* in women who develop UTI. We sought to determine if acquisition of and infection with a UTI-causing, antimicrobial resistant *E. coli* isolate is associated with a woman's dietary habits, specifically her preparation and consumption of retail meat products. *Methods.* Between April 2003 and June 2004, a case–control study was conducted. The dietary habits of women with UTI caused by an antimicrobial resistant *E. coli* (cases) and women with UTI caused by fully susceptible *E. coli* (controls) were compared. Broth microdilution was used to perform antimicrobial resistance testing. All *E. coli* isolates were genotyped by the pulsed-field gel electrophoresis (PFGE) method. *Results.* Ninety-nine women met study criteria. Women who were infected with multidrug-resistant *E. coli* reported more frequent chicken consumption (adjusted OR = 3.7, 95% CI 1.1, 12.4). Women with UTI caused by an ampicillin- or cephalosporin-resistant *E. coli* isolate reported more frequent consumption of pork (adjusted OR = 3.2, 95% CI 1.0, 10.3 and adjusted OR = 4.0, 95% CI 1.0, 15.5, respectively). Frequent alcohol consumption was associated with antimicrobial resistant UTI. *Conclusions*. This study provides epidemiologic evidence that antimicrobial resistant, UTI-causing *E. coli* could have a food reservoir, possibly in poultry or pork.

Introduction

Community-acquired extraintestinal infections caused by *Escherichia coli*, including cystitis, pyelonephritis, and septicemia, are a significant but underappreciated cause of morbidity and mortality. Extraintestinal *E. coli* (ExPEC) refers to *E. coli* that causes disease outside the intestinal tract. Antimicrobial resistance prevalence in these *E. coli* is increasing, which complicates the management of community-acquired extraintestinal infections. The incidence of these infections range from 6 to 8 million cases per year in the United States and result in USD $1–2 billon in direct medical costs (Foxman *et al*., 2000; Russo and Johnson, 2003).

Unlike intestinal pathogenic *E. coli* such as enterotoxigenic, Shiga-toxin producing/enterohemorrhagic, enteropathogenic, enteroinvasive, enteroaggregative, and diffusely adherent *E. coli*, the *E. coli* that cause extraintestinal infections (termed ExPEC) are not associated

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with gastrointestinal disease. ExPEC exist as commensals in the intestinal reservoir and cause disease under certain circumstances such as in the case of compromised or anatomically abnormal hosts, medical instrumentation, or when host defenses are inadequate and the organism can enter a normally sterile extraintestinal site. ExPEC are associated with phylogenetic group B2 or D and possess a broad range of virulence genes such as adhesins, iron-acquisition systems, and toxins, many of which are associated epidemiologically with cases of extraintestinal infections (Johnson and Russo, 2002).

There is mounting evidence that the *E. coli* that cause urinary tract infections (UTIs) and other extraintestinal infections may be responsible for community-wide epidemics. In 1986–1987, *E. coli* O15:K52:H1 caused an outbreak of community-acquired UTI and septicemia in South London (Phillips *et al.*, 1988). The distinctive antibiotic resistance profile of this clonal group contributed to its recognition in London and other areas of Europe and the United States (Johnson *et al.*, 2002b; Prats *et al.*, 2000). Other outbreaks of UTI caused by *E. coli* have been described in Copenhagen (O78:H10) and recently in Calgary (Olesen *et al.*, 1994; Pitout *et al.*, 2005).

In 2001, we reported that a multidrug-resistant *E. coli* clonal group, designated clonal group A (CgA), as defined by enterobacterial repetitive intergenic consensus two (ERIC2) polymerase chain reaction, and characterized by O11, O77, O17, O73:K52:H18 serotypes, caused 11% of all *E. coli* UTIs and 49% of all trimethoprim-sulfamethoxazole (TMP-SMZ)–resistant *E. coli* UTIs in a single California community over a 4-month period (Manges *et al.*, 2001). Members of this clonal group were responsible for antimicrobial resistant UTIs in university communities in Michigan and Minnesota, a community in Colorado (Burman *et al.*, 2003), as well as pyelonephritis in several states (Johnson *et al.*, 2002a). The CgA genotype was also found in 129 (26%) of 495 animal and environmental isolates examined in another study; these CgA were overrepresented among poultry isolates (Johnson *et al.*, 2005b; Ramchandani *et al.*, 2005).

In recent studies by Johnson *et al.* (Johnson *et al.*, 2003, 2005a, 2005b), retail poultry meat sampled from grocery stores in Minnesota exhibited the greatest prevalence of *E. coli* contamination compared to beef, pork, and other foods, as well as the highest levels of antimicrobial resistance and virulence traits. Two of these studies identified strains of *E. coli* in retail foods that exhibited genotypes indistinguishable from those of human extraintestinal infection-causing *E. coli* (Johnson *et al.*, 2005a, 2005b).

The degree of genetic homogeneity of the California CgA isolates, as demonstrated by multiple genotyping methods, suggested that a point source, possibly a contaminated food product, may have been responsible for the dissemination of this clonal group. Identification of candidate food source(s) for these closely related UTI-causing *E. coli* is an important research question and is the focus of this study. We report the results of a case–control study conducted from April 2003 to June 2004 in the same California university community in which CgA was identified, to determine if acquisition of and infection with a UTI-causing, antimicrobial resistant *E. coli* isolate is associated with a woman's dietary habits, specifically her preparation and consumption of retail meat products.

It is important to note that in this study, we are interested in understanding the relationship between diet and the intestinal acquisition of antimicrobial resistant *E. coli*. The risk factors related to the intestinal acquisition of antimicrobial resistant *E. coli* are more relevant in this case than the risk factors that directly trigger the UTI episode. According to studies of *E. coli* population dynamics in the intestinal tract, new *E. coli* strains may be introduced into the intestine and persist for months or may turnover within 2–4 weeks (Manges *et al.*, 2004). This strain may at a later point appear as the cause of a symptomatic infection. The 6-month exposure window was chosen to capture the relevant exposure period when a woman would be at-risk to acquire and develop an infection with an *E. coli* strain. For these reasons, the questionnaire was designed to capture dietary and other exposures often related to foodborne outbreaks and to minimize dietary exposure misclassification due to poor recall.

Materials and Methods

Study design

A case–control study was conducted in collaboration with the University Health Services

at the University of California at Berkeley from April 2003 to June 2004. Eligible women ages 18–45 years, presenting to the health center with a suspected UTI were enrolled into the study. To be eligible, women could not be: (i) pregnant; (ii) diabetic; (iii) catheterized or hospitalized in the previous 30 days; (iv) receiving antimicrobial treatment or prophylaxis in the past 30 days; or (v) have had corrective surgery or have a urinary tract abnormality. UTI was clinically defined as the presence of two or more symptoms suggestive of UTI including, dysuria, increased urinary frequency or urgency, pyuria, and hematuria, and by the presence of $>10^2$ colony-forming units of *E. coli* per milliliter of clean-catch, unspun urine (Hooton and Stamm, 1997). Previous studies have demonstrated that this threshold of bacteriuria corresponds to an optimal sensitivity (95%) and specificity (85%) for the diagnosis of acute cystitis in women (Hooton and Stamm, 1997). If a woman had more than one UTI during the study period, only the first UTI was eligible for inclusion in the analyses. A case was defined as a woman with UTI caused by *E. coli* resistant to at least one of the antimicrobial agents tested. Controls were women with UTI caused by *E. coli* susceptible to all of the antimicrobial agents tested. The study protocol was approved by the University of California at Berkeley, Committee for the Protection of Human Subjects (#2003-2-12).

Survey

The survey questions focused on dietary habits that might be associated with the intestinal acquisition of *E. coli* or that have been linked to *E. coli* associated foodborne outbreaks. We asked women to recall these dietary habits over a period of 6 months prior to the index UTI used as reference for the survey. The survey instrument was devised partly based on the standard questionnaire used by the Centers for Disease Control and Prevention, Foodborne Outbreak Response and Surveillance Unit (based on the Centers for Disease Control and Prevention, Foodborne Outbreak Response and Surveillance Unit; http://www.cdc.gov/foodborneoutbreaks/standard_ques.htm, accessed November 2002) and was partly based on instruments used for nutritional assessments (Block 1998 Food Frequency Questionnaire and the Block Brief 2000 Food Frequency Questionnaire for adults, http://www.nutritionquest.com). Women were asked to estimate their average consumption for each food item or food type according to the following scale: never, a few times a month, 1–3 days per week, 4–6 days per week, or every day. This scale was adopted from the Block 1998 Food Frequency Questionnaire. Responses to these questions were dichotomized to reflect modest consumption versus frequent consumption, where the response category that corresponded to the 90th percentile of responses or above was defined as frequent consumption.

From April 2003 to March 2004, all participants were administered the survey in-person or by telephone. From March 2004 to June 2004, participants were offered the opportunity to complete the survey on-line at the UTI Diet Study website or complete it in-person with a study staff member. Technological innovation and methodological advances in epidemiology have demonstrated that sensitive information may be easier to obtain in a web-based or on-line format; this data collection strategy has been found to be very effective in collecting survey data from university student populations (Baer *et al*., 2002; Kypri *et al*., 2004). For the web-based survey, participants were assigned a unique identifier and were provided the UTI Diet Study website URL. Authentication of the participant's identity was completed through the CalNet Identification website (University of California at Berkeley, official web-based portal), where participants were required to use their university provided user name and passphrase to enter the survey website. Participants were redirected to the informed consent page, where they completed the consent process by reading the consent form and indicating whether they agreed to participate.

E. coli *isolation*

Urine samples were cultured on blood, eosin methylene blue (EMB), and Rose agar plates. Oxidase-negative and lactose- and indole-positive colonies were presumptively identified as *E. coli* (York *et al*., 2000). One putative *E. coli* colony from each urine culture was arbitrarily selected for further analysis.

Antimicrobial susceptibility

Antimicrobial susceptibility to 26 antimicrobial agents was evaluated by the broth microdilution method (Microscan Dade-Behring Inc, Deerfield, IL) and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria (National Committee for Clinical Laboratory Standards, 2000). *E. coli* strain 25922 (American Type Culture Collection) was used as the reference strain.

The following classes of antimicrobial agents *in italics* and specific antimicrobial agents (in parenthesis) were tested: *penicillins* (ampicillin, piperacillin, ampicillin/sulbactam, piperacillin/tazobactam, amoxicillin/clavulanic acid, ticarcillin/clavulanic acid); *cephalosporins* (cephalothin, cefazolin, cefotoxin, cefuroxime, ceftriaxone, ceftazidime, cefepime); *carbapenems* and *monobactams* (imipenem, aztreonam, meropenem); *tetracycline*; *trimethoprim-sulfamethoxazole* (TMP-SMZ); *aminoglycosides* (amikacin, gentamicin, tobramycin); *nitrofurantoin*; *chloramphenicol*; and *fluoroquinolones* (ciprofloxacin, gatifloxacin, levofloxacin).

The outcome, infection with an antimicrobial resistant *E. coli*, was defined in several ways. Resistance to specific antimicrobial agents or antimicrobial classes including, resistance to ampicillin, any cephalosporin, tetracycline, and TMP-SMZ were examined because they are either antimicrobial agents commonly used to treat UTI (TMP-SMZ and cephalosporin) or because a close association between certain resistance phenotypes (TMP-SMZ resistance) and prominent uropathogenic clonal groups has been observed in earlier studies (Manges *et al.*, 2001; Phillips *et al.*, 1988). We also examined ampicillin and tetracycline resistance as separate outcomes because these resistance markers are associated with transposable, multidrug-resistance elements. Finally, we examined women infected by multidrug-resistant isolates of *E. coli*, defined by resistance to $\geq$2 antimicrobial classes.

Pulsed-field gel electrophoresis (PFGE)

*Xba*I PFGE was performed on all isolates (Bender *et al.*, 1997). Clonal group membership was defined if two or more strains shared a PFGE pattern or profile that differed by fewer than six bands. These isolates were considered to be possibly related according to the Tenover criteria (Tenover *et al.*, 1995). Images of PFGE electrophoretic patterns were scanned into a software program (GelCompar II, version 3.5, Applied Maths, Sint-Martens-Latem, Belgium) for analysis. Dendrograms based on PFGE patterns were inferred from the Dice similarity coefficient matrix generated by GelCompar by the unweighted pair group method with arithmetic averages (UPGMA).

Statistical analyses

The primary outcomes of the study were UTI caused by an antimicrobial resistant *E. coli* or *E. coli* clonal group member as defined by PFGE. The outcome, UTI caused by an *E. coli* resistant organism, was stratified into several antimicrobial resistance categories: resistance to (i) ampicillin; (ii) tetracycline; (iii) any cephalosporin; (iv) TMP-SMZ; and (v) two or more classes of antimicrobial agents.

We tested the specificity of relationship between antimicrobial resistance phenotypes and diet in three ways. First, we examined whether specific antimicrobial resistance phenotypes were associated with specific dietary exposures. Second, we looked to see whether our results were consistent with observations of antimicrobial resistance phenotypes in *E. coli* recovered from retail meat products. Third, we created a multinomial outcome variable that represented degree of antimicrobial resistance (i.e., presence of resistance to one, two, three, and four antimicrobial classes for each isolate we studied) and examined the relationship between multiple antimicrobial resistance and diet. We also explored dose-response in our data by examining different consumption levels of certain foods and increased odds of infection with antimicrobial resistant *E. coli*.

The associations between self-reported dietary behaviors and UTI were analyzed by multiple logistic regression models adjusted for age, sexual frequency, and recent UTI history. Age and recent UTI history were included in our multivariate analyses because these factors reflect a woman's past exposure to antimicrobial agents. Frequency of sexual intercourse was included because it is a marker for the possible person-to-person transmission of anti-

microbial resistant *E. coli* (Foxman *et al*., 1997; Johnson *et al*., 1998; Manges *et al*., 2004). Adjusted odds ratios (Adj OR) and 95% confidence intervals (CIs) were estimated. Proportions were compared by a chi square test and means were compared by *t* test. A *p* value of ≤ 0.05 was considered statistically significant. In all analyses, except for the evaluation of dose-response, the exposure variables were dichotomized. All analyses were conducted by Stata version 7.0 (Stata Corporation, College Station, TX).

Based on earlier studies we expected that approximately 40% of the *E. coli* isolates causing UTI would be resistant to at least one antimicrobial agent. We estimated our sample size so that we would have sufficient power to detect a difference of 25% in a prevalent exposure between our cases and controls (based on an $\alpha = 0.05$ and $\beta = 0.90$). We aimed to enroll approximately 200 women with UTI caused by *E. coli*.

Results

Study subjects

Between April 2003 and June 2004, 2,145 urine samples were submitted to the clinical laboratory, representing 1,557 unique women. Aside from age, data were not available on women who did not enroll in the study. A total of 166 women with a suspected UTI in the previous 30 days were enrolled in the study. The mean age of the women enrolled was 22.7 years, range 18–39; this did differ slightly from the mean age of our source population (mean age 23.4 years, range 18–45) ($p = 0.08$). From these 166 women, 161 (97%) had urine specimens that were available for culture. Greater than 10^2 colony-forming units of *E. coli* per milliliter of urine was recovered from 99 (60%) of these women. We suspect that the recovery of *E. coli* from many UTI cases was compromised by the dilution of the urine specimens as the result of women drinking large volumes of fluid to manage their symptoms. These 99 women met the UTI case definition and are the focus of the remaining analyses. Fifty-six (57%) of these women reported no recent recurrent UTIs and 23 (23%) were experiencing their first-ever lifetime UTI.

In-person or telephone surveys were administered to 67 (68%) of the women and 32 (32%) surveys were completed by the web-based format. Women who responded to the web-based survey tended to be younger, to be more racially or ethnically diverse, and to report fewer lifetime UTIs. In a separate analysis, no significant differences were observed in the reported dietary habits between women who responded to the web-based versus the in-person/telephone surveys (data not shown). Accordingly, we chose to analyze the combined dietary data from the two survey types.

Antimicrobial susceptibility

Forty-three women (43%) experienced a UTI caused by *E. coli* resistant to one or more antimicrobial agents. Twenty-seven women (27%) experienced a UTI caused by an *E. coli* isolate resistant to two or more classes of antimicrobial agents. Thirty women (30%) developed a UTI caused by an ampicillin-resistant isolate; 19 (19%) by a tetracycline-resistant isolate; 18 (18%) by a cephalosporin-resistant isolate; and 12 (12%) developed a UTI caused by a TMP-SMZ–resistant isolate (Table 1).

Table 1. Antimicrobial Resistance Phenotypes of Urinary Tract Infection Diet Study *Escherichia coli* Isolates

Antimicrobial class or agent	*Resistant n (%)*
Ampicillin	30 (30)
Tetracycline	19 (19)
Any cephalosporin	18 (18)
Trimethoprim-sulfamethoxazole	12 (12)
Chloramphenicol	3 (3)
Any aminoglycoside	1 (1)
Any fluoroquinolone	1 (1)
Carbapenems and monobactams	0 (0)
Nitrofurantoin	0 (0)
Multidrug-resistance phenotypes (≥1 classes)	43 (43)
Multidrug-resistance phenotypes (≥2 classes)	27 (27)
Multidrug-resistance phenotypes (≥3 classes)	12 (12)
Multidrug-resistance phenotypes (≥4 classes)	4 (4)

Antimicrobial susceptibility testing was completed on all 99 *E. coli* isolates.

Dietary habits and UTI caused by multidrug-resistant E. coli

After adjusting for recent UTI history, age, and sexual intercourse frequency, women with UTI caused by *E. coli* resistant to ≥2 classes of antimicrobial agents were significantly more likely to report frequent consumption (≥4–6 days per week versus less) of roasted or cooked chicken (adjusted OR = 3.7, 95% CI 1.1, 12.4) (Table 2). Although the measure of association for an infection with a multidrug-resistant organism was elevated among women reporting frequent pork consumption; the result was not statistically significant. Frequent personal preparation of pork, chicken or turkey and beef consumption were not associated with multidrug-resistant UTI. The number of subjects who reported personally preparing beef in their homes was too small to include in this and the remaining analyses.

We evaluated the relationship between diet and degree of antimicrobial resistance (Table 3). Although not statistically significant, it appears that the risk estimates for women who reported frequent chicken and pork consumption increased with resistance to multiple antimicrobial agents (Table 3). This trend was not observed for beef or turkey consumption or personal preparation of retail meats.

Dietary habits and UTI caused by E. coli *resistant to specific antimicrobial agents*

To assess the specificity of our results, we examined diet in relation to the four most common resistance phenotypes by antimicrobial agent or class (ampicillin, tetracycline, TMP-SMZ, and cephalosporin) (Table 2). Women with UTI caused by an ampicillin-resistant *E. coli* were more likely to report frequent chicken consumption (adjusted OR = 3.5, 95% CI 1.1, 10.9) (Table 2). The association between frequent consumption of pork (≥1–3 days per week) and UTI caused by ampicillin and cephalosporin-resistant *E. coli* (adjusted OR = 3.2, 95% CI 1.0, 10.3 and adjusted OR = 4.0, 95% CI 1.0, 15.5, respectively) was of borderline significance. Frequent pork consumption was not related to tetracycline or TMP-SMZ–resistant UTI cases. Frequent turkey and beef consumption and personal preparation of pork, chicken, or turkey were not associated with risk of developing a UTI caused by *E. coli* resistant to ampicillin, tetracycline, cephalosporins, or TMP-SMZ (Table 2).

Increasing dietary exposure and UTI caused by antimicrobial resistant E. coli

We did not observe a gradient of risk with increasing exposure to any of the food items we studied. Table 4 presents the results of each level of retail meat consumption or preparation for women infected by an ampicillin and cephalosporin or multidrug-resistant *E. coli*. These results are limited by the available sample size.

Alcohol use and UTI caused by antimicrobial resistant E. coli

Frequent alcohol consumption (≥1–3 days per week) was also associated with the development of a UTI caused by an *E. coli* isolate that was resistant to multiple antimicrobial agents (OR = 3.4, 95% CI 1.1, 10.1) (Table 2). The same statistically significant association was observed for UTI caused by ampicillin- and cephalosporin-resistant *E. coli*, but not for UTI cases caused by tetracycline- or TMP-SMZ–resistant *E. coli*.

Dietary habits and UTI caused by specific E. coli *clonal group members*

Clonal analysis by PFGE showed that six PFGE profiles collectively accounted for 25% of the isolates, whereas each of the remaining 75% of isolates exhibited a unique PFGE profile (data not shown). Clonal group membership was not associated with any antimicrobial resistance patterns. A small sample size of any one clonal group precluded any further analyses.

Other factors

Factors that were not associated with antimicrobial resistant UTI included consumption of organic meats or produce, other dietary items (e.g., fish, raw meat, alfalfa sprouts), location of meals (e.g., home, restaurant, delis, dining halls, fast food restaurants, ready-to-eat foods), work in the food service industry, providing childcare, living with pets, swimming or bathing, use of an antimicrobials agent in the past 6 months, recurrent or lifetime UTI, sexual intercourse

Table 2. Consumption and Preparation of Meat and Other Dietary Habits and Risk of Urinary Tract Infection Caused by Antimicrobial Resistant *Escherichia coli*

Dietary Factor	*Total exposed*	*Resistance to ≥2 antimicrobials classes n = 27*		*Ampicillin n = 30*		*Tetracycline n = 19*		*Cephalosporin n = 17*		*Trimethoprim-sulfamethoxazole n = 12*	
		Adj OR	*95% CI*	*Adj OR*	*95 % CI*	*Adj OR*	*95% CI*	*Adj OR*	*95% CI*	*Adj OR*	*95% CI*
Consume chicken (≥4–6 days/week vs. less)	24	3.7	1.1, 12.4	3.5	1.1, 10.9	2.9	0.7, 11.4	2.8	0.7, 10.7	3.3	0.7, 16.0
Consume pork (≥1–3 days/week vs. less)	20	2.7	0.8, 9.0	3.2	1.0, 10.3	1.9	0.4, 7.9	4.0	1.0, 15.5	2.0	0.4, 11.0
Consume turkey (≥1–3 days/week vs. less)	28	1.2	0.4, 3.6	1.5	0.5, 4.1	1.2	0.4, 4.3	1.6	0.4, 5.7	0.9	0.1, 5.0
Personally prepare pork, chicken or turkey (≥1–3 days/week vs. less)	28	0.8	0.3, 2.4	1.0	0.4, 2.8	1.2	0.3, 4.3	1.0	0.3, 3.8	0.6	0.1, 3.1
Consume ground beef (≥1–3 days/week vs. less)	19	1.2	0.4, 4.3	1.0	0.3, 3.4	1.1	0.3, 4.6	0.8	0.2, 3.5	1.3	0.2, 6.7
Consume whole beef (≥1–3 days/week vs. less)	19	0.6	0.2, 2.0	0.5	0.1, 1.6	0.4	0.1, 2.0	0.9	0.2, 3.5	0.4	0.1, 2.5
Consume alcohol (≥1–3 days/week vs. less)	37	3.4	1.1, 10.1	3.1	1.1, 8.4	2.7	0.8, 9.1	4.5	1.2, 16.2	2.0	0.4, 9.4

The comparison group contains women infected by a fully susceptible *E. coli* isolate (n = 56). These analyses were adjusted for number of UTI in the 12 months prior to study enrollment, sexual frequency in the 6 months prior to study enrollment and age.

Frequent consumers were defined by the category corresponding to the 90th percentile.

Multiple resistance is defined as resistance to ≥ 2 classes of antimicrobials (see Methods).

Adj OR = adjusted odds ratio, 95% confidence interval.

The number of subjects that reported frequently preparing beef in their home was too small to include in these analyses.

Table 3. Relationship Between Diet and Degree of Antimicrobial Resistance

Dietary Factor	*Degree of antimicrobial resistance*	*Adj OR*	*95% CI*	
Consume chicken	1	1.2	0.3	4.8
(≥4–6 days/week vs. less)	2	3.3	0.6	16.3
	3	6.5	1.0	40.7
	4	3.2	0.1	69.4
Consume pork	1	1.4	0.3	5.7
(≥1–3 days/week vs. less)	2	2.5	0.5	12.9
	3	4.3	0.7	24.4
	4	4.2	0.2	90.9
Consume turkey	1	1.7	0.5	5.6
(≥1–3 days/week vs. less)	2	2.0	0.5	8.3
	3	1.1	0.2	6.7
	4	NA	NA	NA
Personally prepare pork, chicken or turkey	1	1.5	0.5	4.8
(≥1–3 days/week vs. less)	2	0.2	0.0	2.0
	3	1.6	0.3	8.7
	4	2.1	0.1	44.3
Consume ground beef	1	0.6	0.1	2.9
(≥1–3 days/week vs. less)	2	1.1	0.2	6.7
	3	2.7	0.5	15.8
	4	NA	NA	NA
Consume whole beef	1	0.4	0.1	1.6
(≥1–3 days/week vs. less)	2	0.5	0.1	3.2
	3	1.0	0.1	6.2
	4	NA	NA	NA
Consume alcohol	1	2.5	0.8	8.0
(≥ 1–3 days/week vs. less)	2	6.0	1.4	25.3
	3	2.2	0.4	12.0
	4	3.8	0.2	86.0

The comparison group contains women infected by a fully susceptible *E. coli* isolate (n = 56).
These analyses were adjusted for number of UTI in the 12 months prior to study enrollment, sexual frequency in the 6 months prior to study enrollment and age.
Frequent consumers were defined by the category corresponding to the 90th percentile.
Adj OR = adjusted odds ratio. 95% CI = 95% confidence interval.
NA = insufficient data for point estimation.

frequency, recent history of diarrhea, race/ethnicity, or housing arrangements (e.g., dormitory, sorority, apartment/studio).

Discussion

A study was carried out among 99 women with UTI caused by *E. coli* to investigate the relationship between dietary habits and the development of a UTI caused by an antimicrobial resistant *E. coli*. Our *a priori* hypothesis, based on previous studies (Johnson *et al*., 2002a, 2005a, 2005b; Manges *et al*., 2001; Ramchandani *et al*., 2005), was that UTI caused by either antimicrobial resistant *E. coli* or *E. coli* clonal group members would be more common among women reporting frequent exposure to or consumption of retail meat. We found that frequent consumption of chicken (≥4–6 days per week) was associated with UTI caused by *E. coli* resistant to multiple antimicrobial agents and specifically to ampicillin. Frequent pork consumption (1–3 days per week) was modestly associated with ampicillin- and cephalosporin-resistant *E. coli* causing UTI. Preparation of meat in the home was not associated with an antimicrobial resistant UTI, although relatively few women reported preparing their own meat. Frequent turkey and beef consumption was not associated with antimicrobial resistant UTI. Together these observations suggest that the consumption of certain retail meats may be an important exposure in the epidemiology of antimicrobial resistant UTI.

Table 4. Frequency of Consumption and Risk of UTI Caused by Antimicrobial Resistant *Escherichia coli*

Meat		*Total Exposed*	*Resistance to ≥2 antimicrobials classes n = 27*			*Ampicillin n = 30*			*Tetracycline n = 19*			*Cephalothin n = 17*		
			Adj OR	*95% CI*		*Adj OR*	*95% CI*		*Adj OR*	*95% CI*		*Adj OR*	*95% CI*	
Chicken	Never	13	Reference			Reference			Reference			Reference		
	A few times in six months	8	NA	NA	NA	2.7	0.1	60.9	9.5	0.5	198.8	NA	NA	NA
	A few times a month	18	4.5	0.6	32.5	3.5	0.5	23.9	9.2	0.7	126.5	1.8	0.2	18.4
	1–3 days per week	36	1.0	0.2	6.1	1.2	0.2	7.2	1.5	0.1	16.3	1.1	0.2	7.1
	4–6 days per week	23	5.6	0.8	37.7	5.9	0.9	37.4	6.7	0.6	74.6	3.7	0.5	27.4
Pork	Never	29	Reference			Reference			Reference			Reference		
	A few times in 6 months	23	0.5	0.1	3.2	0.8	0.2	3.5	1.3	0.2	7.9	0.8	0.1	5.0
	A few times a month	27	2.0	0.5	7.6	1.6	0.4	6.1	2.2	0.5	10.6	0.7	0.1	4.1
	1–3 days per week	18	3.5	0.8	16.3	4.2	1.0	18.4	2.6	0.4	17.3	3.9	0.8	20.0
	4–6 days per week	2	2.2	0.1	45.0	1.8	0.1	34.7	5.1	0.2	121.9	NA	NA	NA
Turkey	Never	22	Reference			Reference			Reference			Reference		
	A few times in six months	28	1.8	0.4	8.9	1.4	0.3	6.3	3.3	0.5	20.6	0.9	0.1	5.9
	A few times a month	21	3.5	0.7	17.8	2.2	0.5	10.1	3.0	0.4	23.6	2.2	0.3	13.7
	1–3 days per week	22	2.1	0.4	11.3	2.3	0.5	10.7	2.1	0.3	15.6	2.5	0.5	13.7
	4–6 days per week	6	2.9	0.3	28.8	1.8	0.2	15.6	6.5	0.6	76.9	NA	NA	NA
Ground beef	Never	28	Reference			Reference			Reference			Reference		
	A few times in six months	21	0.3	0.1	2.1	0.6	0.1	2.5	0.6	0.1	4.0	1.6	0.2	14.9
	A few times a month	30	1.5	0.4	5.6	1.6	0.4	5.6	1.3	0.3	6.1	4.6	0.7	29.4
	1–3 days per week	19	1.3	0.3	5.8	1.1	0.3	4.7	1.2	0.2	6.4	1.9	0.2	14.7
Whole beef	Never	28	Reference			Reference			Reference			Reference		
	A few times in six months	23	1.4	0.3	6.4	1.6	0.4	6.4	1.8	0.4	9.2	0.5	0.0	5.7
	A few times a month	29	1.4	0.4	5.5	1.3	0.3	4.8	1.2	0.3	5.4	1.3	0.3	6.2
	1–3 days per week	16	0.6	0.1	2.9	0.4	0.1	2.3	0.2	0.0	2.6	0.4	0.1	3.2
	4–6 days per week	3	3.2	0.2	64.2	2.9	0.1	56.2	3.9	0.2	79.2	7.9	0.5	124.8

The comparison group contains women infected by a fully susceptible *E. coli* isolate (n = 56). These analyses were adjusted for number of UTI in the 12 months prior to study enrollment, sexual intercourse frequency in the 6 months prior to study enrollment and age.

Frequent consumers were defined by the category corresponding to the 90th percentile.

Multiple resistance is defined as resistance to ≥ 2 classes of antimicrobials (see methods).

Adj OR = adjusted odds ratio, 95% CI = 95% confidence interval.

Sample size for trimethoprim-sulfamethoxazole resistant *E. coli* was too small to analyze by frequency.

NA = insufficient data for point estimation.

We observed a surprising relationship between alcohol consumption and antimicrobial resistant UTI. It is possible that this relationship reflects a false positive association; although it was observed for several antimicrobial resistant outcomes. Frequent alcohol consumption was not associated with sexual intercourse or recurrent UTI in this study population. It is unclear how frequent alcohol use pertains to person-to-person or dietary exposures that would contribute to the acquisition of antimicrobial resistant *E. coli*. We were able to find only one reference to alcohol consumption and antimicrobial resistant infection. Koivisto *et al*. (2004) reported that, among women, alcohol consumption correlated significantly with metronidazole-resistant *H. pylori*. Although in their study, alcohol consumption might have been associated with prior reproductive tract infections (Koivisto *et al*., 2004).

Past exposure to antimicrobials agents is a significant risk factor for the development of an antimicrobial resistant infection. In our statistical analyses, we adjusted for number of recent UTI episodes (UTI in 12 months prior to study enrollment) to control for the effect of antimicrobial agent use on the generation and/or selection of antimicrobial resistant organisms in the gut microbiota of our subjects. Only five (5%) women reported antimicrobial agent use for any other reason in the 6 months prior to study enrollment. Due to sample size constraints we were not able to investigate whether women who were experiencing their first lifetime UTI were more likely to develop an antimicrobial resistant UTI due to frequent consumption of certain food items.

Due to the small sample size of any one PFGE clonal group, we did not observe an association between clonal group membership and any of the dietary or other variables. However, since most of the *E. coli* strains possessed unique PFGE patterns, the association between chicken consumption and antimicrobial resistant UTI cannot be attributed to an outbreak caused by a common contaminated food product(s) introduced into this community during the time of this study.

Intensively raised food animals such as pigs and chickens are exposed to antimicrobials used for veterinary and growth promotion purposes and recent studies have demonstrated high levels of antimicrobial resistance in retail meats from these sources (Johnson *et al*., 2003b, 2005a, 2005b; Schroeder *et al*., 2003). According to data from retail meat surveillance at the U.S. National Antimicrobial Resistance Monitoring System (NARMS), antimicrobial resistant, generic *E. coli* is frequently recovered from retail meats. Table 5 summarizes data extracted from the NARMS 2003 report (U.S. Food and Drug Administration, 2003). All of these antimicrobial agents (ampicillin, cephalosporins, tetracycline, and sulfonamides) are used for the treatment of sick animals and in some cases for growth promotion purposes (http://dil.vetmed.vt.edu/default.htm, accessed July 2006). Data on intensity of antimicrobial agent use in animals are difficult to find. Our results are in partial agreement with the levels of antimicrobial resistance found in generic *E. coli* recovered from retail meat in 2003 (Table 5). *E. coli* isolated from chicken breast and pork chops exhibited the second and third highest levels of antimicrobial resistance; both of these were related to antimicrobial resistant UTI in our study. Ground beef, by contrast, had the lowest levels of antimicrobial resistance and was not related to an-

Table 5. National Antimicrobial Resistance Monitoring System (NARMS) Data 2003: Antimicrobial Resistance of Generic *Escherichia coli* Recovered from Retail Meat Samples

Retail meat type	*Percent recovery of* E. coli *by meat type*	*≥2 Antimicrobial classes*	*Percent resistance for selected classes of antimicrobials*			
			Ampicillin	*Cephalothin*	*Tetracycline*	*TMP-SMZ*
Chicken breast	83	11	25	22	43	7
Ground turkey	75	40	36	19	78	7
Ground beef	66	8	5	8	25	0.3
Pork chop	46	13	13	12	46	3

TMP-SMZ = trimethoprim-sulfamethoxazole.

timicrobial resistant UTI. The exception was ground turkey, which exhibited the highest level of antimicrobial resistance of all the meat types studied, but was not associated with antimicrobial resistant UTI in our study. We also observed a nonsignificant increase in risk with increasing antimicrobial resistance for chicken and pork consumption only (Table 3). The analysis of the dose–response relationship between self-reported frequency of retail meat consumption and antimicrobial resistant UTI was inconclusive due to our limited sample size.

The present study is limited by the number of women enrolled with an *E. coli* UTI and by the fact that these women were self-selected into the study. Although, we had recruited 166 women, only 60% had a UTI for which *E. coli* could be detected; we believe this was largely due to the dilution of their urine. Given this smaller than expected sample size, we conducted a post hoc power calculation for our main finding—multidrug-resistant *E. coli* UTI and frequent chicken consumption. If, in truth, cases differ from controls in their exposure, by approximately 34%, this study would have a 90% chance of detecting a difference without continuity correction.

Accurate recall of diet, especially over a long time period, is likely to have produced misclassification in our dietary measures. However, we believe that the misclassification would be nondifferential. Grouping the data based on frequent versus modest consumption, as we did, may have helped to reduce this misclassification. This study was designed in a similar fashion to an outbreak investigation because of our primary interest in acquisition of UTI-causing *E. coli* to the gut. We evaluated many variables that conceivably could be related to the transmission or acquisition of *E. coli*, including diet, diarrhea, antimicrobial agent use, travel, and sexual activity. Testing of multiple hypotheses may have resulted in some false positive findings. As our primary hypothesis concerned retail meat consumption or preparation, our observation of a possible increased risk of antimicrobial resistant infection and frequent chicken and pork consumption is compelling.

Our observations could also result from the transmission of mobile antimicrobial resistance genes between *E. coli* originating from certain animal food products and UTI-causing *E. coli* strains normally found in the human intestine. This alternative hypothesis could not be ruled out by this study. Nevertheless, exchange of resistance genes between *E. coli* derived from animals and our intestinal *E. coli* still represents a significant public health threat. Larger studies should be undertaken to verify these findings if true and to address whether the acquisition of specific antimicrobial resistant clonal groups of *E. coli* are related to diet.

The number of foodborne outbreaks associated with multidrug-resistant enterobacteria, especially antimicrobial resistant organisms such as *Salmonella typhimurium* DT104 and *E. coli* O157:H7 has grown and the impact of antimicrobial agent use in animal food production in relation to this increase in foodborne disease is being examined (Lederberg, 2000; Threlfall *et al.*, 2000, 2002; Witte, 1998). The evidence for local outbreaks of *E. coli* causing extraintestinal infections raises the possibility that the circumstances that lead to outbreaks of antimicrobial resistant foodborne diarrheal disease might also be relevant to our understanding of community-acquired extraintestinal infections, such as UTI (Manges *et al.*, 2001; Olesen *et al.*, 1994; Phillips *et al.*, 1988; Pitout *et al.*, 2005).

Conclusions

Frequent chicken and pork consumption was associated with community-acquired UTI caused by antimicrobial resistant *E. coli*. To our knowledge this study provides the first epidemiological evidence that extraintestinal infections with an antimicrobial resistant *E. coli* may be associated with an animal food reservoir, possibly poultry and pork. It is still unclear whether diet contributes directly to the acquisition of antimicrobial resistant *E. coli* or indirectly through the exchange of mobile resistance elements between *E. coli* introduced to the gut via the diet and *E. coli* strains normally present in the intestine. Larger epidemiologic studies should be undertaken to confirm, refute, and expand upon these findings. The transmission of enteric bacteria through food to the human intestine is not a novel concept. If a portion of the antimicrobial resistant *E. coli* causing extraintestinal infections were disseminated via

animal food products in an analogous way, the consequences to public health may be substantially greater than previously recognized.

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