

# Antimicrobial-Resistant and Extraintestinal Pathogenic *Escherichia coli* in Retail Foods

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(See the editorial commentary by Jones and Schaffner, on pages 1029–31.)

**Background.** Extraintestinal *Escherichia coli* infections are associated with specialized extraintestinal pathogenic *E. coli* (ExPEC) strains and, increasingly, with antimicrobial resistance. The food supply may disseminate ExPEC and antimicrobial-resistant *E. coli*.

**Methods.** In a prospective survey of 1648 diverse food items from 10 retail markets in the Minneapolis–St. Paul area during 2001–2003, selective cultures and disk-diffusion assays for the isolation and characterization of antimicrobial-resistant *E. coli* and polymerase chain reaction–based assays and O serotyping to define ExPEC-associated traits were performed.

**Results.** *E. coli* contamination exhibited a prevalence gradient from miscellaneous foods (9%), through beef or pork (69%), to poultry (92%;  $P < .001$ ). Among *E. coli*-positive samples, similar prevalence gradients were detected for antimicrobial resistance (27%, 85%, and 94% of samples, respectively;  $P < .001$ ) and ExPEC contamination (4%, 19%, and 46%, respectively;  $P < .001$ ). By multivariate analysis, beef or pork and poultry from natural-food stores exhibited reduced risks of *E. coli* contamination and antimicrobial resistance. Indirect evidence suggested on-farm selection of resistance. Four food-source ExPEC isolates (from pea pods, turkey parts, ground pork, and vegetable dip) closely resembled selected human clinical isolates by O antigen and genomic profile.

**Conclusions.** Retail foods may be an important vehicle for community-wide dissemination of antimicrobial-resistant *E. coli* and ExPEC, which may represent a newly recognized group of medically significant foodborne pathogens.

Extraintestinal infections caused by *Escherichia coli* are responsible for several million episodes of urinary tract infection (UTI), an estimated 36,000 deaths from sepsis, and billions of dollars in increased health-care costs annually in the United States [1]. Emerging resistance to first-line antimicrobial agents increases the clinical impact of these infections and complicates their management [1–3]. Acquired antimicrobial resistance is particularly problematic when it occurs in extraintestinal

pathogenic *E. coli* (ExPEC), the distinctive *E. coli* strains that possess the specialized virulence factors (VFs) required for extraintestinal disease [4]. Improved understanding of the origins and transmission pathways of antimicrobial-resistant *E. coli* and ExPEC is needed.

Several studies have suggested that foods might be a source of human-acquired antimicrobial-resistant *E. coli* and/or ExPEC. The food supply is an established vehicle for certain other antimicrobial-resistant and/or pathogenic bacteria—notably, *Salmonella enterica*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *E. coli* O157:H7 [5–12]. Meat and poultry products at slaughtering operations can be extensively contaminated with *E. coli* of animal origin, including strains that express ExPEC-associated O antigens and/or are antimicrobial resistant [13, 14]. Antimicrobial-resistant *E. coli* strains also occur in some retail meats and poultry [15, 16]. Hospital and cafeteria foods may contain *E. coli*, with possible subsequent transmission to consumers [17, 18]. Food-source organisms can contaminate kitchens during meal preparation [19], and cooks can acquire

Received 3 June 2004; accepted 24 September 2004; electronically published 1 March 2005.

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Potential conflicts of interest: J.R.J. has received grant support and honoraria from Bayer, Wyeth-Ayerst, Ortho-McNeil, Merck, and Rochester Medical.

Financial support: Office of Research and Development, Medical Research Service, Department of Veterans Affairs (to J.R.J.); National Research Initiative (Competitive Grants Program/United States Department of Agriculture grant 00-35212-9408 to J.R.J. and S.T.).

The Journal of Infectious Diseases 2005;191:1040–9

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resistant *E. coli* from poultry carcasses without consuming the food [20]. Diverse other foods, including ready-to-eat foods, apple juice, and sprouts [11, 12, 21], may also contain *E. coli*.

However, few current data are available regarding the contamination of retail foods with *E. coli*, specifically resistant strains and ExPEC, or assessing the impact of store type and organic or antibiotic-free labeling. Such data are needed for consumers to make informed choices and for producers and regulators to establish appropriate public policies [22] and to implement appropriate monitoring systems and/or interventions [5, 23, 24]. Accordingly, we conducted a 2-year retail market survey, systematically sampling diverse foods for antimicrobial-resistant *E. coli* and ExPEC.

## MATERIALS AND METHODS

**Food sampling scheme.** From May 2001 through May 2003, foods were purchased in rotation from 10 retail markets in the Minneapolis–St. Paul area. These included 2 representatives each of large luxury and economy chains (defined based on amenities, ambience, decor, product selection, and pricing), small neighborhood markets, natural-foods markets (including 1 outlet each of a multistate chain and a locally owned cooperative; hereafter, “natural store”), and farmer’s markets (summers only). Each week, 27 items were purchased from a different store, according to a schedule that distributed purchases at that store in strict rotation among 63 food types. The purchaser selected specific items and brands within the specified food type at his or her discretion. Unavailable items were omitted; purchasing resumed with the next available scheduled item. Purchases at a particular store cycled through the entire schedule sequentially, despite interruptions. The schedule was initiated in a staggered fashion at different stores, such that, at all times, purchases were being made from different parts of the schedule. Foods purchased (number of samples) included beef (70), pork (68), chicken (56), turkey (133), and blended chicken and turkey (6) (all raw; variably ground and/or frozen); fresh fruits (399) and vegetables (468); and miscellaneous foods, including fermented or processed items—cheese (70), dry salami (67), cooked turkey franks (61), fish (65), crab (28), shrimp (36), delicatessen items (67), and cream or custard pastries (54). Unwrapped items were placed individually in clean plastic bags by use of disposable gloves. Items were refrigerated or frozen until processing. Items were considered to be organic or antibiotic-free if so characterized by the label, store, or producer. The experimentation guidelines of the authors’ institutions were followed in the conduct of clinical research.

**Culture methods.** By use of a sterile technique, food items were weighed and manually rinsed (produce or poultry parts containing bones) or were mechanically dispersed (other foods; Stomacher blender; Seward Medical) in defined volumes of lauryl-tryptose broth (Difco), 1 mL of which was plated onto

Petrifilm (3M), both immediately (undiluted), to detect high-level contamination, and after incubation with the food for 48 h at 37°C (10<sup>-6</sup> dilution), to detect trace contamination. Blue colonies with gas were counted as presumptive *E. coli* [25] and were confirmed by use of an API 20-E system (bio-Merieux). Bacterial quantification was by done by plate counts or, for samples that tested positive only after amplification, by a 3-tube, 3-replicate most probable number method [25]. From each *E. coli*-positive sample, an arbitrarily selected index *E. coli* isolate, up to 12 additional *E. coli* colonies (which were pooled), and a sweep of mixed bacterial growth were saved. The amplified broth was plated onto modified Mueller-Hinton agar [26] that contained ampicillin (32 mg/L), tetracycline (16 mg/L), nalidixic acid (32 mg/L), ceftazidime (32 mg/L), or trimethoprim (16 mg/L), for overnight incubation at 37°C. Presumptive *E. coli* were confirmed by use of an API-20E system.

**Antimicrobial resistance.** Index isolates and each sample’s most resistant *E. coli* isolate (identified through replica plating onto the above-mentioned antimicrobial-containing agars) underwent standardized disk-diffusion susceptibility testing to ampicillin, amoxicillin-clavulanate, cefazolin, ceftazidime, gentamicin, tetracycline, nitrofurantoin, nalidixic acid, ciprofloxacin, sulfisoxazole, trimethoprim, and trimethoprim-sulfamethoxazole [27, 28].

**Detection of ExPEC.** Lysates of each sample’s index *E. coli* isolate, pooled *E. coli* colonies, mixed growth, and most resistant *E. coli* isolate were tested by polymerase chain reaction (PCR) for *hlyD* (hemolysin) and ExPEC status [29]. On the basis of previous statistical analyses of strain collections within which the extraintestinal virulence capability was determined either experimentally or on the basis of clinical source, ExPEC was defined by detection of  $\geq 2$  of *papA* and/or *papC* (P fimbriae), *sfa/foc* (S/F1C fimbriae), *afa/dra* (Dr-antigen-binding adhesins), *kpsM* (group 2 capsule), and *iutA* (aerobactin) [29]. ExPEC-positive samples were further tested for 35 ExPEC-associated VFs [29]; such testing predicts experimental in vivo virulence and differentiates among various clonal groups of ExPEC [30, 31].

**Serotyping and phylotyping.** O antigens were determined by the *E. coli* Reference Center (University Park, PA) by use of 180 O-specific antisera. O antigens associated with UTI (O-UTI) were defined as O1, O2, O4, O6, O7, O16, O18, O25, and O75 [32]. *E. coli* phylogenetic group (A, B1, B2, and D) was defined by triplex PCR [33].

**Comparison with human clinical ExPEC.** To assess whether foodborne *E. coli* resembles human clinical isolates, food-source ExPEC isolates that expressed O-UTI antigens or O11/O17/O77, which have been associated with the recently described *E. coli* “clonal group A” [34, 35], were compared, according to virulence profile and/or O antigen, with human clinical isolates from the investigator’s collections (J.R.J.). (These collections

include isolates from cystitis and pyelonephritis in women, neonatal meningitis in children, febrile UTI in men, and diverse-source bacteremia in adults.) Food-source isolates were compared with the corresponding human isolates by random amplified polymorphic DNA (RAPD) analysis [36], to assess for genomic similarity.

**Statistical methods.** Three prespecified food classes were defined for analysis: poultry (raw), beef/pork (raw), and miscellaneous foods (including produce and processed foods) (table 1). Aggregate resistance and virulence scores were the number of resistance or virulence markers detected. Year of study was analyzed as a continuous variable (calendar year 1, 2, or 3), to identify secular trends. Unpaired comparisons were tested by use of Fisher's exact or  $\chi^2$  test for proportions or by use of the Mann-Whitney *U* test for scores (all 2-tailed). Paired comparisons were tested by use of McNemar's test for proportions or by use of the Wilcoxon rank sum test for scores. Multiple predictor variables associated with selected microbiological outcomes were identified by use of multiple logistic regression models in which the outcome variable of interest was the dependent variable and all relevant source characteristics for the particular food class were simultaneous predictor variables (see table 2, footnote a, for a list of predictor variables).

## RESULTS

**Prevalence of *E. coli*.** During the 2-year survey, 1648 retail food items were cultured. Of these, 396 (24%) yielded *E. coli*. Contamination with *E. coli* varied by food class, with a significant difference among miscellaneous items (produce and other

nonmeat or poultry items, 9%), meats (beef or pork, 69%), and poultry (92%) ( $P < .001$  for all comparisons of each food class vs. another food class or all other foods combined) (figure 1A). Detection of *E. coli* by direct plating likewise varied significantly by food class (0.7%, 5%, and 16% of miscellaneous, beef or pork, and poultry samples, respectively;  $P \leq .002$  for all comparisons). Overall, *E. coli* counts were significantly higher in samples that tested positive by direct plating, compared with samples that tested positive only after broth amplification (median, 20 vs.  $<1.0$  cfu/g;  $P < .001$ ).

Among the miscellaneous foods, *E. coli* contamination varied by food type. No *E. coli* was detected in iceberg lettuce, cauliflower, plums, strawberries, raspberries or blackberries, grapes, pineapple, kiwi fruit, or cream pastry (overall, 0/231 samples vs. 121 (11%) of 1087 other miscellaneous food samples;  $P < .001$ ). In contrast, *E. coli* was detected in  $>25\%$  of samples each of cucumber/zucchini, spinach, corn, mushrooms, and shrimp (overall, 32% of 153 samples vs. 6% of 1165 other miscellaneous food samples;  $P < .001$ ). Likewise, *E. coli* was directly detectable (indicating more intense contamination) in  $\geq 1$  sample each for cucumber or zucchini, potatoes, green onions, fish, and turkey frankfurters (overall, 9/232 samples [4%] vs. 0/1086 other miscellaneous food samples;  $P < .001$ ).

Because of the marked differences between the main food classes, subsequent analyses were stratified by food class. Within each food class and among classes, complex patterns of association were observed for the various predictor variables according to univariate analysis (table 1). Therefore, multiple logistic regression analysis was used to identify multiple pre-

**Table 1. Predictors of *Escherichia coli*, resistant *E. coli*, and virulence traits among 1648 retail food items, by univariate analysis.**

Food class (no. of samples)	Outcome variable <sup>b</sup> (no. positive)	Predictor variable <sup>a</sup>							Subgroup within food class
		Natural store	Antibiotic free	Farmer's market	Ground	Frozen	Season (summer/ autumn)	Year (secular trend)	
Miscellaneous (1315)	<i>E. coli</i> (121)	—	...	↑	...	...	↑	—	—
	Resistance (31)	—	...	—	...	...	↑	↓	Other (nonproduce) ↑
	ExPEC (5), O-UTI (12)	—	...	—	...	...	—	↓	—
Beef/pork (138)	<i>E. coli</i> (95)	↓	↓	...	↑	—	—	—	Pork ↑
	Resistance (73)	↓	↓	...	—	—	↑	↑	Pork ↑
	ExPEC (18), O-UTI (13)	—	—	...	↑	—	↓	↑	Pork ↑
Poultry (195)	<i>E. coli</i> (180)	↓	↓ <sup>c</sup>	...	↑	↑	↑	↑	—
	Resistance (165)	—	—	...	—	—	↓	↓	Turkey ↑
	ExPEC (83), O-UTI (28)	—	—	...	—	—	↓	↓	Turkey ↑

<sup>a</sup> Predictor variables included natural store (vs. other store), antibiotic-free (vs. other or unknown), farmer's market (vs. other store), ground (vs. not ground), frozen (vs. not frozen), year (for secular trend), produce (vs. other miscellaneous foods), pork (vs. beef), and turkey (vs. chicken). Arrows (↓ and ↑), significant negative and positive associations, respectively ( $P < .05$ ), with a positive trend for "year" indicating an increase over time; ellipses (...), predictor variable not applicable to indicated food class; —, no significant effect detected.

<sup>b</sup> Outcome variables included *E. coli*, antimicrobial-resistant *E. coli* (no. shown is for resistance to  $\geq 1$  drug; associations are noted for resistance to  $\geq 1$  drug and/or to  $\geq 5$  drugs), ExPEC (extraintestinal pathogenic *E. coli*, defined as positivity for  $\geq 2$  of *papA* and/or *papC* [P fimbriae], *sfafoc* [S and F1C fimbriae], *afa/dra* [Dr-family adhesins], *kpsM II* [group 1 capsule], and *iutA* [aerobactin receptor]), and O-UTI (O antigens associated with urinary tract infection, i.e., O1, O2, O4, O6, O7, O16, O18, O25, and O75). Analyses involving *E. coli* included all samples. Analyses of antimicrobial resistance, ExPEC status, and O-UTI status were limited to samples that contained *E. coli*.

<sup>c</sup> The association of "antibiotic free" with *E. coli* changed from negative to positive in the multivariate analysis.

**Table 2. Predictors of *Escherichia coli*, antimicrobial-resistant *E. coli*, and *E. coli* virulence markers in retail foods, by multiple logistic regression analysis.**

Characteristic <sup>b</sup> (no. of samples in analysis) <sup>c</sup>	Food class (no. of samples in analysis) <sup>c</sup>	Significant predictor variables <sup>a</sup>		
		Variable	P	OR (95% CI)
<i>E. coli</i> (1648)	Miscellaneous (1315) Beef/pork (138)	Summer/autumn	.001	2.02 (1.31–3.11)
		Natural store	.02	0.11 (0.02–0.72)
		Ground	<.001	5.93 (2.30–15.3)
	Poultry (195)	Year	.007	5.86 (1.63–21.06)
		Natural store	.002	0.04 (0.005–0.32)
		Antibiotic free	.007	13.13 (2.06–83.91)
		Frozen	.03	3.15 (1.11–8.93)
Resistant <i>E. coli</i> (384)	Beef/pork (93)	Beef	.006	0.15 (0.04–0.59)
		Natural store	.02	0.04 (.003–0.61)
	Poultry (175)	Summer/autumn	.001	0.23 (0.10–0.56)
		Year	.04	0.50 (0.26–0.96)
ExPEC and/or O-UTI (390)	Beef/pork (94)	Summer/autumn	.045	0.27 (0.08–0.97)
		Beef	.03	0.09 (0.01–0.81)
	Poultry (179)	Summer/autumn	<.001	0.20 (0.09–0.41)
		Year	<.001	0.39 (0.23–0.66)

<sup>a</sup> Predictor variables included, for miscellaneous foods, produce (vs. other), organic (vs. other or unknown), natural store (vs. other store), farmer's market (vs. other store), year (for secular trend; odds ratios [ORs] indicate the proportional increase or decrease per year), and season (summer/autumn vs. winter/spring); for beef or pork, beef (vs. pork), natural store (vs. other store), antibiotic-free (vs. other or unknown), ground (vs. not ground), frozen (vs. not frozen), year (for secular trend; ORs indicate the proportional increase or decrease per year), and season (summer/autumn vs. winter/spring); and, for poultry, chicken (vs. turkey), natural store (vs. other store), antibiotic-free (vs. other or unknown), ground (vs. not ground), frozen (vs. not frozen), year (for secular trend; ORs and confidence intervals [CIs] indicate the proportional increase or decrease per year) and season (summer/autumn vs. winter/spring). Only variables yielding  $P < .05$  are shown. Among miscellaneous foods, no significant predictors were identified for "Resistant *E. coli*" or "ExPEC and/or O-UTI."

<sup>b</sup> Outcome variables included *E. coli* (from direct plating and/or broth amplification), antimicrobial-resistant *E. coli* (resistance to  $\geq 1$  drug), ExPEC (extraintestinal pathogenic *E. coli*, defined as positivity for  $\geq 2$  of *papA* and/or *papC* [P fimbriae], *sfa/foc* [S and F1C fimbriae], *afa/dra* [Dr-family adhesins], *kpsM II* [group 1 capsule], and *iutA* [aerobactin receptor]), and O-UTI (O antigens associated with urinary tract infection, i.e., O1, O2, O4, O6, O7, O16, O18, O25, and O75). Antimicrobial resistance, ExPEC markers, and O-UTI antigens were as detected in index *E. coli* isolates or total samples.

<sup>c</sup> Analyses for *E. coli* included all samples. Analyses of antimicrobial resistance, ExPEC status, and O-UTI status were limited to those *E. coli*-containing samples available for susceptibility testing, molecular analysis, and/or serotyping.

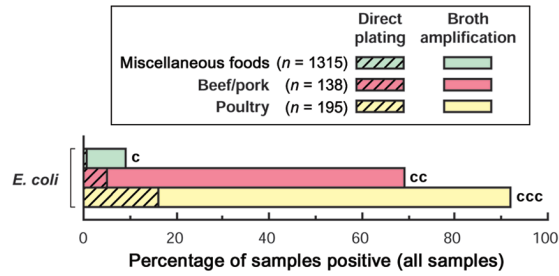
dictors of total and direct *E. coli* contamination (table 2). Among miscellaneous foods, the only significant multivariate predictor of *E. coli* contamination was season, with summer or autumn purchase predicting a higher risk. Among beef and pork items, natural-store source predicted a reduced risk of *E. coli*, whereas being ground was a risk factor. Among poultry items, year of study and being frozen were both risk factors for total *E. coli*, whereas, for direct *E. coli*, natural-store source predicted reduced risk, but antibiotic-free labeling was actually a risk factor (table 2).

**Antimicrobial resistance.** With the analysis limited to *E. coli*-positive food samples, the prevalence of antimicrobial resistance varied significantly by food class, from miscellaneous foods (lowest) to poultry (highest), whether analyzed as resistance to  $\geq 1$  drug, to  $\geq 5$  drugs, or to each drug individually (figure 1B). Ciprofloxacin resistance exhibited a borderline significant association with poultry (3.4% of samples vs. 0.5% for all other foods:  $P = .051$ ). Nalidixic acid resistance also was associated with poultry (30% of samples vs. 2.5% for miscellaneous foods and 3% for beef or pork;  $P < .001$ ) (figure 1B); among nonpoultry items, this was confined to beef (3/47 [6%]

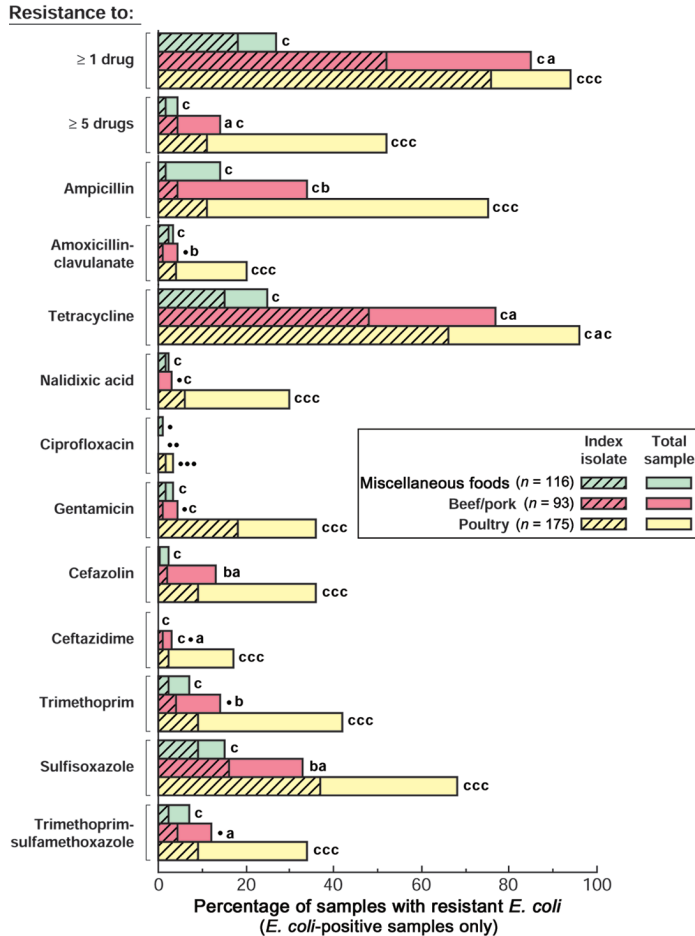
vs. 0/161;  $P = .01$ ). Aggregate resistance scores exhibited a similar by-food-class gradient, with median scores among *E. coli*-positive miscellaneous, beef or pork, and poultry items, respectively, being 0, 1.0, and 2.0 (index isolate); 0, 2.0, and 4.0 (most-resistant isolate); and 0, 1.0, and 5.0 (total sample) ( $P < .001$  for miscellaneous or poultry vs. all others).

Because of these differences, analyses again were stratified by food class. In univariate analyses for resistance to individual drugs (table 3), among *E. coli*-positive miscellaneous items, nonproduce foods exhibited a higher prevalence of tetracycline and sulfisoxazole resistance than did produce, and summer or autumn purchase was associated with increased ampicillin resistance, compared with that for winter or spring purchase. Among *E. coli*-positive beef and pork items, pork exhibited a higher prevalence of ampicillin, tetracycline, and sulfisoxazole resistance than did beef, whereas natural-store source and antibiotic-free purchase predicted decreased tetracycline and ampicillin resistance, respectively. Among *E. coli*-positive poultry items, turkey was associated with nalidixic acid, sulfisoxazole, and trimethoprim-sulfamethoxazole resistance; chicken with resistance to  $\beta$ -lactam agents; winter or spring purchase with

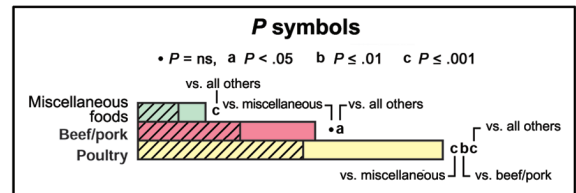
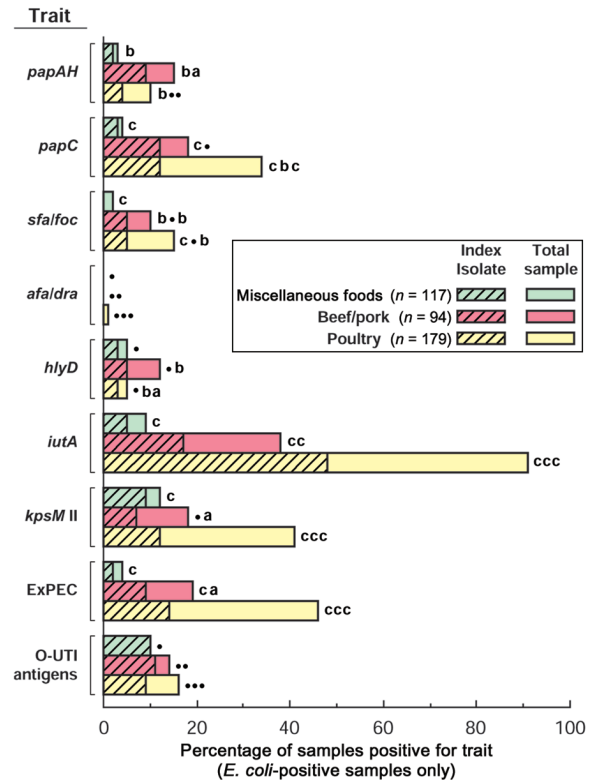
## A *Escherichia coli*



## B Antimicrobial resistance



## C Virulence traits



**Figure 1.** Prevalence of *Escherichia coli*, antimicrobial-resistant *E. coli*, and *E. coli* virulence-associated traits in retail foods. **A**, Prevalence of *E. coli* (as detected by direct plating, reflecting high-grade contamination, or by broth amplification, reflecting even trace contamination) among 1648 retail food items, stratified by food class (miscellaneous foods, beef or pork, and poultry). **B**, Prevalence of *E. coli* antimicrobial resistance (as detected in the index isolate, reflecting the sample's predominant strain, or in the total sample), both overall and to 11 individual drugs, among 384 *E. coli*-positive food samples, stratified by food class. (No resistance to nitrofurantoin was detected.) **C**, Prevalence of *E. coli* virulence-associated traits (as detected in the index isolate, reflecting the sample's predominant strain, or in the total sample) among 390 *E. coli*-positive food samples, stratified by food class. ExPEC, extraintestinal pathogenic *E. coli*, defined as detection of  $\geq 2$  of (*papA* and/or *papC*), *sfafoc*, *afa/dra*, *kpsM II*, and *iutA*; O-UTI, O antigens associated with urinary tract infection (O1, O2, O4, O6, O7, O16, O18, O25, and O75) [32]. *P* symbols (from  $\chi^2$  test or Fisher's exact test) are for comparisons between food classes for the particular outcome variable and apply only to comparisons involving broth amplification (**A**) or total sample (**B** and **C**). The position of the *P* symbol indicates which groups were compared. (The total prevalence of resistant or virulent *E. coli* can be derived by multiplying the overall prevalence values for *E. coli* [**A**] by the prevalence values shown for antimicrobial resistance [**B**] and virulence characteristics [**C**], because the latter were calculated for *E. coli*-positive samples only.) ns, not significant.

**Table 3. Prevalence of resistance to individual antimicrobial agents by food group among 384 *Escherichia coli*-positive retail food items.**

Food class	Comparison groups <sup>a</sup>		Resistance to <sup>b</sup>	Prevalence of resistance (%) <sup>b</sup>		P <sup>c</sup>
	Group 1	Group 2		Group 1	Group 2	
Miscellaneous	Other foods	Produce	Tetracycline	37	20	.04
			Sulfisoxazole	26	10	.03
Beef/pork	Summer/autumn	Winter/spring	Ampicillin	18	3	.04
			Pork	Beef	Ampicillin (ii) <sup>b</sup>	22
	Natural store	Other	Tetracycline (ii) <sup>b</sup>	64	32	.002
			Sulfisoxazole (ii) <sup>b</sup>	24	9	.04
Poultry	Antibiotic free	Other	Tetracycline	47	65	.002
			Ampicillin	15	40	.04
	Turkey	Chicken	Nalidixic acid	34	16	.02
			Sulfisoxazole (ii) <sup>b</sup>	43	23	.02
	Summer/autumn	Winter/spring	Trimethoprim-sulfamethoxazole (ii) <sup>b</sup>	12	0	.01
			Amoxicillin-clavulanate	12	36	<.001
			Cefazolin	23	67	<.001
			Ceftazidime	12	26	.02
			Ampicillin	64	84	.003
			Gentamicin (ii) <sup>b</sup>	9	24	.009
Natural store	Other	Sulfisoxazole (ii) <sup>b</sup>	29	44	.049	
		Tetracycline	98	87	.045	
Antibiotic free	Other	Nalidixic acid (ii) <sup>b</sup>	0	8	.03	

<sup>a</sup> Comparison groups (5 categories for miscellaneous foods and 6 categories each for beef/pork and poultry) were based on the variables shown in table 2, excluding year. Only comparisons that yielded  $P < .05$  are shown. For each food type, the no. of index isolates (no. of samples) was nonproduce miscellaneous foods, 31 (35); produce, 81 (82); pork, 45 (47); beef, 47 (47); turkey, 119 (121); and chicken, 47 (49).

<sup>b</sup> Comparisons were made for 11 antimicrobial agents. (No resistance was detected to nitrofurantoin.) Data shown are for total sample, or index isolate (ii) if so indicated.

<sup>c</sup> Fisher's exact test or  $\chi^2$  test (2-tailed).

ampicillin, gentamicin, and sulfisoxazole resistance; natural-store source with tetracycline resistance; and antibiotic-free labeling with reduced nalidixic acid resistance. In analyses of aggregate resistance scores among *E. coli*-positive samples, for beef and pork items, pork outscored beef, whereas natural-store source and antibiotic-free purchase predicted reduced resistance; for poultry items, turkey outscored chicken (table 4).

By multiple logistic regression analysis for predictors of resistance to  $\geq 1$  drug or to  $\geq 5$  drugs, among miscellaneous foods, no significant predictors were identified. However, for beef/pork items both beef and natural-store source predicted reduced risk, whereas for poultry items both season and year predicted reduced risk (table 2).

**Virulence-associated traits.** Among *E. coli*-positive samples, the prevalence of virulence-associated traits again was usually lowest among miscellaneous foods, intermediate in beef and pork, and highest in poultry (e.g., 4%, 19%, and 46% of *E. coli*-positive samples, respectively, were ExPEC positive;  $P < .001$ ) (figure 1C). Among miscellaneous foods, multivariate analysis identified no significant predictors of ExPEC or O-UTI status. For beef and pork, significant multivariate predictors of ExPEC or O-UTI status included summer or autumn purchase and beef (both with a reduced risk), whereas for poultry, sig-

nificant predictors included summer or autumn purchase and year of study (both with a reduced risk) (table 2).

Extended virulence genotyping of the ExPEC-positive samples detected all but 3 of 35 VFs sought. Fourteen markers were significantly distributed by food class, including 7 at the  $P \leq .001$  level (not shown), which provides evidence of food-class-specific ExPEC populations.

Seventeen isolates, all from phylogenetic groups B2 or D, met molecular criteria for ExPEC and exhibited O-UTI antigens or O11/O17/O77 antigens, consistent with possible human pathogenic potential. Two were from miscellaneous foods, 5 from beef or pork, and 10 from poultry, thus constituting 0.2%, 3.6%, and 5.1% of all samples in their food classes ( $P < .001$ ). Four of these isolates (from pea pods, turkey parts, ground pork, and vegetable dip) were indistinguishable according to RAPD profiling from selected human clinical isolates from the investigators' collections (figure 2). One of these food isolates corresponded with the recently described *E. coli* clonal group A, a disseminated cause of multidrug-resistant cystitis and pyelonephritis that, within one community, exhibited unexplained point-source spread [34, 35].

**Comparison of resistant and susceptible *E. coli*.** To assess their degree of commonality, the resistant and susceptible *E.*

**Table 4. Aggregate antimicrobial resistance scores by food group among 379 *E. coli*-containing retail food samples.**

Food class (no.)	Comparison groups (no.) <sup>a</sup>		Aggregate resistance score, median <sup>b</sup>		<i>P</i> <sup>c</sup>
	Group 1	Group 2	Group 1	Group 2	
Miscellaneous (116)	Produce (81)	Other (35)	0.3 <sup>d</sup>	1.1 <sup>d</sup>	.04
Beef or pork (93)	Pork (46)	Beef (47)	1.0	0	.003
	Natural store (17)	Other stores (76)	0	2.0	.01
	Antibiotic-free (20)	Other/unknown (73)	1.0	2.0	.01
Poultry (170) <sup>e</sup>	Turkey (121)	Chicken (49)	2.3 <sup>d</sup>	1.6 <sup>d</sup>	.04

<sup>a</sup> Comparison groups (5 categories for miscellaneous foods and 6 categories each for beef/pork and poultry) were based on the variables shown in table 2, excluding year. Only comparisons that yielded *P* < .05 are shown.

<sup>b</sup> Results shown are for comparisons of most-resistant isolates or index isolates, whichever yielded the lowest *P* value for the particular comparison.

<sup>c</sup> Mann-Whitney *U* test for nonparametric continuous data.

<sup>d</sup> Result shown is 5% trimmed mean (i.e., the mean after excluding the outlying 5% of values), because both groups exhibited the same median value.

<sup>e</sup> Five poultry samples (of 175 with resistance scores) were mixed (turkey and chicken) patties and so could not be analyzed for turkey vs. chicken.

*coli* isolates within each food class were compared for phylogenetic background, VFs, and O antigens. Only 7 (3.8%) of the resulting 180 comparisons yielded *P* < .05, and only 2 (1%) yielded *P* ≤ .01—evidence of considerable intrinsic similarity between the resistant and susceptible populations within each food class. In contrast, with the same by-food-class stratification, when phylogenetic group B2/D *E. coli* isolates were compared with non-B2/D *E. coli* isolates, according to VFs and O antigens, 26 (29%) of the resulting 90 comparisons yielded *P* < .05, whereas 16 (21%) yielded *P* ≤ .01, and 10 (11%) yielded *P* ≤ .001, which demonstrates extensive diversity within each food-class-specific population, despite the near absence of differences according to resistance status.

## DISCUSSION

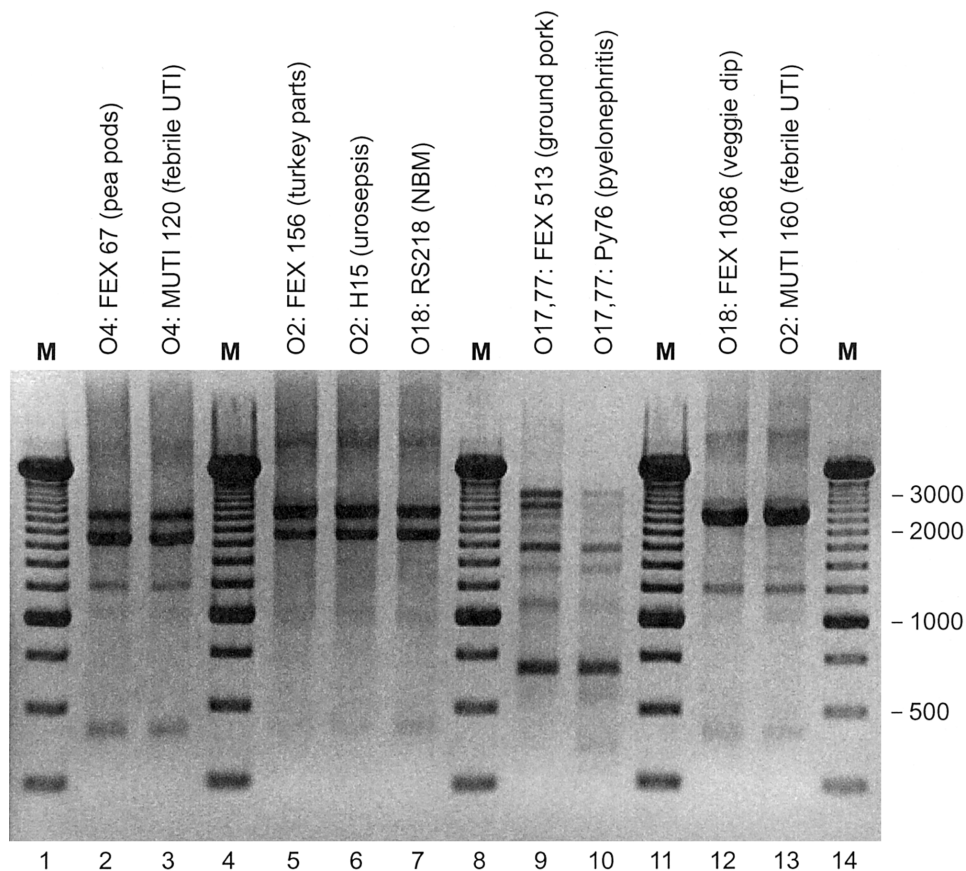
In our 2-year prospective market survey, we found that many retail foods, particularly poultry but also beef or pork and certain ready-to-eat items, were contaminated with antimicrobial-resistant *E. coli* and ExPEC. This is particularly alarming, given the rising prevalence of antimicrobial resistance among clinical *E. coli* isolates, the evidence of transmission of other foodborne bacteria to consumers and food preparers, and the recent unexplained dissemination of multidrug-resistant ExPEC clones [2, 20, 34, 35].

Extraintestinal *E. coli* are responsible for millions of UTI episodes, an estimated 36,000 deaths from sepsis, and billions of dollars in health-care costs annually in the United States [1]. This dwarfs the disease burden associated with the notorious *E. coli* O157:H7, which, as a foodborne pathogen, causes an estimated 62,458 infections and 52 deaths annually in the US [5]. Thus, if even a small fraction of extraintestinal *E. coli* infections involve foodborne ExPEC or resistance elements, a

possibility that is supported by our demonstration of a close resemblance between certain foodborne and human clinical ExPEC isolates, ExPEC may rival (or exceed) *E. coli* O157:H7 as a foodborne pathogen. Our findings therefore have considerable potential public-health and medical significance.

The highest prevalences and densities of resistant *E. coli* and ExPEC were found in meat products. This is consistent with contamination of animal carcasses with the host's fecal flora during slaughter and processing and with use of antimicrobial agents in food-animal production [13, 14]. In contrast, produce and other miscellaneous food items (including cheeses, salami, delicatessen items, turkey franks, and pastry products) were comparatively devoid of *E. coli*. Thus, although produce has been associated with antimicrobial-resistant gram-negative bacilli [37], and the other miscellaneous foods sometimes carry different pathogens and/or generic *E. coli* [8, 10, 21, 38], our data suggest that these foods represent relatively less important vehicles for antimicrobial-resistant *E. coli* or ExPEC than do meat and poultry. However, because ready-to-eat foods are consumed without being cooked, even infrequent or low-level contamination may pose a substantial risk. Of note, 2 of the food-source *E. coli* that we matched to human clinical isolates were from ready-to-eat foods. The bacterial inoculum size required for establishing colonization with ingested ExPEC or transfer of foodborne resistance elements to endogenous human gut *E. coli* is undefined but may not be large, because the ingestion of <100 viable cells of *Shigella dysenteriae*, an *E. coli* variant, is sufficient to cause disease in humans.

The present study provides an assessment of the microbial content of organic and natural foods, which have been presumed to be less likely to contain antimicrobial-resistant bacteria [39]. By multivariate analysis, natural-store source did



**Figure 2.** Random amplified polymorphic DNA (RAPD) profiles of selected *Escherichia coli* isolates from retail foods and infected humans. Profiles were done by use of arbitrary decamer primer 1283 (5'-gcatcccca-3') [36]. Lane nos. are shown below the gel image. Food-source isolates (lanes 2, 5, 9, and 12) have FEX designations. Each isolate's O antigen and ecological source (i.e., food type or clinical syndrome) are shown above the gel image. FEX 1086 exhibited ampicillin, tetracycline, gentamicin, sulfisoxazole, trimethoprim, and trimethoprim-sulfamethoxazole resistance; the other FEX isolates were susceptible to all agents tested. Serotypes of human clinical isolates are: O4:K2:H5 (strain MUTI 120), O2:K1:H7 (strain H15), O18:K1:H7 (strain RS218); O17,77:K52:H18 (strain Py76, which is a representative of *E. coli* clonal group A); and O2:H<sup>-</sup> (strain MUTI 160) (serotype data are from Peter Ulleryd, personal communication, and [35, 47, 48]). Markers (lanes 1, 4, 8, 11, and 14) are a 250-bp ladder (Gibco). UTI, urinary tract infection; NBM, neonatal bacterial meningitis.

predict a reduced risk of total and antimicrobial-resistant *E. coli* in beef or pork and of total *E. coli* in poultry. Paradoxically, antibiotic-free labeling actually was a multivariate risk factor for *E. coli* in poultry and, although it was negatively associated with certain resistance markers by univariate analysis, it was not a significant multivariate predictor of net resistance.

That natural-store source was a more potent (negative) predictor than antibiotic-free status for total and antimicrobial-resistant *E. coli* may be of immediate interest to consumers and raises questions as to underlying mechanisms. Presumably, undefined aspects of natural-store production and/or distribution are beneficial, which warrants further study. The lesser effect of antibiotic-free labeling should not be interpreted as indicating that on-farm antimicrobial use does not significantly influence local *E. coli* resistance patterns. Indeed, the favorable results of Denmark's ban on antimicrobial growth promoters suggest the opposite (<http://www.who.int/salmsurv/>

[links/gssamrgrowthreportstory/en/](http://www.who.int/salmsurv/)) [22]. More probably, on-farm effects may be obscured by downstream contamination—for example, in processing plants or from retail food handlers [23]. Likewise, labeling may misrepresent true on-farm antimicrobial use [40]. Indeed, information provided by certain producers of ostensibly antibiotic-free meats and poultry suggested that antimicrobial agents actually are administered to an unspecified proportion of their animals (J.R.J., unpublished data).

Two findings indirectly supported the hypothesis of on-farm resistance selection. The paucity of differences between the resistant and susceptible *E. coli* populations within each food class, in contrast to the marked differences between phylogenetic groups and food types, suggested that resistant and susceptible isolates within a given food class derive from a common source population, with resistance plausibly emerging on the farm [29]. Likewise, the (statistically or borderline significant) associations of ciprofloxacin and nalidixic acid resistance with



poultry and/or beef correspond with the approved agricultural use of fluoroquinolones in the United States only in these animals.

Our findings cause concern and indicate a need for further study to determine whether foodborne *E. coli* present a significant human health threat and, if so, to define the source of the problem. Additional studies are needed that compare food-source and human clinical isolates for resistance elements [41], genomic background [42], and virulence profiles [43] and that assess food-to-human transmission [9, 44]. The results would help establish the extent of commonality between food-source and human clinical isolates and estimate the contribution of foods to drug-resistant and/or ExPEC infections in humans. Studies of upstream food production steps are also needed, to determine the source(s) of the contamination and antimicrobial resistance [14]. Such information is required for root causes to be addressed—for example, through modified animal husbandry or distribution practices [22, 23] and/or by irradiating foods to eliminate pathogens and resistance elements before they reach consumers [45, 46].

In this regard, our finding that multiple variables (e.g. natural store, season, year, ground or frozen status, beef vs. pork, and chicken vs. turkey) were associated with significant differences in the prevalence of foodborne *E. coli*, antimicrobial resistance (including to specific drugs), and ExPEC suggests that these characteristics may provide clues to the origins of the observed contamination and selection of resistance. Discovery of the underlying causal links conceivably could identify opportunities for preventive interventions.

Limitations of the study include that foods were from 1 locale and 10 markets, which possibly limits generalizability, although the distributed nature of the food supply mitigates this concern. The sampling scheme, although highly structured, still allowed for possible bias. Because laboratory methods were not 100% sensitive, the results represent minimum estimates. Multiple comparisons allowed for possible type I errors (such that certain less statistically significant associations could represent chance findings), and small numbers within certain subgroups limited statistical power. Finally, food-to-human transmission was not directly studied, the source of the contaminating *E. coli* and antimicrobial resistance was not defined, and virulence potential was inferred from molecular and serological data rather than from experimentation.

In summary, we found that retail foods, particularly poultry products but also beef or pork items and certain ready-to-eat foods, are frequently contaminated with antimicrobial-resistant *E. coli* and/or ExPEC, in patterns that were significantly predicted by store type, specific food type, frozen or ground status (for meats), season, and year. Thus, the food supply may represent a significant but underrecognized vehicle for the dissemination of important pathogens and resistance elements. Clar-

ification of the health significance and underlying mechanisms of these findings is needed to allow rational selective purchasing and appropriate remediation and control efforts.

## Acknowledgments

We thank Dave Prentiss (Veterans Affairs Medical Center) for preparing the figures; Peter Ulleryd (University of Göteborg, Sweden) for providing strains MUTI 20 and MUTI 160; and Kwang Sik Kim (Johns Hopkins University) for providing strain RS218.

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