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Evaluation of drug-resistant *Enterobacteriaceae* in retail poultry and beef

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ABSTRACT There has been increasing concern on the emergence of multidrug-resistant foodborne pathogens from foods of animal origin, including poultry. The current study aimed to evaluate antibiotic-resistant *Enterobacteriaceae* from raw retail chicken/turkey parts (thigh, wings, breast, and ground) and beef meat (ground and chunks) in Middle Tennessee. Resistance of the collected *Enterobacteriaceae* to a panel of antibiotics was determined by the Kirby-Bauer disk diffusion test. Retail meats were also assayed for the presence of *Salmonella* spp. and *Escherichia coli* O157:H7. Two hundred thirty-seven samples representing 95.2% of the total of 249 samples tested were positive for *Enterobacteriaceae*. The level of contamination with *Enterobacteriaceae* in raw meats ranged from 3.26 $\log_{10}$ cfu/g to 4.94 $\log_{10}$ cfu/g with significant differences in counts among meat types ($P < 0.05$). Contamination was significantly greater ($P < 0.05$) in ground beef, beef chucks, ground chicken, chicken breast, and turkey wings (4.92, 4.58, 4.94, 4.75, 4.13 $\log_{10}$ cfu/g, respectively) than ground turkey and chicken wings (3.26 and 3.26 $\log_{10}$ cfu/g, respectively). *Klebsiella oxytoca*, *Serratia* spp., *E. coli*, and *Haffnia alvei* were most prevalent contaminants at 27.4, 14.3, 12.1, and 11.4%, respectively. Resistance of the *Enterobacteriaceae* to antimicrobials was most frequent with erythromycin, penicillin, and ampicillin at 100, 89, and 65.8%, respectively. Few (2.7%) of the *Enterobacteriaceae* were resistant to chloramphenicol. *Salmonella* spp., *E. coli* O157:H7, *Morganella morganii*, *Yersinia enterocolitica*, and *Vibrio parahemolyticus* exhibited multiple drug resistance. This investigation demonstrates that raw poultry and beef are potential reservoirs of antibiotic-resistant *Enterobacteriaceae*.

Key words: *Enterobacteriaceae*, antibiotic resistance, poultry, beef

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INTRODUCTION

Antimicrobial resistance is increasing in several species of *Enterobacteriaceae* (Karlowsky et al., 2003), and this has been a major concern with both clinical and commensal bacteria (Chikwendu et al., 2008). *Enterobacteriaceae* is distributed widely in nature and in the gastrointestinal tract of humans, other mammals, and birds. Previous studies (Barham et al., 2002; Fluckey et al., 2007; Mainali et al., 2009) suggest that increased shedding of enteric bacteria is associated with stress factors during transportation of animals and change of diet before slaughter. At some point in the carcass processing and handling, enteric bacteria in the animal's gut may contaminate meats and other surfaces with which these meats come into contact (Madden et al., 2004; Rasschaert et al., 2007).

It is a widespread practice to use antimicrobials as feed supplements in livestock production, but the use of antibiotics in agricultural practices has been implicated in the increase of these antibiotic-resistant foodborne pathogens (McEwen and Fedorka-Cray, 2002; Shea, 2004). These antimicrobial agents in livestock and poultry feed, which are intended to prevent and control infections, are suggested to create selective pressure favoring the emergence of antibiotic-resistant bacteria (Aarestrup et al., 2001). Evidently, contamination of food with antibiotic-resistant foodborne pathogens continues to be a major risk to public health and potentially compromises the treatment of severe bacterial infections (Van et al., 2007). Price et al. (2007) cited evidence that antibiotic-resistant zoonotic pathogens can funnel to human exposure and infection through various pathways, including meat and poultry products. According to Schroeder et al. (2003) and Dunowska et al. (2006), generic *Escherichia coli*, which is commonly found in raw meats, has the potential to transfer antibiotic resistance to other intestinal organisms. Other reports have also shown that enteric bacteria develop resistance to common antibiotics used in human and veterinary medicine such as tetracycline, gentamycin, kanamycin, and streptomycin (Kim et al., 2005).

Antimicrobial-resistant bacteria in animals are a growing concern because of their potential for trans-

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mission to humans as foodborne pathogens (Welton et al., 1998; Witte, 1998). Therefore, surveillance for antimicrobial susceptibility in *Enterobacteriaceae* is imperative because species of this family are among the most significant and prevalent human pathogens (Karlowsky et al., 2003). Previous reports indicate that the pathogenic *E. coli* serotype (O157:H7) and *Salmonella* account for most of foodborne illnesses caused by species in *Enterobacteriaceae* and are often transmitted through raw meats (Gorman et al., 2002; Kennedy et al., 2005; Kim et al., 2005). The presence of *Salmonella* in raw poultry meat and *E. coli* serotype O157:H7 in raw beef is a major public health concern (Doyle and Schoeni, 1987; Chen et al., 2010). Through unsafe raw meat handling practices and preparation, foodborne pathogens might also be transferred to ready-to-eat foods. An earlier report demonstrated that *Salmonella* potentially spread on kitchen surfaces during preparation of contaminated poultry (Cogan et al., 1999). Because food consumption is, therefore, a significant path for bacteria to gain entry into humans, the presence of antimicrobial-resistant bacteria in poultry and beef warrants attention. The spread of foodborne pathogens, especially the antibiotic-resistant ones, threaten the successful treatment of infectious diseases (Andersson, 2003). It is essential to evaluate the emergence and diffusion of antibiotic-resistant foodborne pathogens and commensal bacteria in raw meats.

The resistance of bacteria to antimicrobials will continue to threaten the therapeutic use of antibiotics in clinical medicine if massive use of antibiotics is not restricted. McGowan (2001) estimated the annual cost for treating infections caused by antibiotic-resistant bacteria to be approximately $4 to 5 billion. Several studies have been conducted to evaluate antibiotic resistance of clinical bacterial isolates (Fernandes et al., 2009), but a more limited number of comparable studies has been conducted to evaluate antibiotic-resistant bacteria in foods (Simeoni et al., 2008). Because most foodborne outbreaks are associated with the consumption of contaminated animal-derived products, studies on the occurrence of antimicrobial-resistant *Enterobacteriaceae* in raw meats are significant. These studies may provide valuable data needed for logical assessment of the relative risks of handling raw meats and also elucidate the role of foods in the transmission of antibiotic-resistant strains to human populations. Therefore, in this study, raw chicken, turkey, and beef sold at retail stores in Middle Tennessee were investigated for the presence of antibiotic-resistant *Enterobacteriaceae*.

MATERIALS AND METHODS

Sample Collection

Raw meats were purchased from 25 retail stores in Davidson County, Tennessee, and evaluated for possible contamination with antibiotic-resistant *Enterobacteriaceae*. The meats consisted of chicken (n = 93; 32.5%), beef (n = 99; 34.6%), and turkey (n = 94; 32.9%). The samples were stored in an ice chest and transported to the laboratory and were processed on the day of purchase or after 1 d of storage at 4°C. Meat samples were collected from 3 types of grocery stores classified by high, middle, and low income areas. Differences in occurrence of *Enterobacteriaceae* and their antimicrobial resistance among the 3 types of grocery stores were not significant ($P > 0.05$).

Enterobacteriaceae Enumeration and Identification

All meat types were processed for *Enterobacteriaceae* counts. Two 25-g samples were removed aseptically from each package of meat and added to 225 mL of sterile buffered peptone water (**BPW**; Fisher Scientific, Hanover Park, IL) contained in a mesh-lined stomacher bag. The mixture was pummeled in bag 400 Circulator (Seward Limited, London, UK) at 230 rpm for 2 min. Ten-fold serial dilutions up to 10^{-6} were prepared and subsequently plated on Petrifilm plates (3M Microbiology, St. Paul, MN) for *Enterobacteriaceae* counts and incubated at 35°C for 24 to 48 h. The colonies were enumerated manually and recorded after incubation. One randomly selected isolate from each positive sample were recultured 3 times to increase the likelihood of clonality and was then identified biochemically. Gram staining and oxidase tests were performed on fresh isolated colonies. Subsequently, presumptive *Enterobacteriaceae* were identified using the API 20E system (Bio-Merieux, Durham, NC) and according to the manufacturer's instructions.

Isolation of Salmonella spp.

Preenrichment was performed by 1:10 dilution of 25 g of meat sample in 225 mL of sterile BPW followed by incubation at 35°C for 20 h. After incubation, each enriched sample was pummeled in 400 Circulator as previously described. The enriched BPW cultures (1.0 mL) were transferred into 10 mL tetrathionate broth and incubated at 42°C for 24 h for selective enrichment. Loops of tetrathionate enrichment cultures were streaked onto selective Xylose Lysine Tergitol 4 agar (XLT4, Difco, Becton Dickinson, Sparks, MD) and selective CHROMagar Salmonella agar and incubated at 35°C for 24 h. The plates were evaluated for colonies typical of *Salmonella* species after 24 h of incubation. In addition, *Salmonella* isolation was also performed by using Reveal for *Salmonella* Complete System-SC (Neogen, Lansing, MI). Briefly, 25 g of each sample (the same meat samples) was added to Reveal reconstituted media and incubated at 42°C for 2 h. Following the incubation period, the mixture was enriched for 18 h in a selective concentrate of Selenite Cystine and subsequently tested for *Salmonella* with Neogen's Reveal for *Salmonella* test system. A colony showing *Salmonella*

characteristics was confirmed by biochemical test triple sugar iron and lysine iron agar. *Salmonella* colonies were also subjected to biochemical characterization using an API 20E kit (BioMerieux, Marcy l'Etoile, France) following the manufacturer's instructions. The strips were read, and final identification was secured using API LAB PLUS computer software (Bio-Merieux, France). *Salmonella* isolates were further tested with *Salmonella* O grouping antisera.

Isolation of E. coli O157:H7

For enrichment, 25 g of each meat sample was homogenized in 225 mL of modified tryptone soy broth (Becton Dickinson and Co.) supplemented with novobiocin (20 mg/L) and incubated at 37°C for 18 to 24 h. The enrichment broth was streak-cultured on Sorbitol MacConkey agar (CT-SMAC; Difco Laboratories, Detroit, MI) containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) and incubated at 37°C for 24 h. Colorless colonies on CT-SMAC were subcultured on tryptic soy agar with 0.6% yeast extract and incubated at 37°C for 18 to 24 h. Additionally, samples were subjected to Reveal *E. coli* O157:H7, 20-h system (Neogen) for *E. coli* O157:H7 isolation. Briefly, 25 g of each sample was added to the Reveal *E. coli* reconstituted media and incubated at 36°C for 20 h. Samples from 20-h enrichment cultures were tested for the occurrence of *E. coli* O157:H7 with lateral flow immunoassay for *E. coli* O157:H7.

In this study only 73 isolates; beef (n = 24), chicken (n = 28), and turkey (n = 21) were subjected to an antimicrobial susceptibility test.

Determination of Antimicrobial Susceptibility

The antimicrobial susceptibility test was determined using the Bauer and Kirby disk diffusion technique on Mueller–Hinton Agar (Becton Dickinson Microbiological Systems, Cockeysville, MD). To determine the *Enterobacteriaceae* antibiogram, isolates from selected meat samples were subjected to an antimicrobial susceptibility test. About 3 to 4 colonies were chosen per plate and a total of 3 plates per selected meat samples were evaluated for antibiotic resistance. In this case, only 73 isolates [beef (n = 24), chicken (n = 28), and turkey (n = 21)] were tested.

Further, these colonies were identified. For the identification of individual *Enterobacteriaceae* colonies such as *Salmonella*, *E. coli*, *Klebsiella oxytoca*, *Enterobacter cloacae*, and so on, only one single isolate per plate was chosen for the antimicrobial sensitivity test. The choice to analyze the individual *Enterobacteriaceae* species was based on their importance clinically and included *Salmonella*, *Shigella*, *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Morganella*, Y*ersinia*, and so on (Stiles and Ng, 1981a,b; Doyle and Erickson, 2006). Some of these genera of *Enterobacteriaceae* such as *Salmonella*, *E. coli, Shigella*, and *Yersinia* are usually associated with gastroenteritis, foodborne diseases, and have the potential to develop antimicrobial resistance (Carattoli, 2009; Li and Wang, 2010; Iwabuchi et al., 2011).

The antimicrobial agents used in this study included tetracycline (30 μg), streptomycin (10 μg), ampicillin (10 μg), erythromycin (15 μg), gentamycin (10 μg), kanamycin (30 μg), penicillin (10 μg), and chloramphenicol (30 μg). Antimicrobial resistance to erythromycin was evaluated primarily because both tylosin and erythromycin are classified as macrolide drugs with the potential for cross-resistance between these 2 antimicrobials (Berrang et al., 2007). In the United States, tylosin phosphate is an antimicrobial drug approved for use in broiler feed at subtherapeutic levels to promote growth. It is generally accepted that bacteria exposed to these subtherapeutic levels of the drugs can develop resistance to those drugs (Singer and Hofacre, 2006), of which erythromycin is used to treat human infections. According to Belanger and Shryock (2007), the use of macrolide antibiotics in food animals has the potential to select for macrolide-resistant strains of resident bacterial flora.

Bacteria cultures were grown with shaking in 5 mL of Luria-Bertani (Difco, Becton Dickinson) broth at 37°C for 24 h. Each overnight culture was spread evenly onto Mueller-Hinton agar and incubated at 37°C for 24 h. To achieve the antibiotic susceptibility of bacteria, the measurement for the zones of inhibition was based on the breakpoints of the zone diameters for individual antibiotic agents. Categorical interpretations were made according to Clinical and Laboratory Standards Institute (**CLSI**, 2009). Results were interpreted as sensitive, intermediate resistant, or resistant based on CLSI guidelines. Reference strain *E. coli* ATCC 25922 were used to validate the results of the antimicrobial discs.

Data Analysis and Interpretation

Bacterial count results were transformed to log form before analysis. Data were compared using one-way ANOVA using SPSS software for Windows, version 12 (Chicago, IL). Treatment means were compared using the *t*-test and chi square analysis. Statistical significance were defined at $P < 0.05$.

RESULTS

Enterobacteriaceae in Retail Poultry and Beef

The average *Enterobacteriaceae* viable counts for chicken, turkey, and beef were between 3.26 to 4.94 $\log_{10}$ cfu/g (Table 1). With the exception of turkey, ground meats were inclined to harbor greater *Enterobacteriaceae* contamination levels compared with chunks of the corresponding ground meat types. For example, the average *Enterobacteriaceae* population was significantly

Table 1. *Enterobacteriaceae* contamination levels in retail meats

Sample ID	Sample size	Log_{10} cfu/g	SEM
Ground beef	43	4.92[a]	0.336
Ground chicken	22	4.94[a]	0.392
Ground turkey	45	3.26[b]	0.170
Turkey wings	22	4.13[a]	0.275
Beef steak	40	4.58[a]	0.354
Chicken wings	14	3.26[b]	0.170
Chicken breast	41	4.75[a]	0.329
Turkey breast	22	3.89[ab]	0.400
Total samples	249	—	—
P-value	—	≤0.05	—

[a,b]Means with no common superscript differ significantly ($P < 0.05$).

greater ($P < 0.05$) in ground chicken (4.94 log_{10} cfu/g) than in chicken wings (3.25 log_{10} cfu/g). Differences in mean *Enterobacteriaceae* count among ground beef, ground chicken, turkey wings, steak, and chicken breast (4.92, 4.94, 4.13, 4.58, and 4.75 log_{10} cfu/g, respectively) were not different ($P > 0.05$), but they were significantly greater ($P < 0.05$) than those of ground turkey and chicken wings (3.26 log_{10} cfu/g).

The occurrence of *Enterobacteriaceae* in retail meats is presented in Table 2. As shown, out of 281 bacteria isolates from raw meat samples, 34 (12.1%) were identified as *E. coli* and only one sample was positive for *E. coli* O157:H7. Other potentially pathogenic isolates were *Morganella morgani* 3 (1.1%), *Vibrio parahemolyticus* 1 (0.4%), and *Yersinia enterocolitica* 1 (0.4%). Only 16 samples (5.7%) were positive for *Salmonella* spp. Among the 16 *Salmonella* spp. isolates, *Salmonella* Arizonae, *Salmonella* Pullorum, *Salmonella* Gallinarum, and *Salmonella* Choleraesuis were identified. According to our study, commensal bacteria including *Proteus mirabilis* 3 (1.1%), *Enterobacter aerogenes* 18 (6.4%), *Klebseiella oxytoca* 77 (27.4%), and *Citrobacter freundii* 5 (1.7%) were also isolated from retail meats. The occurrence of *Klebseiella oxytoca* in retail meats was highest ($P < 0.05$) among all other pathogens. Significant occurrences that were lower ($P < 0.05$) than those of *Klebseiella oxytoca*, but statistically greater ($P < 0.05$) than other microorganisms, were observed in *E. coli* (11.4%), *Hafnia alvei* (11.4%), and *Serratia* ssp. (14.3%). Other notable and significant occurrences were *Enterobacter aerogenes* (6.4%), *Kluvyera* spp. (5.6%), *Pantoea* spp. (3.6%), and *Salmonella* spp. (5.7%). The occurrence of *Klebseiella oxytoca* was more than 2-fold greater than *E. coli*, *Hafnia alvei*, and *Serratia* spp. and 4 to 60 times greater than other microorganisms detected in the retail meats. In this study, there was high prevalence of *Klebseiella oxytoca* in chicken, turkey, and beef meats. Hence, prevalence of *Klebseiella oxytoca* might be a useful marker for the identification of contaminated raw retail meats.

Drug-Resistant Enterobacteriaceae

The prevalence of drug-resistant *Enterobacteriaceae* isolated from retail meats is represented in Table 3. Generally, our results indicate that the *Enterobacteria-*

Table 2. Occurrence (%) of *Enterobacteriaceae* isolated from retail meats[1]

Bacteria	Number of isolates (n = 281)		*P*-value[2]
	Detected	Not detected	
Acinetobacter baumanii	11 (3.9)[cd]	270 (96.1)	NS
Aeromonas hydrophila	6 (2.1)[d]	275 (97.9)	NS
Battiauxella agrestis	1 (0.4)[d]	280 (99.6)	NS
Citrobacter freundii	5 (1.7)[d]	276 (98.3)	NS
Escherichia coli spp.	34 (12.1)[b]	247 (87.9)	0.00
Enterobacter aerogenes	18 (6.4)[c]	263 (93.6)	0.01
Flavimonas oryzihabitans	1 (0.4)[d]	280 (99.6)	NS
Hafnia alvei	32 (11.4)[b]	249 (88.6)	0.00
Klebsiella oxytoca	77 (27.4)[a]	204 (72.6)	0.00
Klebsiella pneumoniae	1 (0.4)[d]	280 (99.6)	NS
Klebsiella terrigena	1 (0.4)[d]	280 (99.6)	NS
Kluyvera spp.	16 (5.6)[c]	265 (94.4)	0.05
Lecrercia adecarboxylata	1 (0.4)[d]	280 (99.6)	NS
Morganella morganii	3 (1.1)[d]	278 (98.9)	NS
Ochrobactum antropi	1 (0.4)[d]	280 (99.6)	NS
Pantoea spp.	10 (3.6)[c]	271 (96.4)	0.05
Proteus mirabilis	3 (1.1)[d]	278 (98.9)	NS
Providencia struartii	1 (0.4)[d]	280 (99.6)	NS
Rahnella aquatilis	1 (0.4)[d]	280 (99.6)	NS
Salmonella spp.	16 (5.7)[c]	265 (94.3)	0.01
Serratia spp.	40 (14.3)[b]	241 (85.7)	0.00
Vibrio parahaemolyticus	1 (0.4)[d]	280 (99.6)	NS
Yersinia enterocolitica	1 (0.4)[d]	280 (99.6)	NS
PSEM[3]	1.36	—	—

[a–d]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Chicken, turkey, and beef.

[2]Probability that mean number of isolates of a specific microorganism detected is significantly greater than that of other listed microorganisms.

[3]Pooled SEM.

ceae tested was resistant to erythromycin (100%), penicillin (89%), ampicillin (65.8%), streptomycin (43.8%), tetracycline (28.8%), and kanamycin (17.8%). Antimicrobial resistance was lowest in *Enterobacteriaceae* incubated in chloramphenicol- (2.7%) and gentamycin- (9.6%) containing plates. It was evident that in the retail meats evaluated, all (100%) of the *Enterobacteriaceae* isolates were resistant to erythromycin and such prevalence was significantly greater ($P < 0.05$) than that of all other antimicrobial agents evaluated. Significant statistical differences ($P < 0.05$) in prevalence of drug resistance of the *Enterobacteriaceae* was such that erythromycin > penicillin > ampicillin > streptomycin = tetracycline > kanamycin = gentamycin > chloraphenicol. On the other hand, the proportion of isolated from retail chicken and turkey which were resistant to tetracycline, streptomycin, kanamycin, and gentamycin were significantly greater ($P < 0.05$) than those isolated from retail beef. The *Enterobacteriaceae* isolates that were resistant to ampicillin were greater ($P < 0.05$) in retail beef than in chicken and turkey. Although differences in the proportion of *Enterobacteriaceae* isolated from beef and chicken that were resistant to chloraphenicol were not significant ($P > 0.05$), they were significantly greater ($P < 0.05$) than those isolated from retail turkey. All *Enterobacteriaceae* isolates were resistant to at least one of the antimicrobial agents evaluated. Overall, 84.9% of the isolates displayed microbial drug resistance (**MDR**) to 3 or more antimicrobials, whereas 19.2% (14 of 73) of the 73 isolates evaluated displayed MDR to 5 or more antimicrobials.

Table 4 presents antibiotic resistance patterns for foodborne pathogens isolated from retail meats. Various strains of *Salmonella* were resistant to at least one or more of the antibiotics evaluated, except chloramphenicol with which intermediate resistance was noted in *Morganella morganii*. *Morganella morganii* was isolated only from retail chicken and displayed resistance to tetracycline, ampicillin, erythromycin, and penicillin, but none was seen in streptomycin, kanamycin, and gentamycin. *Salmonella* Arizonae isolated from retail turkey exhibited the highest level of MDR (87.5%), which was statistically greater ($P < 0.001$) than all other pathogenic *Enterobacteriaceae* isolated from retail beef and chicken. Other notable MDR pathogenic *Enterobacteriaceae* were isolated from retail chicken, turkey, and beef and include *Morganella morganii*, S*almonella* spp., and *Yersinia enterocolotica*, respectively. The MDR of these microorganisms ranged from 50 to 62% and was significantly lower ($P < 0.05$) than that of *Salmonella* Arizonae isolated from retail turkey, but greater ($P < 0.05$) than that of other pathogenic *Enterobacteriaceae* isolated from retail beef turkey and chicken. On the other hand, the lowest ($P < 0.05$) mean MDR of 12.5% was observed in *Salmonella* Pullorum isolated from retail chicken. These mean MDR were not different ($P > 0.05$) from each other but were significantly lower ($P < 0.001$) than those of other *Enterobacteriaceae* isolated from retail beef, turkey, and chicken.

The detailed presentation of MDR patterns of *Enterobacteriaceae* isolated from retail poultry are presented in Table 5. The highest MDR was observed for *K. oxytoca* in retail chicken and *E. coli* I in retail turkey where the microorganisms isolated were resistant to 87.5% of the antimicrobial agents evaluated. These mean MDR values were significantly greater ($P < 0.05$) than those observed in all other *Enterobacteriaceae* isolated from both retail chicken and turkey. Other notable *Enterobacteriaceae* that exhibited significantly high MDR than other microorganisms include *E. coli*, which was isolated from retail chicken and turkeys and *Serratia liquifaciens* isolated from retail turkey. These microorganisms were resistant to about 75% of the antimicrobial drugs evaluated. *Aeromonas hydrophila* and *Hafnia alvei*, which were isolated from retail chicken, exhibited resistance to the least number of antimicrobi-

Table 3. Prevalence of drug-resistant *Enterobacteriaceae* (%) from raw meat sampled from retail stores[1]

Antimicrobial agent (μg)	Concentration (μg)	Beef (n = 24)	Chicken (n = 28)	Turkey (n = 21)	Total (n = 73)	*P*-value
Tetracycline	30	2 (8.3)^{b}	11 (39.3)^{a}	8 (38.1)^{a}	21 (28.8)^{wx}	<0.01
Ampicillin	10	19 (79.2)^{a}	15 (53.6)^{b}	14 (66.7)^{b}	48 (65.8)^{v}	<0.05
Streptomycin	10	7 (29.2)^{b}	12 (42.9)^{ab}	13 (61.9)^{a}	32 (43.8)^{w}	<0.01
Kanamycin	30	3 (12.5)^{b}	5 (17.9)^{ab}	5 (23.8)^{a}	13 (17.8)^{xy}	<0.05
Gentamycin	10	0 (0)^{c}	2 (7.1)^{b}	5 (23.8)^{a}	7 (9.6)^{y}	<0.05
Erythromycin	15	24 (100)^{a}	28 (100)^{a}	21 (100)^{a}	73 (100)^{t}	NS
Penicillin	10	23 (95.8)^{a}	23 (82.1)^{b}	19 (90.5)^{a}	65 (89.0)^{u}	<0.01
Chloramphenicol	30	1 (4.1)^{a}	1 (3.8)^{a}	0 (0)^{b}	2 (2.7)^{z}	<0.05
DR[2] ≥1		24 (100)^{a}	28 (100)^{a}	21 (100)^{a}	73 (100)^{x}	NS
MDR[3] ≥3		22 (91.7)^{a}	20 (71.4)^{b}	19 (90.5)^{a}	62 (84.9)^{x}	<0.05
MDR[4] ≥5		1 (4.1)^{c}	6 (21.4)^{b}	7 (33.3)^{a}	14 (19.2)^{y}	<0.05

$^{a–c}$Means within a row with no common superscript differ significantly ($P < 0.05$).

$^{t–z}$Means within a column with no common superscript differ significantly ($P > 0.05$).

[1]n = number of isolates tested. Numbers in parentheses are the number of total resistant isolates.

[2]Drug resistance to one or more antimicrobial (DR).

[3]Microbial drug resistance to 3 or more antimicrobials.

[4]Microbial drug resistance to 5 or more antimicrobials.

Table 4. Multiresistance (%) patterns of foodborne pathogens from retail meats[1]

Pathogenic *Enterobacteriaceae*	Meat type	Tet	Amp	Str	Kan	Gen	Ery	Pen	Chl	MDR[2]	*P*-value[3]
Escherichia coli O157:H7	Beef		I				R	R		3 (37.5)[c]	<0.05
Salmonella Arizonae	Beef	R					R	R		3 (37.5)[c]	<0.05
Salmonella spp.	Beef		R				R	R		3 (37.5)[c]	<0.05
Yersinia enterocolitica	Beef	I			R		R	I		4 (50.0)[b]	<0.01
Salmonella Arizonae	Turkey	R	I	I	R	I	R	R		7 (87.5)[a]	<0.001
Salmonella spp.	Turkey	R		R			R	R		4 (50.0)[b]	<0.01
Salmonella spp.	Turkey			I			R	R		3 (37.5)[c]	<0.05
Salmonella spp.	Turkey			I			I	S		2 (25.0)[d]	<0.05
Salmonella spp.	Turkey						R	R		2 (25.0)[d]	<0.05
Salmonella Choleraesuis	Chicken			I	R		R	R		4 (50.0)[b]	<0.01
Salmonella Gallinarum	Chicken						R	R		2 (25.0)[d]	<0.05
Salmonella Pullorum	Chicken						R			1 (12.5)[e]	NS
Salmonella Pullorum	Chicken		I				R	R		3 (37.5)[c]	<0.05
Salmonella spp.	Chicken			R			R			2 (25.0)[d]	<0.05
Salmonella spp.	Chicken	R					I	I		3 (37.5)[c]	<0.05
Salmonella spp.	Chicken						R	I		2 (25.0)[d]	<0.05
Salmonella spp.	Chicken						R			1 (12.5)[e]	NS
Salmonella spp.	Chicken						R			1 (12.5)[e]	NS
Morganella morganii	Chicken	I	R				R	R		4 (50.0)[b]	<0.01
M. morganii	Chicken	R	R				R	R	I	5 (62.5)[b]	<0.001
M. morganii	Chicken	R	R				R	R		4 (50.0)[b]	<0.01
PSEM[4]		—	—	—	—	—	—	—	—	3.88	—

[a–e]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Tet: tetracycline, Amp: ampicillin, Str: streptomycin, Kan: kanamycin, Gen: gentamycin, Ery: erythromycin, Pen: penicillin, Chl: chloramphenicol, R, resistant; I, intermediate; S, susceptible.

[2]Microbial drug resistant (MDR) isolates.

[3]Probability that mean number of isolates of a specific microorganism detected is significantly greater than that of other listed microorganisms.

[4]Pooled SEM.

al agents evaluated (25%). Overall, *Enterobacteriaceae* isolated from retail chicken were *Aeromonas hydrophila*, *E. coli* I*, Hafnia alvei* I, and *Klebsiela oxytoca* and were resistant to 25 to 87.5% of the antimicrobial agents evaluated. On the other hand, *Enterobacter eurogenes*, *E. coli* I, *Klebsiela oxytoca*, and *Serratia liquifaciens* were isolated from retail turkey and were resistant to 37.5 to 87.5% of the antimicrobial agents evaluated.

Detailed presentation of MDR patterns of *Enterobacteriaceae* isolated from retail beef are presented in Table 6. *Escherichia coli* was resistant to 75% of the antimicrobial agents evaluated, a proportion that was significantly greater ($P < 0.05$) than all other *Enterobacteriaceae* in retail beef. Although differences in proportion of MDR among most *E. coli*, *K. oxytoca*, and *Enterobacter cloacae* were not significant, they were significantly greater than those of *Klebsiella terrigena* (50 vs. 25%, respectively).

DISCUSSION

In this study, *Enterobacteriaceae* was collected from retail chicken, turkey, and beef to determine their antimicrobial susceptibility. Overall, there was a significant difference in *Enterobacteriaceae* contamination levels among different meat types, with the most contamination seen in chicken. These findings are supported by previous studies (Harrison et al., 2001; Wong et al., 2004; Meldrum et al., 2005; Wong et al., 2007), where raw poultry had significantly ($P < 0.05$) greater bacterial counts than other meat types. In this study, ground chicken exhibited a greater contamination level than corresponding whole chicken pieces. These results were also in agreement with previous studies that demonstrated that ground meats tend to be heavily contaminated as a result of more surface area being exposed to contamination from food processing equipment and meat handlers (Schroeder et al., 2003, 2004). The combination of meat tissues from several animals is also documented as one of the reasons for increased contamination of ground meat (Troutt and Osburn, 1997; LeJeune and Christie, 2004).

The *Enterobacteriaceae* contamination levels in ground turkey did not differ significantly ($P > 0.05$) from those of turkey breast (3.26 vs. 3.89 $\log_{10}$ cfu/g, respectively). Because the presence of *Enterobacteriaceae* is an indicator of hygiene and postprocessing contamination of retail meats, *Enterobacteriaceae* contamination of retail meats observed in this study clearly highlights a possible breakdown of hygienic handling practices at different stages of the meat processing and distribution chain. The finding that raw retail meats were contaminated with *Enterobacteriaceae* suggests that more weight must be placed on hygiene and handling practices in the manufacturing and distribution to guarantee the safety of retail meats.

Escherichia O157:H7, *Salmonella* spp., *Morganella morganii*, *K. oxytoca*, and *Klebsiella* spp. are potential pathogens isolated from raw retail meats in this study. In our observations, *E. coli* O157:H7 was de-

Table 5. Multiresistance (%) patterns of *Enterobacteriaceae* from raw retail poultry[1]

Pathogenic *Enterobacteriaceae*	Meat type	Tet	Amp	Str	Kan	Gen	Ery	Pen	Chl	MDR[2]	*P*-value[3]
Aeromonas hydrophila	Chicken	I		R			R	R		4 (50.0)^{cd}	<0.05
A. hydrophila	Chicken	R					R			2 (25.0)^{e}	NS
Escherichia coli 1	Chicken	R		R	R		R	R		5 (62.5)^{c}	<0.05
E. coli 1	Chicken	R		R	I	I	R	R		6 (75.0)^{b}	<0.02
E. coli 1	Chicken	R	R	R			R	R		5 (62.5)^{c}	<0.05
E. coli 1	Chicken	R		R	I		R	R		5 (62.5)^{c}	<0.05
E. coli 1	Chicken	R	R	R			R	R		5 (62.5)^{c}	<0.05
Hafnia alvei 1	Chicken		S				R	R		2 (25.0)^{e}	NS
Klebsiella oxytoca	Chicken		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Chicken		R		I		R	R		4 (50.0)^{cd}	<0.05
K. oxytoca	Chicken		R	I			R	R		4 (50.0)^{cd}	<0.05
K. oxytoca	Chicken		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Chicken		R	I			R	R		4 (50.0)^{cd}	<0.05
K. oxytoca	Chicken		I	I			R	R		4 (50.0)^{cd}	<0.05
K. oxytoca	Chicken		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Chicken		R	I			R	R		4 (50.0)^{cd}	<0.05
K. oxytoca	Chicken	R	R	R	I	R	R	R		7 (87.5)^{a}	<0.01
Enterobacter aerogenes	Turkey		R				I	R		3 (37.5)^{d}	<0.05
Escherichia coli 1	Turkey	R		I			R	R		4 (50.0)^{cd}	<0.05
E. coli 1	Turkey	R		I			R	R		4 (50.0)^{cd}	<0.05
E. coli 1	Turkey	R	R	R	I	R	R	R		7 (87.5)^{a}	<0.01
E. coli 1	Turkey	R	R	I	I		R	R		6 (75.0)^{b}	<0.02
E. coli 1	Turkey	R	R	R		I	R	R		6 (75.0)^{b}	<0.02
E. coli 1	Turkey			I			R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Turkey		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Turkey		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Turkey		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Turkey		R	I			R	R		4 (50.0)^{cd}	<0.05
K. oxytoca	Turkey		R				R	R		3 (37.5)^{d}	<0.05
K. oxytoca	Turkey		R				R	R		3 (37.5)^{d}	<0.05
Serratia liquifaciens	Turkey	R	R	R		R	R	R		6 (75.0)^{b}	<0.02
PSEM[4]		—	—	—	—	—	—	—	—	3.17	—

[a–e]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Tet: tetracycline, Amp: ampicillin, Str: streptomycin, Kan: kanamycin, Gen: gentamycin, Ery: erythromycin, Pen: penicillin, Chl: chloramphenicol, R, resistant; I, intermediate; S, susceptible.

[2]Microbial drug resistant (MDR) isolates.

[3]Probability that mean number of isolates of a specific microorganism detected is significantly greater than that of other listed microorganisms.

[4]Pooled SEM.

Table 6. Multiresistance (%) patterns of *Enterobacteriaceae* from raw retail beef[1]

Pathogen	Tet	Amp	Str	Kan	Gen	Ery	Pen	Chl	MDR[2]	*P*-value[3]
Escherichia coli 1	R	R	R	R		R	R		6 (75.0)^{a}	<0.001
E. coli 1		R				R	R		3 (37.5)^{bc}	<0.05
E. coli 1		R	I			R	R		4 (50.0)^{b}	<0.01
E. coli 1		R				R	R		3 (37.5)^{bc}	<0.05
E. coli 1		R				R	R		3 (37.5)^{bc}	<0.05
E. coli 1			I	I		R	R		4 (50.0)^{b}	<0.01
Klebsiella oxytoca		R	I			R	R		4 (50.0)^{b}	<0.01
K. oxytoca		R				R	R		3 (37.5)^{bc}	<0.05
K. oxytoca		R				R	R		3 (37.5)^{bc}	<0.05
K. oxytoca		R	I			R	R		4 (50.0)^{b}	<0.01
K. oxytoca		R				R	R		3 (37.5)^{bc}	<0.05
K. oxytoca		R				R	R		3 (37.5)^{bc}	<0.05
K. oxytoca		R				R	R		3 (37.5)^{bc}	<0.05
Enterobacter aerogenes		R				R	R		3 (37.5)^{bc}	<0.05
E. aerogenes		R				R	R		3 (37.5)^{bc}	<0.05
E. cloacae		I				R	R	I	4 (50.0)^{b}	<0.01
Klebsiella pneumoniae			I			R	R		3 (37.5)^{bc}	<0.05
Klebsiella terrigena		R				R	R		3 (37.5)^{bc}	<0.05
K. terrigena						I	I		2 (25.0)^{c}	NS
PSEM[4]	—	—	—	—	—	—	—	—	2.12	—

[a–c]Means within column with no common superscript differ significantly ($P < 0.05$).

[1]Tet: tetracycline, Amp: ampicillin, Str: streptomycin, Kan: kanamycin, Gen: gentamycin, Ery: erythromycin, Pen: penicillin, Chl: chloramphenicol, R, resistant; I, intermediate; S, susceptible.

[2]Microbial drug resistant (MDR) isolates.

[3]Probability that mean number of isolates of a specific microorganism detected is significantly greater than that of other listed microorganisms.

[4]Pooled SEM.

tected in only one of the beef samples tested. Colonies of this organism on SMAC medium were colorless and hence readily recognizable. The presence of foodborne pathogens in raw retail meats, as reported by Wong et al. (2004), could be due to fecal contamination at the time of meat processing. *Escherichia coli* contamination in meats is possibly due to bowel rapture during the slaughter process (Mead et al., 1999; Schroeder et al., 2004). Schroeder et al. (2004) cited evidence that *E. coli* occurs naturally in the digestive tract of all warm-blooded animals, but some strains are pathogenic and cause diseases. *Salmonella* Arizonae, *Salmonella* Pullorum, *Salmonella* Gallinarum, and *Salmonella* Choleraesuis were among the *Salmonella* spp. isolated from raw poultry in this study. *Salmonella* Choleraesuis has an elevated predilection for causing systemic infection in humans (Foley et al., 2008). Previous reports have shown that *Salmonella* occur in the gut and can cause carcass contamination during slaughter and processing of poultry (Wong et al., 2004). Although not in high numbers, *Yersinia enterocolitica* and *Morganella morganii* were also isolated from raw beef and chicken. Our findings are in agreement with a previous study (Poppe et al., 2006) in which *Yersinia* spp. and *Morganella* spp. were isolated from fresh meats.

Poultry and retail meats are frequently tainted with gastrointestinal flora, which could possibly be foodborne pathogens (Kegode et al., 2008). Therefore, the spread of foodborne pathogens from retail meats within the home is anticipated. The potential and implications for contamination with microorganisms such as *Salmonella* and *E. coli*, among others within the domestic kitchen environment, have been reviewed (Scott et al., 1982; Spiers et al., 1995). It is therefore critical to educate consumers on effective procedures to sanitize kitchen surfaces, utensils, and hands, especially after handling raw meats, a premise that has also been supported by Gorman et al. (2002) and Mattick et al. (2003).

Enterobacteriacea recovered from the poultry and beef were resistant to multiple antimicrobials, which can be transmitted to humans through food products. Essentially all tested poultry and beef in the present study were resistant to erythromycin (100%) and other tested antibiotics. Erythromycin is used in global livestock production (Massé et al., 2000) and was frequently detected in surface waters in the United States (Kolpin et al., 2002). According to Pothuluri et al. (1998), erythromycin has been used expansively in livestock, poultry, and fish as a growth promotant and to control bacterial diseases. In poultry production (chickens and turkeys), erythromycin is incorporated in feed as an aid in the prevention of chronic respiratory diseases during periods of stress to prevent infectious coryza and in prevention and reduction of lesions (Lundeen, 2008). The increased use of erythromycin has had consequences too; for instance, Kim et al. (2006) reported erythromycin resistance in *Campylobacter coli* strains from turkeys.

Resistance to the antimicrobial penicillin in retail meats was also significantly greater ($P < 0.05$) than in all other tested antimicrobials except erythromycin. Tetracycline and penicillin are routinely used in poultry feeds as antimicrobial agents, and resistance to these antimicrobials has previously been demonstrated to be linked to poultry production areas (Hayes et al., 2004; Castanon, 2007). Shea (2004) suggested that prolonged exposure to therapeutic doses of antimicrobial agents is the primary cause of antimicrobial resistance. In the present study, the least antimicrobial resistance (2.7%) was observed in chloramphenicol and only in beef and chicken isolates. The low resistance toward chloramphenicol is probably due to the restricted use in slaughtered animals (LeJeune and Christie, 2004).

Contamination of retail meats with antibiotic-resistant foodborne pathogens including *Salmonella*, *Morganella morgani*, *Vibrio parahemolyticus*, and *Yersinia enterocolitica* could mainly suggest carriage of these organisms by food animals. Commensal bacteria, particularly enteric bacteria, are regularly exposed to antibiotics and develop resistance, thus becoming a reservoir for resistance genes (Knezevic and Petrovic, 2008). They may transfer resistance genes to other bacteria, including foodborne pathogens (Sorum and Sunde, 2001; Catry et al., 2003). The data presented here indicate that raw retail meats may contribute to the spread of antibiotic-resistant enteric bacteria. Mitigation efforts should therefore center primarily on reducing the number of pathogens present on farms and in slaughterhouses (White et al., 2001).

Ninety-six percent of isolates (70 of 73) displayed resistance to at least one antibiotic, and 86.3% (63 of 73) displayed MDR. The MDR was also determined in *Salmonella* isolates from turkey and beef. These results are supported by the report of Zhao et al. (2002), which suggested MDR in *Salmonella* isolates from retail meats. Multiple drug-resistant isolates account for 20 to 25% of human *Salmonella* infections in the United States (Holmberg et al., 1984). Food contamination with MDR bacteria is a major problem for public health and could be transferred to bacteria of clinical significance. According to our data, all the *E. coli* and *Morganella morganii* isolates showed MDR. The MDR strains have arisen in *Enterobacteriaceae*, and this is a great concern because of their potential for widespread diffusion and complications in remedial management of infected patients (Karlowsky et al., 2003). According to the National Academy of Sciences Committee on Drug Use in Food Animals (1999), the use of antibiotics in food animals could enhance the development of antibiotic resistance and its transfer to human pathogens. It is also documented that the use of antimicrobials in agriculture can potentially pilot to extensive diffusion of antimicrobial resistant bacteria (Gomez-Lus, 1998; Witte, 1998). Consumers should therefore evade the consumption of rare meats and cross-contamination of foods during food handling and preparation.

In summary, this study suggests MDR *Enterobacteriaceae* has spread in retail chicken, turkey, and beef meats. Resistant zoonotic bacteria reach the human population not only by direct contact, but also via food products of animal origin. Strict observance of hygiene policies plays an important role in ensuring food safety and controlling the transmission of resistant bacteria from retail meats to humans. Poultry and beef meats need to be cooked thoroughly to prevent human infection because proper cooking at high temperatures destroys pathogenic bacteria. Unless intervention strategies are in place, formerly treatable antimicrobial-resistant foodborne pathogens could emerge as untreatable. The increasing prevalence of resistance in the isolates of animal origin may have important therapeutic implications. Fundamental hygienic measures and cautious and more rational antibiotic use of antimicrobials in food animals should be fostered.

Although this study suggests the occurrence of antibiotic-resistant *Enterobacteriaceae* in meats, further susceptibility test in larger populations is needed to verify the occurrence of MDR *Enterobacteriacea* in retail meats.

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