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Prevalence and Antimicrobial Resistance of Pathogenic Bacteria in Chicken and Guinea Fowl

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PROCESSING, PRODUCTS, AND FOOD SAFETY

Prevalence and Antimicrobial Resistance of Pathogenic Bacteria in Chicken and Guinea Fowl

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ABSTRACT This study was conducted to compare the presence and antimicrobial susceptibility of *Campylobacter*, *Salmonella* spp., and other enteric bacteria between chickens and guinea fowls. Birds were reared on enclosed concrete floor housing covered with pine wood shavings litter material. Chicken (n = 40) and guinea fowl (n = 40) carcasses, drinking water (10 mL; n = 40), and litter (10 g; n = 40) were aseptically collected randomly from a poultry farm and analyzed within 1 h of collection. Individual pens served as experimental units and were replicated twice. *Campylobacter* spp., *Salmonella* spp., and other enterobactericeae were isolated and identified using standard selective media and biochemical tests. Isolates were tested for sensitivity to tetracycline, ampicillin, streptomycin, kanamycin, nalidixic acid, gentamicin, erythromycin, ciprofloxacin, cefoxitin, and colistin using the Kirby-Bauer disk diffusion test. *Campylobacter* spp. and *Salmonella* spp. were isolated from 28 and 35% of whole carcass rinses of chickens and from 18 and 23% of whole carcass rinses of guinea fowl, respectively. Although only *Salmonella* spp. were recovered from drinking water, both *Salmonella* and *Campylobacter* spp. were recovered from litter material. C*ampylobacter upsaliensis* was recovered only in the guinea fowl, whereas *Klebsiella oxytoca* and *Enterobacter sakazakii* were recovered only in chickens. Although no antibiotic resistance was determined in *Campylobacter upsaliensis*, most *Campylobacter*, *Salmonella*, and *Escherichia coli* isolates from both chickens and guinea fowl were resistant to antibiotics such as ampicillin, kanamycin, erythromycin, and nalidixic acid.

Key words: chicken, guinea fowl, antimicrobial resistance

INTRODUCTION

Significance of Antimicrobial Resistance

There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Van Looveren et al., 2001). Recent reports have shown that different types of food and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-producing animals (Anderson et al., 2003; Schroeder et al., 2004).

Annual cost of treating infections caused by antibiotic-resistant bacteria is estimated to be $4 to $5 billion (McGowan, 2001). International and US public health agencies have targeted antibiotic resistance as an emerging public health concern (Barza and Travers, 2002) and one of the most pressing public health needs.

Contaminated food of animal origin is one source of human bacterial infections; therefore, the presence of antibiotic-resistant strains in food animals such as poultry has raised concerns that the treatment of human infections will be compromised.

Antimicrobials Used in Poultry Management

Antibiotics are used for control and treatment of bacterial diseases in poultry. Common antibiotics are bacitracin, chlortetracycline, erythromycin, and penicillin. The fluoroquinolones are important members of the quinolone group of antibiotics licensed to treat diseases in humans and animals, and their use in livestock animals can contribute to increased resistance in foodborne bacteria (such as *Campylobacter* and *Salmonella*), which may infect humans. The fluoroquinolones are important for the treatment of invasive *Salmonella* and *Campylobacter* infections in humans, and an increase in the resistance in these bacteria is therefore of concern. In addition, when antibiotics are administered to birds over a long period, particularly at a low level, certain species of bacteria become resistant, and finally the resistance renders the antibiotic ineffective.

Antimicrobial Resistance in Chickens

Billions of chickens and millions of specialty poultry products enter the US market annually (McCrea et al., 2005). Consequently, poultry have been implicated as an important source of human infections (Stern and Robach, 2003). Although many of these pathogenic bacteria recovered from poultry have been monitored, several published studies have reported on antimicrobial resistance in pathogenic bacteria found in poultry, particularly *Salmonella* and *Escherichia coli* (Chung et al., 2004).

In the modern poultry industry, antibiotics are used for the treatment and prevention of infectious diseases in farm animals intended for food production and to protect public health from foodborne diseases. Mishandling of raw poultry and consumption of undercooked poultry are potential contamination sources of *Campylobacter* (Nadeau et al., 2002) and *Salmonella*. It is well documented that *Campylobacter* and *Salmonella* infections in humans have been associated with raw chicken (Harrison et al., 2001; Hernandez et al., 2005). Birds appear to be an important reservoir for *Campylobacter lari*, which has been isolated from gulls (Glunder and Petermann, 1989) and chickens (Tresierra-Ayala et al., 1995; Shih, 2000). Fresh chicken carcasses have been indicated to contain high numbers (approximately 10^5 cfu/g) of *Campylobacter* spp. (Cogan et al., 1999). *Salmonella* and *Campylobacter* have also been isolated in chicken feed and water (Padungtod and Kaneene, 2005*)*.

Antimicrobial Resistance in Guinea Fowl

Guinea fowl does not comprise a large portion of the poultry meat market in the United States; however, it is sold year round in supermarkets and served as a special delicacy in some restaurants and hotels in large cities within the United States, Canada, Europe, Africa, and many other parts of the world. Although efforts to establish industrial production of guinea fowl in the United States are under way (Phillips and Ayensu, 1991), guinea fowl production is a viable enterprise in European markets. Recent reports indicate that guinea fowl are also raised commercially on farms in Canada (Nova Scotia Department of Agriculture, 1997) and Australia (Embury, 1998). In a commercial setting, guinea fowl are kept in confinement using methods similar to those for raising chickens (Phillips and Ayensu, 1991). These conditions predispose guinea fowl to microbial infection although previous reports have shown that they adapt well to harsh environmental conditions and are less susceptible to poultry diseases (Mathis and McDougald, 1987). However, there is a paucity of information pertaining to antimicrobial resistance in guinea fowl.

It has been observed that antibiotic usage over a long period can induce antibiotic resistance in bacteria (Gautier-Bouchardon et al., 2002). Although many pathogenic bacteria recovered from poultry have been monitored, few published studies have reported on antimicrobial resistance in poultry. Therefore, the objectives of this study were to 1) characterize pathogenic bacteria in the poultry housing environment; 2) investigate antibiotic resistance of pathogenic bacteria isolated from chicken and guinea fowl carcasses and the poultry housing environment; and 3) differentiate prevalence and antibiotic resistance of pathogenic bacteria between chickens and guinea fowls.

MATERIALS AND METHODS

Management of Experimental Birds

Eighty each of Pearl Grey guinea fowl obtained from Ideal Poultry Breeding Farms (Cameron, TX) and Hyline Single Comb White Leghorn (**SCWL**, Hyline International, Warren, IN) chickens were weighed and randomly assigned to electrically heated, temperature-controlled brood units (Petersime Brood Units, Model 2SD12, Petersime Incubator Company, Gettysburg, OH) equipped with raised wire floors for the first 3 wk of age (**WOA**). The battery cages measured 99 × 66 × 26 cm and each housed 10 birds. During the first WOA, the brooder temperature was maintained at 32.2°C and reduced gradually by 2.8°C every week until reaching 21.1°C; from this point on, no artificial heating was provided to the birds. At 4 WOA the birds were transferred onto floor pens covered with pine wood shavings where they were raised until 47 WOA. The concrete floor pens (240 × 150 cm) were covered with pine wood shavings litter to a depth of 10 cm. Each pen (which served as a replicate) housed 20 birds; each treatment was replicated twice and the experiment was repeated 2 times. Therefore, the total number of birds per treatment was 80. The birds were reared under standard conditions (Bell and Weaver, 2002a) and were fed standard Leghorn diets (NRC, 1994; Bell and Weaver, 2002a) and Pearl Grey guinea fowl diets (Nahashon et al., 2006) from hatch to 47 WOA. The diets were provided in mash form for ad libitum consumption. Water was provided in hanging bell water fountains for ad libitum consumption throughout the experimentation period. The birds received 23, 12, and 16 h of constant lighting from hatch to 8, 9 to 14, and 15 to 47 WOA, respectively. Ventilation within the battery holding room and the floor pens was maintained by thermostatically controlled exhaust fans.

The experimental house is part of Tennessee State University's poultry research facilities, which include grower/breeder (floor) and layer (cage) houses. The poultry houses are about 100 feet apart and house chickens (layers, replacement pullets, and broilers) and sometimes guinea fowl. The floor house in which the experimental birds were reared is usually populated

with breeder birds or replacement pullets. The house is usually depopulated, cleaned, and disinfected before repopulation with a new flock. This was the case before assigning the experimental birds in this study to their respective house. During the experimental period, antibiotics were not used in the experimental facilities and birds. However, before this study, antimicrobials such as erythromycin, chlortetracycline, and fluoroquinolones had been used to treat bacterial infections in poultry flocks that occupied these housing facilities. The anti-coccidia drug amprolium (at 0.0125% of diet) was administered continuously through feed to the experimental birds.

Birds used in this study were not verified to be germ free. Furthermore, even with strict biosecurity measures that include cleaning and disinfecting, there are always some microorganisms present in the housing environment. On the other hand, microorganisms such as salmonella can be transmitted vertically and spread to other flocks horizontally. Birds also pick up microorganisms from litter and water because these are shared in the poultry house. It has been documented that air, water, supplies, and materials brought into the poultry houses can contribute to microbial levels (Bell and Weaver, 2002b). In the present study, the assumption was that the experimental birds would harbor or pick up pathogenic microorganisms from the housing environment even without inoculation of these microorganisms into individual birds.

Processing Procedures

At 47 WOA, 50% of experimental SCWL chickens (n = 40) and Pearl Grey guinea fowl (n = 40) were randomly selected and evaluated for presence of pathogenic microorganisms. Feed and water were withdrawn 12 h before slaughter. The birds were then manually caught and crated in plastic coops such that each coop contained 8 birds. All crates and equipment used in bird processing were cleaned in chlorinated water to ensure sanitized conditions. Crated birds were immediately transported and slaughtered. These birds were transported less than 100 m to the processing facility. While hanging by their feet, all 40 birds from each treatment group (bird type) were electrically stunned by passing their heads through 1% NaCl solution charged with electrical current (14V, 60 Hz) for 18 s. The birds were killed by hand using a conventional unilateral neck cut to sever the carotid artery and jugular vein and bled for 180 s. Birds were scalded for 120 s at 63°C in an air-agitated commercial scalder (Cantrell Model SS300CF, Cantrell Machine Co. Inc., Gainesville, GA) and picked for 30 s in a commercial in-line picker (Cantrell Model CPF-60, Cantrell Machine Co. Inc.). After the head, shanks, feet, and feathers were removed, the carcass was eviscerated manually by cutting around the vent to remove all of the viscera including the kidneys.

Sample Collection

The SCWL chicken (n = 40) and Pearl Grey guinea fowl (n = 40) carcasses were aseptically collected immediately after processing, refrigerated and transported to the laboratory for analysis. The samples were kept chilled (<4°C) and assayed within 1 h of collection. Each carcass was placed separately in a sterile bag containing 300 mL of buffered peptone water (**BPW**) and manually rinsed for 2 min, ensuring that all surfaces, internal and external, had contact with the rinse. Environmental samples from the farm included drinking water (10 mL; n = 40) and litter (10 g; n = 40). For environmental samples, 90 mL of BPW was added and pummeled in a stomacher 400 circulator (Seward Limited, London, UK) at 230 rpm for 2 min. The carcass rinse and the environmental homogenate from the samples were analyzed for the presence of *Campylobacter*, *Salmonella*, and other enteric bacteria. Drinking water samples were evaluated immediately after the bell water fountains were cleaned (fresh drinking water) and after 7 d (7 d-old drinking water). All samples were collected between December 2005 and August 2006, and between October and December 2007. All bird carcasses passed inspection and appeared healthy.

Isolation of Campylobacter spp.

Campylobacter spp. isolation and identification was achieved using selective media and biochemical tests. Carcass rinses (20 mL) and homogenate from litter and drinking water were placed in 20 mL of blood-free Bolton broth base (CM983, Oxoid, Basingstoke, UK), which had selective supplement (CR208E, Oxoid). The culture tubes were incubated at 42°C for 48 h. Microaerophilic conditions were generated by using Campygen sachets (CampyGen, Oxoid). After incubation, enrichment cultures were subcultured directly to Campylobacter blood-free selective agar plates (CM739, Oxoid) containing selective supplement (SR 155E, Oxoid). The plates were incubated microaerobically at 42°C for 48 h. Each suspected isolate was examined for catalase and oxidase production (Food and Drug Administration, 2005). The catalase- and oxidase-positive isolates were confirmed by API-Campy (BioMerieux, Durham, NC).

Isolation of Salmonella spp.

Salmonella spp. were also isolated using selective media and biochemical tests. Carcass rinses (BPW, 20 mL) and 20-mL homogenates from litter and drinking water were incubated at 37°C for 24 h. After incubation, 1.0 mL of enrichment broth was transferred into 9.0 mL of tetrathionate broth and incubated at 42°C for 24 h. A loopful of tetrathionate broth was streaked onto xylose-lysine-tergitol 4 agar (Difco) and incubated at 37°C for 24 h. Presumptive *Salmonella* colonies on

Table 1. Antimicrobials and resistance breakpoints used in antimicrobial susceptibility test for *Campylobacter*, *Salmonella*, and other enteric bacteria[1]

Antimicrobial agent	Disc potency (μg)	Resistant	Intermediate	Susceptible
Tetracycline	30	≤14	15−18	≥19
Ampicillin	10	≤11	12−14	≥15
Streptomycin	10	≤11	12−14	≥15
Kanamycin	30	≤13	14−17	≥18
Nalidixic acid	30	≤13	14−18	≥19
Gentamicin	10	≤12	13−14	≥15
Erythromycin	15	≤15	16−20	≥21
Ciprofloxacin	5	≤15	16−20	≥21
Cefoxitin	30	≤14	15−17	≥18
Colistin	10	≤8	9−10	≥11

[1]Antimicrobial susceptibility was performed according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2000). *Campylobacter jejuni* ATCC 33560 was used as a quality control organism for *Campylobacter*; *Staphylococcus aureus* ATCC 29212 and *Escherichia coli* ATCC 25922 were used for *Salmonella* and other enteric bacteria.

xylose-lysine-tergitol 4 agar plates were further tested. The identities of *Salmonella* isolates were confirmed by use of the oxidase test and biochemical strips (API20E, BioMerieux).

Enumeration of Other Enterobacteriaceae

Carcass rinses (BPW) were enriched at 37°C for 20 h and 200 μL was streaked onto MacConkey agar (Oxoid) with incubation at 37°C for 24 h. Isolates were identified by oxidase tests and biochemical strips (API20E, BioMerieux) following the manufacturer's recommendations. For each of the samples, typical colonies were selected to make a bacterial suspension, which was used to inoculate the strips. Biochemical tests were used to identify these isolates to the species or genus level.

Testing for Antimicrobial Susceptibility

The disk diffusion assay was performed according to the method described by the National Committee for Clinical Laboratory Standards [NCCLS, now Clinical and Laboratory Standards Institute] (CLSI, 2000). Cultures were tested for sensitivity to 10 antimicrobials (Table 1). *Staphylococcus aureus* ATCC 29212 and *Escherichia coli* ATCC 25922 were used as quality control strains.

Campylobacter colonies were selected from agar plates and transferred into Luria-Bertani broth (Difco, Becton Dickinson, Sparks, MD) with incubation at 42°C for 24 h under microaerobic conditions. After the incubation period, a sterile cotton swab was dipped into the suspension and streaked evenly on to the entire surface of Müller-Hinton agar (5% sheep blood) and incubated at 42°C for 48 h. Colonies of *Salmonella* spp. and other enteric bacteria were also picked and cultured in Luria-Bertani broth with incubation at 37°C for 24 h; a sterile cotton swab was dipped into the suspension and streaked evenly on to the entire surface of a Müller-Hinton plate and incubated at 37°C for 24 h. When available, inhibition zones were measured and interpreted as resistant according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2000).

Statistical Analysis

The experimental design was a completely randomized assignment of birds onto floor pens. Data were analyzed using the SAS/STAT software (SAS Institute,

Table 2. *Campylobacter* spp. and *Salmonella* spp. isolated from chicken, guinea fowls, and environmental samples[1]

Sample	*Campylobacter*	*Salmonella*
Litter	5/40[b] (13)	9/40[b] (23)
Drinking water	0/40[c] (0)	5/40[c] (13)
Chickens (carcass)	11/40[a] (28)	14/40[a] (35)
Guinea fowl (carcass)	7/40[b] (18)	9/40[b] (23)

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

[1]Number of positive samples/number of total samples (percentage) evaluated.

Table 3. Microorganisms isolated from chicken, guinea fowls, and environmental samples

Microbial identity	Location of microbial isolation[1]			
	CC	GFC	DW	L
Campylobacter jejuni	+	+	−	+
Campylobacter lari	+	+	−	−
Campylobacter upsaliensis	−	+	−	−
Salmonella spp.	+	+	+	+
Klebsiella pneumoniae	+	+	+	+
Klebsiella oxytoca	+	−	−	−
Enterobacter sakazakii	+	−	−	−
Enterobacter cloacae	+	+	+	+
Pantoea spp.	+	+	+	+
Pseudomonas aeruginosa	+	−	−	−
Enterobacter aerogenes	+	+	−	−
Escherichia coli	+	+	+	+
Citrobacter youngue	+	+	−	−

[1]CC = chicken carcasses; GFC = guinea fowl carcasses; DW = drinking water; and L = floor litter material.

Table 4. Incidence of resistance[1] to drugs tested in microorganism*s* isolated from chicken

Species	Antimicrobial drug[2]									
	A	Ci	Ce	C	E	G	K	Nx	S	T
Campylobacter jejuni	R	R	N	N	R	N	N	R	N	N
Campylobacter lari	N	N	N	N	N	N	R	R	N	N
Salmonella spp.	R	N	N	N	N	N	N	N	R	R
Escherichia coli	R	N	N	N	N	N	N	R	N	N
Klebsiella pneumonia	R	N	R	N	R	N	N	R	R	N
Klebsiella oxytoca	R	N	N	N	R	N	N	N	N	N
Enterobacter sakazakii	R	N	N	N	N	R	N	N	N	N
Pseudomonas aeruginosa	N	N	N	N	N	N	N	N	N	N

[1]R = resistant; N = not resistant.

[2]A = ampicillin; Ci = ciprofloxacin; Ce = cefoxitin; C = colistin; E = erythromycin; G = gentamycin; K = kanamycin; Nx = nalidixic acid; S = streptomycin; T = tetracycline.

1999). Differences in prevalence of *Campylobacter* and *Salmonella* among chickens, guinea fowl, and environmental samples were analyzed using the chi-square method. Significance implied $P < 0.05$ unless specified otherwise.

RESULTS

Presentation of Pathogenic Bacteria in Chickens, Guinea Fowl, and Environmental Samples

Campylobacter spp. and *Salmonella* spp. were isolated from 28 and 35% of whole chicken carcass rinses, respectively (Table 2). However, the number of *Campylobacter* spp. and *Salmonella* spp. isolated from guinea fowl (18 and 23%, respectively) was significantly lower ($P < 0.05$) when compared with those isolated from chickens. The litter (floor covering) material on which the birds were reared was also contaminated with *Campylobacter*. *Campylobacter* spp. and *Salmonella* spp. were isolated in 13 and 23% of the litter samples collected from the floor pens housing both chickens and guinea fowl. Apparently, the numbers of positive isolates of these pathogens obtained from pens that housed chickens were not different ($P > 0.05$) from those obtained from pens that housed the guinea fowl. Although *Campylobacter* was not recovered from the drinking water, about 13% of the samples tested were positive for *Salmonella* spp. The *Salmonella* spp. were isolated from 7-d-old drinking water but not from fresh water. Although *Campylobacter jejuni* and *C. lari* were the 2 common *Campylobacter* species isolated from chickens and guinea fowls carcasses and litter materials, *Campylobacter upsaliensis* was recovered only in the guinea fowl carcasses (Table 3). Overall, *Salmonella* were present in chickens and guinea fowl carcasses, and in the environmental samples.

All other enteric bacteria isolated from chicken, guinea fowl, and environmental samples are presented in Table 3. *Klebsiella pneumoniae* were isolated from carcasses of chickens and guinea fowl and from all environmental samples evaluated. *Klebsiella oxytoca* and *Enterobacter sakazakii* were isolated only in chicken carcasses and not in guinea fowl carcasses or environmental samples. On the other hand, *E. coli* was found in carcasses of broilers and guinea fowls and in environmental samples, whereas *Pseudomonas aeruginosa* was found only in the chicken carcasses. *Enterobacter aerogenes* and *Citrobacter youngue* were only recovered in chickens and guinea fowl carcasses, but not in environmental samples.

Antibiotic Resistance of Pathogenic Bacteria in Chickens and Guinea Fowls

Expressions of antibiotic resistance by microorganisms isolated from chickens and guinea fowls carcasses are presented in Tables 4 and 5, respectively. *Campylobacter jejuni* isolated from chickens and guinea fowl

Table 5. Incidence of resistance[1] to drugs tested in microorganism*s* isolated from guinea fowls

Species	Antimicrobial drug[2]									
	A	Ci	Ce	C	E	G	K	Nx	S	T
Campylobacter jejuni	R	R	N	N	R	N	N	R	N	N
Campylobacter lari	R	N	N	N	N	N	N	R	N	N
Campylobacter upsaliensis	N	N	N	N	N	N	N	N	N	N
Salmonella spp.	R	N	N	N	N	N	N	N	N	R
Escherichia coli	R	N	N	N	N	N	N	N	N	N
Klebsiella pneumonia	R	N	R	N	N	N	N	R	N	N

[1]R = resistant; N = not resistant.

[2]A = ampicillin; Ci = ciprofloxacin; Ce = cefoxitin; C = colistin; E = erythromycin; G = gentamycin; K = kanamycin; Nx = nalidixic acid; S = streptomycin; T = tetracycline.

were resistant to ampicillin, ciprofloxacin, erythromycin, and nalidixic acid. *Campylobacter lari* was only resistant to ampicillin, kanamycin, and nalidixic acid. However, isolates of *C. upsaliensis* from guinea fowls were not resistant to any of the antibiotics evaluated. *Salmonella* isolates were resistant to ampicillin, streptomycin, and tetracycline, whereas *E. coli* isolates were resistant to ampicillin and nalidixic acid. *Klebsiella pneumoniae* was resistant to ampicillin, erythromycin, cefoxitin, streptomycin, and nalidixic acid. On the other hand, *K. oxytoca* isolated from chickens were resistant to ampicillin and erythromycin (Table 4). *Enterobacter sakazakii* also isolated from chicken was resistant to ampicillin and gentamicin. *Pseudomonas aeruginosa* isolated from chicken was not resistant to any of the antibiotics tested. Out of 80 isolates, from environmental samples, chicken, and guinea fowl, only 30 of the isolates (37.5%) were resistant to antibiotics.

DISCUSSION

In the present study, *Salmonella* was not isolated in 1-d-old (fresh) drinking water, but it was recovered in 7-d-old drinking water, which contained visible droppings and feathers. In instances where birds are raised on litter floor houses, sharing of water troughs or fountains is common and such practices increase the probability of microbial contamination to a greater number of housed poultry flocks. These observations suggest that drinking water in poultry houses should be changed more often to avoid such contamination (Doyle and Erickson, 2006).

Several pathogenic bacteria including *C. jejuni*, *C. lari*, *Salmonella* spp., and *K. pneumoniae* were isolated from carcasses of both chickens and guinea fowl. Although *C. jejuni* and *C. lari* were the 2 common *Campylobacter* species isolated in chicken and guinea fowl carcasses as well as litter materials, *C. upsaliensis* was recovered only in guinea fowls (Table 3). On the other hand, *Salmonella* were present in chickens and guinea fowl carcasses and in the environmental samples. Previous reports show that chickens are an important reservoir for *C. lari* (Shih, 2000). Fresh chicken carcasses have been indicated to contain high numbers (approximately 10^5 cfu/g) of *Campylobacter* spp. (Cogan et al., 1999). *Salmonella* and *Campylobacter* have also been isolated in chicken feed and water (Pedungtod and Kaneene, 2005*).

Multidrug-resistance (ampicillin, ciprofloxacin, erythromycin, kanamycin, and nalidixic acid) was observed in *Campylobacter* spp. that were isolated from chickens and guinea fowl (Tables 4 and 5, respectively). Findings of significance in this study include the confirmation of the existence of antimicrobial resistance of *Campylobacter* to ciprofloxacin and erythromycin, antibiotics commonly used for treatment of campylobacteriosis in humans. Recent reports (Larkin et al., 2006; Threlfall et al., 2006) have cited evidence for an increase in the incidence of ciprofloxacin-resistant *Campylobacter* throughout the world. A recent survey of *Campylobacter* from raw poultry products indicated that 35% of isolates were resistant to ciprofloxacin (Ge et al., 2003). In this study, antimicrobial drugs for which *Salmonella* isolates exhibited resistance were ampicillin, streptomycin, and tetracycline. *Salmonella* spp. isolated from chickens have also been reported to be resistant to ampicillin, tetracycline, and gentamycin (Wilson, 2004). The antibiotic resistance in *Salmonella* from chickens should be considered a great risk for treatment of human salmonellosis. *Escherichia coli* isolates from chicken carcasses were only resistant to ampicillin and nalidixic acid (Table 4), whereas similar isolates from guinea fowl were only resistant to ampicillin (Table 5). Recent reports (Schroeder et al., 2004) have shown that *E. coli* isolated from meat and poultry demonstrated resistance to at least one antimicrobial drug. The housing environment in which the experimental birds were housed was previously populated with flocks that were treated with antibiotics. However, antibiotics were not fed to experimental birds in this study. Therefore, any microorganisms, antibiotic resistant or not, that were isolated from the housing environment and carcasses may have been introduced through the birds, air, supplies, and objects brought into the poultry house.

Resistance to the multidrugs ampicillin, cefoxitin, nalidixic acid, and streptomycin were observed in *K. pneumoniae* isolates (Tables 4 and 5, respectively). This observation was consistent with previous reports (Kim et al., 2005) that multidrug-resistant *K. pneumoniae* was isolated in farm environments and retail poultry and beef products. *Klebsiella pneumoniae* is resistant to several antibiotics such as ampicillin, streptomycin, gentamicin, chlorolphenicol, tetracycline, and ofloxacin (Rasool et al., 2003). *Klebsiella pneumoniae* is therefore an increasing problem in hospitals because of the evolution of antibiotic-resistant strains. In the present study, *K. oxytoca* isolates from chickens were resistant to ampicillin and erythromycin (Table 4). The *E. sakazakii* isolates recovered from chickens were resistant to ampicillin and gentamycin (Table 4). These findings are consistent with previous reports that *E. sakazakii* was resistant to multiple antibiotics, including ampicillin, gentamicin, and cefotaxime (Dennison and Morris, 2002). *Enterobacter sakazakii* is considered a foodborne pathogen that can cause severe illness and death in newborns, particularly in premature newborns or infants with weakened immune systems (Lai, 2001).

In conclusion, these data indicate that chicken and guinea fowl are reservoirs of antibiotic-resistant *Salmonella*, *C. jejuni*, *C. lari*, *E. coli*, and *Klebsiella* spp. There is potential for these antibiotic-resistant bacteria to be transferred to humans through contaminated poultry. Multidrug resistance of foodborne pathogens is certainly a public health concern and reinforces the need for more prudent use of antibiotics by farmers,

veterinarians, and physicians. Therefore, a continued development of methods to reduce risk of foodborne pathogens in poultry is critical.

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