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Antimicrobial Resistance Patterns of *Salmonella* from Retail Chicken

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Abstract: *Salmonella* is frequently reported as a cause of food-borne illness. The emergence of antimicrobial resistant *Salmonella* associated with meat products has heightened concerns regarding antimicrobial use in food animal production. Eighty *Salmonella* isolates recovered from fresh whole chicken carcasses purchased at retail outlets were examined for susceptibility to 18 antimicrobials. Fifteen serotypes were identified; the top five included; *S. Heidelberg* (25%), *S. Typhimurium* 5- (formerly var. Copenhagen) (18.75%), *S. Kentucky* (17.5%), *S. Berta* (11.25%), and *S. Hadar* (8.75%). Overall, resistance was most commonly observed to tetracycline (25%), ampicillin (22.5%), streptomycin (21.25%) and cephalosporin derivatives (cephalothin 18.75%, ceftiofur 16.25%, and cefoxitin 15%). Of all isolates, 43.75% were resistant to one or more antimicrobial and 36 % were identified as multi-drug resistant (MDR, resistant to 2 or more antimicrobials). Fourteen resistance patterns were observed and among isolates showing resistance, 22.5% were resistant to 1-3 antimicrobials, 16.25% were resistant to 4-6 antimicrobials, and 5.0% were resistant to = 7 antimicrobials. The prevalence of antimicrobial resistance varied by serotype. All 7 *S. Hadar* isolates were resistant to 1-2 antimicrobials, 4 of 20 *S. Heidelberg* isolates were resistant to 1-3 antimicrobials, 10 of 15 *S. Typhimurium* 5- isolates were resistant to 4-5 antimicrobials, 7 of 14 *S. Kentucky* isolates were resistant to 1-7 antimicrobials, and 3 of 9 *S. Berta* isolates expressed resistance to 9-11 antimicrobials. These data indicate that *Salmonella* recovered from retail poultry carcasses may be resistant to multiple antimicrobials, and that resistance among these isolates varies by serotype.

Key words: *Salmonella*, antimicrobial resistance, chicken

Introduction

The incidence of *Salmonella* associated with poultry and poultry meat products is well documented, having both public health and economic implications. Nontyphoidal *Salmonella* spp. are estimated to account for 2.4 million cases of human gastroenteritis annually in the United States (Mead *et al.*, 1999). Most cases of gastroenteritis resulting from these organisms manifest as self-limiting diarrheal disease. However, extended duration of illness, cases resulting in septicemia, or cases involving immuno-compromised individuals, may warrant antimicrobial therapy. Under these circumstances, infections caused by antimicrobial resistant strains may result in increased morbidity and mortality.

The emergence of antimicrobial resistance in bacteria associated with food producing animals and evidence of human infections from animal sources (Holmberg *et al.*, 1984; Cohen and Tauxe, 1986; Lee *et al.*, 1994) have compelled the scientific community and public health officials to reassess antimicrobial use in food animal production (Levy, 1992; WHO, 1997; NRC, 1998; Angulo, 1999; Tollefson and Miller, 2002). In meat animal production, antimicrobials are used both therapeutically and non-therapeutically. The role that each of these practices plays in the development of resistance, is currently under debate.

There are some published studies on antimicrobial resistance of salmonellae isolated from poultry products. Logue *et al.* (2003) examined salmonellae collected from turkey processing plants and other studies have examined salmonellae from poultry related samples (Roy *et al.*, 2002). Surveys of the prevalence of antimicrobial resistant *Salmonella* on retail poultry meat are relatively limited. Wilson (2004) examined the antimicrobial resistance of *Salmonella* collected from 434 chicken meat samples in the UK; however, only 23 of the samples were positive for *Salmonella*. A survey of retail chicken in Maryland found that although organically raised broilers had higher prevalence of *Salmonella*, more antimicrobial resistant *Salmonella* were found on carcasses of conventionally raised broilers than on carcasses from organic production (Cui *et al.*, 2005). Other information on *Salmonella*, antimicrobial resistance and retail meat can be found through the National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS; www.fda.gov/cvm/narms_pg.html). The current study was conducted to determine the serotypes and antimicrobial susceptibility of *Salmonella* isolated from commercial broiler carcasses produced under conventional conditions by several national (U. S.) integrated broiler companies and purchased from retail outlets in Northeast Georgia.

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Materials and Methods

Salmonella isolates: Eighty *Salmonella* isolates previously recovered from fresh whole broiler carcasses purchased from retail outlets in Northeast Georgia (Simmons *et al.*, 2003) were included in this study. Frozen stock cultures of the isolates were resuscitated and streaked on nutrient agar (Becton Dickinson, Sparks, MD) slants to facilitate isolate characterization. Isolates were serogrouped using serogroup specific antisera (Becton Dickinson, Sparks, MD) and were sent to National Veterinary Services Laboratory (Ames, IA) for serotyping. Prior to susceptibility testing, isolated colonies were subcultured twice on tryptic soy agar plates with 5 % sheep blood (Becton Dickinson, Sparks, MD) incubated 24 h at 37°C.

Antimicrobial susceptibility testing: All *Salmonella* isolates were evaluated for susceptibility to 18 antimicrobials used by the NARMS program (http://www.fda.gov/cvm/narms_pg.html accessed 3/30/2006). Minimal inhibitory concentrations were determined by broth-micro dilution method utilizing a semi-automated system (Sensititre, Trek Diagnostics, Westlake, OH) and custom designed 96 well plates. The antimicrobials and corresponding breakpoints were as follows: amikacin (64 µg/ml), amoxicillin/clavulanic acid (32/16 µg/ml), ampicillin (32 µg/ml), apramycin (32 µg/ml), cefoxitin (32 µg/ml), ceftiofur (8 µg/ml), ceftriaxone (64 µg/ml), cephalothin (32 µg/ml), chloramphenicol (32 µg/ml), ciprofloxacin (4 µg/ml), gentamicin (16 µg/ml), imipenem (16 µg/ml), kanamycin (64 µg/ml), nalidixic acid (32 µg/ml), streptomycin (64 µg/ml), sulfamethoxazole (512 µg/ml), tetracycline (16 µg/ml), and trimethoprim/sulfamethoxazole (4/76 µg/ml). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 were included as control strains. Susceptibility results were interpreted in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2004), when available. Intermediate results were considered susceptible to avoid over estimation of the prevalence of resistance among these isolates.

Results

Serotypes: Overall, 15 different *Salmonella* serotypes (*S. enterica* subsp. *enterica*) were identified (Table 1). *Salmonella* Heidelberg was identified most often (25 %), followed by *S. Typhimurium* 5- (formerly var. Copenhagen) (19 %) and *S. Kentucky* (17.5 %). The five most prevalent serotypes: Heidelberg, Typhimurium 5-, Kentucky, Berta, and Hadar accounted for over 80 % of the isolates evaluated.

Antimicrobial resistance: Fifty-six percent of the *Salmonella* isolates were susceptible to all the antimicrobials tested. The susceptible proportion of isolates included less frequently recovered serotypes

Table 1: Serotypes and antimicrobial resistance of *Salmonella* recovered from whole broiler carcasses collected from retail outlets

Serotype	Number identified		No. expressing resistance	
	#	(% of total)	#	(% of serotype)
<i>S. Heidelberg</i>	20	(25)	4	(20)
<i>S. Typhimurium cop</i> ¹	15	(19)	10	(67)
<i>S. Kentucky</i>	14	(17.5)	7	(50)
<i>S. Berta</i>	9	(11)	3	(33)
<i>S. Hadar</i>	7	(9)	7	(100)
<i>S. Typhimurium</i>	3	(4)	0	(0)
<i>S. untypeable</i> ²	3	(4)	1	(33)
<i>S. Senftenberg</i>	2	(2.5)	1	(50)
<i>S. Enteritidis</i>	1	(1)	0	(0)
<i>S. Mbandaka</i>	1	(1)	1	(100)
<i>S. Montevideo</i>	1	(1)	0	(0)
<i>S. Ohio</i>	1	(1)	1	(100)
<i>S. Schwarzengrund</i>	1	(1)	0	(0)
<i>S. Thompson</i>	1	(1)	0	(0)
<i>S. untypeable</i> ³	1	(1)	0	(0)
Total	80	(100)		

¹*S. Typhimurium* var. *co. penhagen*. *Salmonella*² untypeable 4,5,12:i monophasic. ³ *Salmonella* untypeable 4,12:i monophasic

Table 2: Percentage of *Salmonella* isolates expressing resistance by Antimicrobial

Antimicrobial ¹	% Resistant ²	n
Amoxicillin/Clavulanic acid	13.75	14
Ampicillin	22.50	18
Cefoxitin	15.00	12
Ceftiofur	16.25	13
Cephalothin	18.75	15
Chloramphenicol	3.75	3
Gentamicin	10.00	8
Kanamycin	3.75	3
Nalidixic Acid	2.50	2
Streptomycin	21.25	17
Sulfamethoxazole	11.25	9
Tetracycline	25.00	20

¹No isolates were resistant to amikacin, apramycin, ceftriaxone, ciprofloxacin, imipenem, or the combination of sulfamethoxazole and trimethoprim. ²n=80

(Enteritidis, Montevideo, Schwarzengrund, Thompson, and untypeable isolates 4,12:i-monophasic and 4,5,12:i monophasic) as well as some members of serotypes in which resistance was observed (Table 1). None of the 80 isolates were resistant to amikacin, apramycin, ceftriaxone, ciprofloxacin, imipenem, or the combination of sulfamethoxazole and trimethoprim. Resistance was most commonly observed to tetracycline (25 %), ampicillin (22.5 %), and streptomycin (21.25 %) (Table 2).

Overall, fourteen resistance patterns were observed among the 80 *Salmonella* isolates (Table 3). Forty seven percent of isolates belonging to the five most prevalent serotypes were resistant to at least one antimicrobial. Different serotypes had differing prevalence of resistance; only 20% of *S. Heidelberg*

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Table 3: Antimicrobial resistance patterns of *Salmonella* isolates recovered from whole chicken carcasses collected from retail outlets

Antimicrobial Resistance Patterns ¹	Serotype(s)
TC	Kentucky (2) ² ; Hadar (2); Mbandaka (1)
AM	Heidelberg (1)
AM-CP	Heidelberg (1)
ST-TC	Heidelberg (1); Kentucky (2); Hadar (5)
A/C-AM-CP	Heidelberg (1)
GE-ST-SU	Senftenberg (1); untypeable ³ (1)
AM-GE-ST-SU	Typhimurium var. copenhagen (1)
AM-CL-SU-TC	Typhimurium var. copenhagen (2)
GE-NA-ST-SU	Ohio (1)
A/C-AM-CF-CT-CP	Typhimurium var. copenhagen (7)
A/C-AM-CF-CT-CP-TC	Kentucky (2)
A/C-AM-CF-CT-CP-ST-TC	Kentucky (1)
A/C-AM-CT-CP-GE-KA-ST-SU-TC	Berta (1)
A/C-AM-CF-CT-CP-CL-GE-KA-ST-SU-TC	Berta (2)

¹Amoxicillin/clavulanic acid (A/C), ampicillin (AM), cefoxitin (CF), ceftiofur (CT) cephalothin (CP), chloramphenicol (CL), gentamicin (GE), kanamycin (KA), nalidixic acid (NA), streptomycin (ST), sulfamethoxazole (SU), tetracycline (TC), trimethoprim/sulfamethoxazole (T/S).

² number of isolates of this serotype expressing specified resistance profile. ³S. untypeable 4,5,12:i-monophasic

isolates were resistant to antimicrobials while 100% of the *S. Hadar* isolates displayed resistance. *Salmonella* Heidelberg was the most commonly isolated serotype in this study but had relatively low prevalence of antimicrobial resistance and when resistance was noted it was to only 1, 2 or 3 drugs. In contrast, *S. Typhimurium* 5-, the second most prevalent serotype, expressed resistance to as many as 5 drugs and *S. Kentucky*, the third most prevalent serotype, expressed resistance to anti-microbials with patterns ranging from 1 to 7 drugs. Although every *S. Hadar* isolate recovered expressed antimicrobial resistance, the resistance patterns included only tetracycline, or tetracycline and streptomycin. Resistance patterns to 9-11 antimicrobials were observed for one third of the *S. Berta* isolates that displayed any resistance.

Discussion

Salmonella serotypes identified in this study were typical of those reported in the U.S. broiler industry. In a study conducted by The Food Safety Inspection Service (FSIS) of the USDA in 1999, Kentucky, Heidelberg, Typhimurium Copenhagen (now designated as 5-), Typhimurium, Hadar, and Monophasic were the six most common serotypes recovered from large broiler plants (FSIS, 1999); all but Monophasic were among the six most prevalent serotypes detected in the current study. Serotypes Kentucky and Heidelberg were also among the most common recovered from poultry products by Roy *et al.* (2002) and Rigney *et al.* (2004). However, serotypes Agona and Hadar were the most prevalent from turkey carcasses sampled in two U.S. processing plants (Logue *et al.*, 2003). Byrd *et al.* (1999) reported *S. Heidelberg* and *S. Kentucky* as predominant serotypes recovered from broiler grow-out houses which may help explain the source of these serotypes to broiler processing facilities and fully processed carcasses at retail.

When compared to resistance levels observed from

chicken carcass rinses by the National Antimicrobial Resistance Monitoring System in recent years (<http://www.ars.usda.gov/Main/docs.htm?docid=6750>, accessed April 2006), the current data showed more resistance to beta-lactams, including the broad spectrum aminopenicillins: ampicillin and amoxicillin (used in combination with the beta-lactamase inhibitor clavulanic acid) and cephalosporin derivatives: cephalothin, cefoxitin, and ceftiofur. While the proportions of isolates expressing resistance to amino glycosides (gentamicin, kanamycin, and streptomycin), chloramphenicol, and tetracycline were similar to earlier reports. The differences may be attributed to higher prevalence of resistant *S. Typhimurium* 5- (formerly var. Copenhagen) among the collection of isolates examined in the current study.

The most common resistances encountered in this study were to tetracycline (25%), ampicillin (22.5 %) and streptomycin (21.25 %). These results are similar to some of those published earlier. Roy *et al.* (2002) reported 27% of *Salmonella* isolates from a variety of poultry related samples were resistant to tetracycline. Resistance to tetracycline and streptomycin were prevalent in a collection of salmonellae isolates from turkey carcasses (Logue *et al.*, 2003). Similar levels of resistance to these drugs were also noted in *Salmonella* isolates collected from retail chicken in the UK where resistance to streptomycin, tetracycline and ampicillin were 26%, 22% and 17% respectively (Wilson, 2004). However, unlike the current study, Wilson (2004) found 52 % of *Salmonella* from retail chicken meat resistant to sulfonamide. Broiler chickens can be exposed to antimicrobial drugs during grow-out. Perhaps, as Logue *et al.* (2003) suggest, resistance to drugs such as tetracycline could be expected since members of this class (chlortetracycline and oxytetracycline) are approved for use in broiler feeds for purposes of growth promotion (Jones and Ricke, 2003). Thirteen of 80 isolates (16 %) in the current study were resistant to five or more drugs. Wilson (2004) found 3 of

23 (13%) isolates from British chicken samples resistant to 4 or more drugs. In Maryland, all 12 *Salmonella* isolates from conventionally raised broilers were found to be resistant to five or more drugs (Cui *et al.*, 2005). The data sets collected from chicken show a higher prevalence of multi drug resistance than was reported in isolates from a broad range of foods sampled by the US FDA where just 12 of 502 isolates (2.3 %) resistant to 5 or more drugs (Kiessling *et al.*, 2002).

The current data show that antimicrobial resistant *Salmonella* can be readily recovered from fully processed broiler carcasses at retail. Despite the fact that no resistance was noted to ciprofloxacin, a fluoroquinolone which could be used to treat humans with salmonellosis (<http://www.who.int/mediacentre/factsheets/fs139/en/>), resistance, including multi-drug resistance was observed. Therefore, consumers should apply proper food handling procedures to avoid contracting *Salmonella* which may be resistant to antimicrobials.

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