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%, cefotiofur 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/narmsg.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.
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 8% 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, MD) incubated 24 h at 37°C. Antimicrobial susceptibility testing was performed at the National Veterinary Services Laboratory (Ames, IA) for serotyping. Prior to susceptibility testing, isolated colonies were subcultured twice on tryptic soy agar plates with 8% sheep blood (Becton Dickinson, Sparks, MD) incubated 24 h at 37°C.

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 (Enteritidis, Montevideo, Schwartzengrund, Thompson, and untypeable isolates 4,12:i monophagic and 4,5,12:i monophagic) as well as some members of serotypes in which resistance was observed (Table 1). None of the 80 isolates were resistant to amikacin, apramycin, cephalothin, 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 isolates were resistant to all 18 antimicrobials tested.
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 antimicrobials 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 commonly 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: cephalexin, cefozolin, and cefotaxim. 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 (chlorotetracycline 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
that no resistance was noted to ciprofloxacin, a poultry from US Midwestern processing plants. J. Cohen, M.L. and R.V. Tauxe, 1986. Drug-Resistant resistance was observed. Therefore, consumers should 1999. Food-related illness and death in the United factsheets/fs139/en/), resistance, including multi-drug Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, with salmonellosis (http://www.who.int/mediacentre/Mead, P.S., L. Slutsker, V. Dietz, L.F. McCraig, J.S. fluoroquinolone which could be used to treat humans Appl. Microbiol., 94: 16-24. processed broiler carcasses at retail. Despite the fact resistant 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.

References


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