Antimicrobial Resistance in *Salmonella* and *Escherichia coli* Isolated from Commercial Shell Eggs

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ABSTRACT The development of antimicrobial resistance in bacteria has become a global problem. Isolates of *Salmonella* and *Escherichia coli* recovered from shell egg samples, collected at 3 commercial plants, were analyzed for resistance to 16 antimicrobial agents (n = 990). Eggs were sampled by rinsing in a saline solution. Pooled samples were preenriched in buffered peptone water and then selectively isolated using standard broths and agars. *Salmonella*-positive isolates were serogrouped immunologically before being serotyped. *Enterobacteriaceae* were enumerated from individual samples using violet red bile glucose agar plates. *Escherichia coli* were identified biochemically from presumptive *Enterobacteriaceae* isolates. *Salmonella* and generic *E. coli* antimicrobial-susceptibility testing was conducted using a semiautomated broth microdilution system. More resistance was observed in the *Salmonella* isolates (n = 41) than in the *E. coli* isolates (n = 194). *Salmonella* Typhimurium was the most prevalent (69.0%) serotype and demonstrated the greatest multiple resistance. *Salmonella* Kentucky, the least prevalent (5.0%) serotype recovered, was the most susceptible. Although 34.1% of the *Salmonella* serotypes were susceptible to all antimicrobial agents, 60.1% were resistant to 11 or more compounds. Many *Salmonella* isolates exhibited resistance to tetracycline (63.4%), nalidixic acid (63.4%), and streptomycin (61.0%). Most *E. coli* isolates (73.2%) were susceptible to all antimicrobial drugs. Many *E. coli* isolates exhibited resistance to tetracycline (29.9%), streptomycin (6.2%), and gentamicin (3.1%). Only 1% of the *E. coli* isolates were resistant to 4 antimicrobial agents. These data indicate that shell eggs can harbor resistant foodborne and commensal bacteria; among *Salmonella* isolates, resistance was serotype-dependent.

Key words: *Salmonella*, *Escherichia coli*, egg, antimicrobial resistance

INTRODUCTION

Antimicrobial resistance is an increasingly global problem, and emerging antimicrobial resistance has become a public health issue worldwide (Kaye et al., 2004). A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production (Bager and Helmuth, 2001; Anderson et al., 2003; Schroeder et al., 2004). Though many bacteria recovered from poultry or poultry-related samples have been monitored, few published studies have reported on antimicrobial resistance in bacteria, particularly *Salmonella* and *Escherichia coli* recovered from shell eggs (Yang et al., 2002; Antunes et al., 2003; Bajaj et al., 2003; Dargatz et al., 2003; Zhao et al., 2003; Busani et al., 2004; Chung et al., 2004; Del Cerro et al., 2003; Dias de Oliveira et al., 2005).

Studies were conducted in 2003 to monitor microbial populations, including *Salmonella* and other *Enterobacteriaceae*, along the shell egg-processing chain (Musgrove, 2004; Musgrove et al., 2005a,b). This paper reports on the antimicrobial susceptibility profiles of *E. coli* and *Salmonella* recovered in that study.

MATERIALS AND METHODS

Description of Shell Egg Processing Plants

A survey of in-line egg-processing facilities in the southeastern United States was conducted. Three plants were selected for sampling on 3 separate processing days. These plants were designated as X, Y, and Z to protect the anonymity of the participating companies. A more detailed description of the plants has been previously published (Musgrove et al., 2005b).
Shell Egg-Sample Collection

Eggs were collected from commercial plants at the following points of processing: at the accumulator, at prewash wetting, after the first washer, after the second washer, at the sanitizing rinse, at drying, at oiling, at check detection and weighing, at packaging (at 2 different packer head belts), at the entrance of the rewash belt, and at the exit of the rewash belt. Eggs were collected after the line had been operating for at least 2 h but during the midmorning break so as not to interfere with processing. This also allowed samples to be taken simultaneously from all sampling sites. Twelve eggs from each collection site were aseptically placed into clean foam cartons, packed into half-cases, and transported back to the laboratory.

Shell Egg-Sampling Methodologies

As described in Musgrove et al. (2005b), 10 of the 12 eggs collected at each site were sampled using a shell rinse technique. Each egg was placed into a sterile Whirl-Pak bag (Nasco Modesto, Modesto, CA) with 10 mL of sterile PBS and rinsed by shaking for 1 min. Rinsate from the rinse and the crush method for every egg was then subjected to microbiological analyses as previously described (Musgrove et al., 2005b).

Water-Sampling Methodology

Water samples were collected from the washer tanks in each of the 3 shell egg-processing plants and analyzed for pH, temperature, chlorine, protein, solids, and minerals, including iron, using procedures described previously (Northcutt et al., 2005). Samples were enriched for Salmonella. Collection procedures and other details are described in a previously published article (Northcutt et al., 2005).

Direct Plating Microbiology

Microbial populations from the individual samples described above were enumerated for Enterobacteriaceae on violet red bile glucose agar (Becton, Dickinson and Co., Franklin Lakes, NJ) plates with overlay (purple-red colonies) as previously described. Presumptive colonies were counted and reported as log cfu/mL of egg rinsate. Up to 5 isolates for each positive sample were randomly selected for further analysis. An isolate from the third streak plate was saved on brain heart infusion agar slants at 37°C and Protect beads (Technical Service Consultants Ltd., Heywood, Lancashire, UK) at −20°C until further analyses for identification could be performed. Identification of isolates A more detailed description of sampling methodology has been published previously (Musgrove, 2004). Each stored isolate was streaked onto plate count agar and incubated overnight at 37°C. A cultural suspension using 5 mL of physiological saline was prepared from each isolate. BioMérieux API 20 E strips (bioMérieux, Marcy l’Etoile, France) were inoculated, incubated, handled, and analyzed according to the manufacturer’s instructions. Reactions were recorded, and identifications were determined using Apilab Plus software (bioMérieux).

Salmonella Enrichment

For each of the 12 collection sites, 2 pooled samples were formed by combining shell egg rinses or crushed shells and membranes from 5 eggs. Samples were preenriched in buffered peptone water at 35°C for 18 to 24 h, followed by enrichment in TT broth (Becton, Dickinson and Co.) and Rappaport-Vassiliadis broth (Becton, Dickinson and Co.) overnight at 42°C. Enriched samples were plated onto BG Sulfa (Becton, Dickinson and Co.) and XLT-4 (Becton, Dickinson and Co.) agar plates and incubated at 37°C for 24 h. Presumptive positive colonies were inoculated into lysine iron agar (Becton, Dickinson and Co.) and triple sugar iron slants (Becton, Dickinson and Co.) and incubated at 35°C for 18 to 24 h. Those samples giving presumptive results on each of these media were confirmed using serogrouping antisera (Becton, Dickinson and Co.). Confirmed isolates were then streaked for purity and stocked onto agar slants and ceramic beads in cryogenic protective media. A copy of each isolate was provided to the National Veterinary Services Laboratories (Ames, IA) for serotyping. A sample was recorded as positive if it was confirmed and serotyped from either the shell rinse or crushed shell and membrane composite samples.

Antibiogram Methodology

Salmonella and generic E. coli were tested for antimicrobial susceptibility using a semiautomated broth microdilution system (Sensititre, TREK Diagnostic Systems Inc., Cleveland, OH). Custom-made panels of 16 antimicrobial drugs were configured in 96-well plates (National Antimicrobial Resistance Monitoring System, 2005). The minimum inhibitory concentration for each isolate was determined according to Clinical and Laboratory Standards Institute guidelines were not available (NARMS, 2005).

RESULTS AND DISCUSSION

Since their discovery in the 1940s, antibiotics have been widely used in both human and veterinary medical practice (Salyers and Whitt, 2005). However, many important human and animal pathogens have developed resistance to these compounds (Walsh, 2003). Resistant bacteria are routinely isolated from a variety of foods, including poultry meat and eggs (NARMS, 2005). Since 1997, pathogenic and commensal organisms, such as Salmonella and E. coli,
Table 1. Antimicrobial resistance patterns for *Escherichia coli* isolated from shell eggs at commercial shell egg processing plants (n = 194)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>No. of resistant isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>142 (73.2)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Cephalothin-tetracycline</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Gentamicin-streptomycin-sulfamethoxazole-tetracycline</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Gentamicin-tetracycline</td>
<td>4 (2.1)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>5 (2.6)</td>
</tr>
<tr>
<td>Streptomycin-tetracycline</td>
<td>5 (2.6)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>33 (17.0)</td>
</tr>
</tbody>
</table>

are monitored by the NARMS. However, little attention has been given to antimicrobial resistance of bacteria isolated from commercial shell eggs or the egg-processing environment.

Antibiogram patterns for *E. coli* isolates are summarized in Table 1. Most of the 194 isolates in this study (73.2%) were pan susceptible. Antimicrobial agents for which many *E. coli* isolates exhibited resistance were tetracycline (29.9%), streptomycin (6.2%), and gentamicin (3.1%). Only 1% (n = 2) of the *E. coli* isolates were resistant to 4 compounds. Many *E. coli* isolated from meat and poultry have demonstrated resistance to at least one antimicrobial drug (Schroeder et al., 2004; NARMS, 2005). Lanz et al. (2003) analyzed *E. coli* isolates from veterinary clinical sources in Switzerland, including from laying hens. Of the 16 antimicrobial drugs tested, the isolates in the Swiss study were most resistant to sulfonamides, tetracycline, and streptomycin. Sulfonamides, tetracycline, and streptomycin are the oldest drugs used in infectious disease, and it is not surprising that some level of resistance would have emerged over time (Salysers and Whitt, 2005). In a NARMS summary of antimicrobial resistance in *E. coli* collected from a variety of species from 1998 to 2003, gentamicin resistance ranged from 14.7 to 26.5%, streptomycin resistance ranged from 35.4 to 62.2%, and tetracycline resistance ranged from 36 to 79.9% (NARMS, 2005). Levels of resistance for these compounds were considerably lower in the *E. coli* isolates analyzed in the present study compared with the published reports. In our study, prevalence of multiple resistances was 20.6% for 1 compound, 5.2% for 2 compounds, and 1.0% for 4 compounds.

*Salmonella* antibiogram results are summarized in Table 2. From the perspective of total numbers, more resistance was observed in the *Salmonella* isolates than in the *E. coli* isolates. However, resistance in *Salmonella* is largely serotype-dependent (NARMS, 2005). *Salmonella Typhimurium* was the most prevalent (69.0%) serotype and demonstrated the greatest multiple resistance. *Salmonella Kentucky*, the least prevalent (5.0%) serotype recovered, was also the most susceptible. Although 34.1% of the *Salmonella* isolates was susceptible to all compounds, 60.1% was resistant to more than 11 compounds. However, multiple resistances were predominately observed among Typhimurium isolates. Antimicrobial drugs for which many *Salmonella* isolates exhibited resistance were tetracycline (63.4%), nalidixic acid (63.4%), and streptomycin (61.0%). Few studies have reported on antimicrobial resistance of *Salmonella* isolates collected from eggs or the egg-processing environment. In a study conducted in India, all of the 66 *Salmonella* isolates were susceptible to at least one of the compounds tested (Bajaj et al., 2003). Highest resistance was observed for penicillin (96.9%), vancomycin (83.3%), and erythromycin (81.8%). Resistance was also noted for trimethoprim (42.4%), streptomycin (24.2%), tetracycline (9.0%), and gentamicin (6.0%). Bajaj et al. (2003) did not report on the serotype(s) of *Salmonella* isolated in their study. In the NARMS summary report for 2003, when considering all *Salmonella* serotypes, 27.1% were resistant to tetracycline, 0.4% were resistant to nalidixic acid, and 19.5% were resistant to streptomycin (NARMS, 2005).

Figure 1 depicts the distribution of resistance in *Salmonella* by serotype. In a study conducted in India of 38 salmonellae isolated from eggs and egg packing materials, 95% were *Salmonella* Enteritidis (Suresh et al., 2005). All of the isolates were resistant to tetracycline, and 40% were resistant to nalidixic acid. There were 5,353 *Salmonella* isolates in the 2003 NARMS report (2005). The most frequently isolated serotypes were Kentucky (n = 555, 10.4%), Typhimurium-Copenhagen (n = 533, 10.0%), Newport (n = 483, 9.0%), Heidelberg (n = 439, 8.2%), and Typhimurium (n = 411, 7.7%). Serotypes Typhimurium, Heidelberg, and Kentucky are 3 of the most commonly isolated serotypes collected from poultry clinical and slaughter samples (NARMS, 2005), and they were the most commonly identified isolates in our study. No *S. Enteritidis* were recovered in the current study, whereas only 2.5% of *S. Enteritidis* (n = 139) was reported to NARMS in 2003 (NARMS, 2005). An Italian survey recovered antimicrobial-resistant *Salmonella* Typhimurium, Enteritidis, and Infantis from humans, food, and animal samples (Busani et al., 2004). In the Italian study, the highest rates of multiple resistances were detected for *S. Typhimurium*, as was the case with the current study. In a Turkish study involving *S. Enteritidis* isolated from chicken, eggs, and humans, 58 of 82 isolates (71%) were...
resistant to one or more antimicrobial agents, and multidrug resistances were observed in 35% of the isolates (Icgen et al., 2002).

Resistance was serotype-dependent in the current study. Salmonella Typhimurium isolates demonstrated the greatest degree of multiple resistances, followed by Heidelberg isolates. In the 2003 NARMS summary, only 2.6, 0.8, and 0.1% of 5,353 isolates were resistant to 11, 12, and 13 antimicrobial drugs, respectively, over all serotypes (NARMS, 2005). However, in the current study, most of the S. Typhimurium isolates were recovered from a single shell egg-processing plant and on the same sample collection day, increasing the likelihood that the isolates would be related. However, further characterization by pulsed-field gel electrophoresis will be required to confirm this suspicion.

Escherichia coli and Salmonella isolates analyzed in the current study displayed resistance to antimicrobial drugs. However, as expected, Salmonella resistance was serotype-dependent. Salmonella isolates were more likely to be resistant to antimicrobial drugs and displayed a larger number of multiple resistances. This observation was influenced by the fact that many of the isolates were S. Typhimurium, a serotype that has been previously reported as exhibiting multiple resistances. These data indicate that shell eggs and shell egg processing water can harbor resistant foodborne and commensal bacteria.

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**REFERENCES**


Kaye, K. S., J. J. Engemann, H. S. Fraimow, and E. Abruyn. 2004. Pathogens resistant to antimicrobial agents: Epidemiology,


