Emerging Antimicrobial Resistance in Foodborne Pathogens

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Summary: Foodborne microbial illnesses are an important public health issue worldwide. Although these illnesses are usually a mild to moderate self-limiting gastroenteritis, invasive diseases and complications may occur. Many foodborne bacteria (pathogenic and commensal varieties) colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption. Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, and preparation. An additional concern is the growing incidence of antimicrobial-resistant foodborne pathogens. This paper will focus on antimicrobial resistance among three of the most relevant foodborne bacterial pathogens, Salmonella, Campylobacter, and E. coli.

Keywords: Salmonella; Campylobacter; E. coli; Multiple antimicrobial resistance; food animals

Introduction: The fact that microbial ecosystems are interconnected must be underscored. Bacteria present today in a pig's intestine may a week later be in packaged pork products, and two months thereafter, in a community reservoir. Antimicrobial resistance in foodborne bacteria, therefore, should not necessarily be considered distinct from that in isolates from humans, food animals, or other niches. Yet because food consumption is an important pathway for bacteria to enter humans, the presence of antimicrobial-resistant bacteria in foods warrants particular attention.

Antimicrobial-resistant bacteria have been recovered from a wide variety of foods, and several thorough reviews have been written on the broad subject of antimicrobial-resistant bacteria in the food production
Antimicrobial resistance among *Salmonella* isolates is increasing on a global scale. A recent 7-year study in Spain revealed that ampicillin resistance in *Salmonella* species has increased from 8% to 44%, tetracycline resistance from 1% to 42%, chloramphenicol resistance from 1.7% to 26%, and nalidixic acid resistance from 0.1% to 11% (Prats et al. 2000). Similarly, in Great Britain, the reported rates of antimicrobial resistance for *S. Typhimurium* more than doubled between 1981 and 1989 (Threlfall et al. 1993). In the United States, resistance to tetracycline has increased from 9% in 1980 to 24% in 1990 and ampicillin resistance increased from 10 to 14% (Lee et al. 1994). Fluoroquinolones and expanded-spectrum cephalosporins, the primary antimicrobials for treating human infections caused by multidrug-resistant strains (Cherubin & Eng 1991; Fey et al. 2000), are also showing decreased activity against *Salmonella* species (Fey et al. 2000; Winokur et al. 2000).

An important factor in this increase in resistance has been the epidemic spread through food animals and humans over the past 10 years of multidrug resistant *S. Typhimurium DT104* (Glynn et al. 1998; Threlfall et al. 1993). DT104 is characterized by a resistance to five agents: ampicillin, chloramphenicol/florfenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). This strain type was first isolated from sea gulls, then cattle and later in humans in England. Since then, it has been isolated from multiple animal species including poultry, cattle, pigs, sheep, and non-domestic birds (Besser et al. 2000). Some strains in Great Britain have acquired additional resistance to trimethoprim, aminoglycosides as well as decreased susceptibility to fluoroquinolones (Threlfall et al. 1996). The majority of resistant DT104 isolates have a unique multidrug resistance chromosomal gene cluster encoding the complete spectrum of the ACSSuT phenotype (Threlfall et al. 1996). This gene cluster typically consists of a chromosomal locus 12.5 kb in size with flanking integrons (Briggs & Fratamico 1999). The first integron possess the *aadA2* gene, conferring resistance to streptomycin and spectinomycin; the second contains the *b-lactamase gene blaPSE-1* encoding resistance to ampicillin. A gene encoding sulfonamide resistance (*sul-1*) is present within conserved sequences of both integrons. Flanked by these two integron structures are the flo efflux gene, conferring cross-resistance to chloramphenicol and florfenicol, and the tetracycline resistance genes *tetR* and *tetA* (1999; Briggs & Fratamico 1999). The DT104-like resistance gene cluster has also been described in poultry strains of *S. Agona*, suggesting that it is transmittable between serotypes. It has been shown experimentally that the DT104 MDR cluster can be efficiently transduced by P22-like phages. In addition, upstream of the first integron in the MDR locus is a gene encoding a putative resolvase enzyme, which demonstrates greater than 50% identity with the Tn3 resolvase family. This suggests that the MDR antibiotic resistance gene cluster could be part of a much larger transposon.

The presence of resistant *Salmonella* in retail meats has been assessed in numerous studies. In a pilot survey by White et al., (White et al. 2001) two hundred ground meat samples (51 chicken, 50 beef, 50 turkey, and 49 pork) were purchased from retail stores representing three different supermarket chains in the greater Washington DC area between June and September of 1998. Products were fresh, prepackaged meats coming from four poultry, and one pork processing plants, and store-ground and packaged beef. *Salmonellae* were recovered from 41 of 200 (21%) ground meat samples. *Salmonella* was isolated more frequently from poultry (33% of chicken and 24% of turkey samples) than red meats (18% of pork and 6% of beef samples). All *Salmonella* were susceptible to amikacin, apramycin, ciprofloxacin and nalidixic acid. Eighty-four percent (38/45) of isolates displayed resistance to at least one antibiotic. The most common resistance observed was to tetracycline (80%), streptomycin (73%), sulfamethoxazole (69%), and to a lesser extent ampicillin (27%). In addition, 16% displayed resistance to amoxicillin/clavulanic acid, cephalothin, cefotiofur, and ceftriaxone. Cefotiofur- and ceftriaxone-resistant *Salmonella* were isolated from ground turkey, chicken, and beef.
Antimicrobial resistant _Salmonella_ have also been reported from imported foods. Zhao et al. reported the antimicrobial susceptibilities of 187 _Salmonella_ isolates, representing 80 serotypes, recovered from imported foods by FDA field laboratories in 2000 (Zhao et al. 2003). Fifteen (8%) were resistant to at least one antimicrobial, and five (2.7%) were resistant to three or more antimicrobials. Nine isolates exhibited resistance to tetracycline. Four isolates also demonstrated resistance to nalidixic acid - all were isolated from imported catfish or tilapia from Taiwan or Thailand. All four nalidixic acid resistant _Salmonella_ isolates possessed amino acid substitutions at the Ser83 or Asp87 position in DNA gyrase. One S. Derby isolated from frozen anchovies imported from Cambodia was resistant to 6 antimicrobials including ampicillin, amoxicillin/clavulanic acid, chloramphenicol, trimethoprim/sulfamethoxazole, sulfamethoxazole, and tetracycline.

Studies conducted outside the U.S. have also examined foods for the presence of antimicrobial resistant _salmonellae_. One study from Spain, determined the extent of antimicrobial resistance among one hundred and twelve _Salmonella_ isolates recovered from 691 samples of frozen and fresh chicken meat (Hernandez et al. 2002). Almost half of the isolates tested (46%) were susceptible to all antimicrobials tested. However, resistance was commonly seen to chloramphenicol (45%), ampicillin (35%), and tetracycline (34%). Resistance to multiple antimicrobials was observed in 44% of isolates, whereas single resistance was seen in 11%. _S. Typhimurium_ isolates tended to be more resistant than other serotypes tested. Another study from Europe authored by Mammina et al., investigated the distribution of serotypes and patterns of drug resistance of 206 strains of _Salmonella_ isolated in southern Italy. _Salmonella_ were obtained between 1998-2000 from 172 samples of raw foods of animal origin, 22 fecal samples from food animals and 12 animal feed samples (Mammina et al. 2002). _Salmonellae_ resistant to three or more antimicrobials were considered multi-resistant. Among non-Typhimurium isolates tested, 46 of 122 (38%) strains were categorized as multi-drug resistant. _S. Typhimurium_ was the predominant serotype recovered with 35 of 67 (52%) isolates displaying multidrug resistance. The characteristic DT104 antimicrobial resistance phenotype of ACSSuT was identified in 17 of these isolates.

Recently, there has been a national emergence of strains of _S. Newport_ in the U.S. known as Newport-MDRAmpC, which are resistant to at least nine antimicrobials, including extended-spectrum cephalosporins. In the U.S., the prevalence of Newport-MDRAmpC among _S. Newport_ isolates from humans increased from 0% during 1996-1997 to 26% in 2001. At least 26 states have isolated these strains either from humans, cattle, or ground beef. Like DT104, these strains were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. In addition, Newport-MDRAmpC isolates were resistant to amoxicillin/clavulanic acid, cephalothin, cefoxitin, and ceftiofur, and exhibited decreased susceptibility to ceftriaxone (MIC ≥16 μg/ml). In 2001, Newport-MDRAmpC strains were responsible for 2.6% of the more than 1 million estimated _Salmonella_ infections in 2001 (Anonymous 2002b).

_Campylobacter_

_Campylobacter_ has been recovered from the intestinal tracts of both wild and domestic animals. In food-producing animals such as cattle, poultry and swine, fecal _C. jejuni/C. coli_ can be regarded as a commensal organism. As a result of fecal contact during processing, enteric bacteria frequently contaminate foods derived from animals. This is the case with _Campylobacter_ (_C. jejuni_), which are present in high numbers in the poultry intestine. Consequently, _Campylobacter_ can be readily cultured from most retail chicken carcasses.

In a Minnesota survey, 91 chicken meat products were purchased in the Minneapolis-St. Paul area from September to November, 1997 (Smith et al. 1999). These products came from 15 poultry-processing plants in nine states. All chicken meat products were cultured for _Campylobacter_, which were tested for susceptibility to nalidixic acid and ciprofloxacin. _Campylobacter_ was isolated from 88% of the meat samples tested: _C. jejuni_ was isolated from 74% of chicken samples and _C. coli_ was recovered from 21% of samples tested. Ciprofloxacin resistant _Campylobacter_ (in this study; MIC > 32 μg/ml) were recovered from 20% of
tested chicken samples (n=18). Using molecular subtyping methods (fla-RFLP), they further identified six of seven subtypes among Campylobacter isolates from both retail chicken products and quinolone-resistant C. jejuni isolates from humans. This data demonstrated that domestic chicken obtained from retail markets had high rates of contamination with ciprofloxacin-resistant C. jejuni. Furthermore, the authors identified an association between molecular subtypes of quinolone-resistant C. jejuni strains that were acquired domestically in humans and those found in retail chicken products.

Zhao et al. (Zhao et al. 2001a) examined 719 retail raw meat samples (chicken, turkey, pork, and beef) obtained from 59 stores of four supermarket chains during 107 sampling visits in the Greater Washington D.C. area, from June 1999 to July 2000. Campylobacter was detected in 71% of 184 chicken samples, and a large percentage of the stores visited (91%) had Campylobacter-contaminated chicken. Approximately 14% of the 172 turkey samples were culture-positive, whereas few pork (1.7%) and beef (0.5%) samples were positive. Approximately one-half (53.6%) of the isolates were identified as C. jejuni, 41.3% were identified as C. coli, and 5.1% were identified as other species. C. coli was more often recovered from retail turkey samples than C. jejuni. The most common resistance observed among the Campylobacter poultry isolates was to tetracycline (82%), followed by erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%)(Ge et al. 2003). C. coli isolates displayed higher resistance rates to ciprofloxacin and erythromycin than C. jejuni. Turkey isolates, regardless of species, showed elevated resistance rates to ciprofloxacin and erythromycin than Campylobacter isolates from retail chickens. Eighty-seven percent of samples contained Campylobacter resistant to at least 2 antimicrobial agents, and 22% to at least 5 antimicrobials.

Co-resistance to ciprofloxacin and erythromycin was found in Campylobacter from 26% of the meat samples. Multi-drug resistance, including co-resistance to fluoroquinolones and erythromycin, has been reported previously in Campylobacter isolated from retail meat product (Saenz et al., 2000) and from humans (Engberg et al., 2001). The finding of co-resistance to fluoroquinolones and erythromycin in Campylobacter is undesirable, as these two antimicrobials are generally advocated as first-line drugs for treatment of human campylobacteriosis.

In 2002, The National Antimicrobial Resistance Monitoring System (NARMS) expanded into surveillance of retail meats to determine the prevalence of antimicrobial resistance among Salmonella, Campylobacter, E. coli and Enterococcus. As of January, 2003, 9 FoodNet participating sites are involved (California, Colorado, Connecticut, Georgia, Minnesota, New York, Tennessee, Maryland, and Oregon). Each FoodNet site purchases a total of 40 food samples per month, consisting of 10 samples each of chicken breast, ground turkey, ground beef, and pork chops. Preliminary data show that 58% of 356 chicken breasts, 8% of 372 ground turkey, 3% of 373 ground beef samples, and 2% of 343 pork chop samples cultured positive for Campylobacter (unpublished data). This surveillance is ongoing and being expanded to include additional state labs.

Escherichia coli
Antimicrobial-resistant E. coli have been recovered from a variety of foods, including minced meat, vegetables, cakes and confectionary, custards and desserts, and milk and milk products. A number of studies outside the U.S. from the early 1980s looked for the presence in foods of antimicrobial resistant Gram-negative bacteria, including E. coli. One of the first studies was described by Persson et al. (Persson et al. 1980). E. coli was the predominant bacterium identified and was most often isolated from raw meat and egg products. All isolates were considered susceptible to streptomycin, neomycin, and trimethoprim-sulfamethoxazole. Fifty-four percent (166/308) of bacteria tested, however, were resistant to at least one of the 9 antibiotics tested, with 40% displaying multi-resistance. Resistance to nitrofurantoin and sulphaisodimidine was most frequently observed (30% and 25% respectively). The lowest numbers of resistant strains identified were recovered from custards and desserts and from raw meat products (42% and 46% respectively) and the highest from ice-cream (89%). Multi-resistant strains were recorded most frequently from pasteurized milk products, custards and desserts.
Bensink et al. examined Australian beef and pig carcasses, meat products, and frozen chickens for the presence of antibiotic resistant coliforms (Bensink et al. 1981). Escherichia coli was isolated from 18 of 50 beef carcasses, and only resistance to tetracycline was detected. The situation was different with E. coli recovered from pig carcasses, meat products, and chickens, where numerous resistance phenotypes were observed, including multidrug resistant strains. A later study by the same researchers investigated antibiotic resistance in coliforms isolated from poultry carcasses immediately after slaughter and at retail (Bensink & Botham 1983). Approximately 85% of the total of 13,858 isolates examined were found to be resistant to at least one antibiotic. Highly significant differences were found in the levels of antibiotic resistance from the 2 sources; ampicillin, chloramphenicol, and sulphonamide resistance was found more frequently in isolates from poultry at retail, while resistance to streptomycin and neomycin occurred more frequently in isolates from poultry at slaughter.

Several more recent studies have investigated specifically the prevalence of antimicrobial resistant E. coli in retail foods. Meng et al. determined antimicrobial susceptibilities of 118 E. coli O157:H7 and 7 O157:NM isolates from animals, foods, and humans in the U.S. (Meng et al. 1998). Among the 125 isolates tested, 30 (24%) were resistant to at least one antibiotic and 24 (19%) were resistant to three or more antibiotics. Cattle strains were more often resistant than other isolates tested (34%). The seven resistant food E. coli isolates were all recovered from ground beef. Two E. coli/O157:NM isolates from cattle were resistant to six antibiotics: ampicillin, kanamycin, sulfisoxazole, streptomycin, tetracycline, and ticarcillin. Streptomycin was the most common antibiotic to which E. coli O157:H7 and O157:NM were resistant (29/30 isolates), followed by tetracycline (26 isolates). Overall, the most frequent resistance phenotype observed was to streptomycin-sulfisoxazole-tetracycline, which accounted for over 70% of the resistant strains.

Sáenz et al. investigated the prevalence of antimicrobial resistant E. coli from animals, foods and humans in La Rioja, Spain (Saenz et al. 2001). Food products of animal origin sampled for the presence of antimicrobial resistant E. coli included hamburger, sausage, chicken, and turkey. Using disk diffusion, they reported that among 47 E. coli isolates recovered from foods, 53% were resistant to nalidixic acid, 47% to ampicillin, and 40% to kanamycin. Resistance was also observed, but to a lesser extent, to gentamicin (17%), ciprofloxacin (13%), and amoxicillin-clavulanic acid.

Schroeder et al. (2003), reported antimicrobial susceptibilities among 472 generic Escherichia coli isolates recovered from ground and whole retail beef, chicken, pork, and turkey obtained from greater Washington, DC, during the years 1998 and 2000. Isolates displayed resistance to tetracycline (59%), sulfamethoxazole (45%), streptomycin (44%), cephalothin (38%) and ampicillin (35%). Lower resistance rates were observed for gentamicin (12%), nalidixic acid (8%), chloramphenicol (6%), ceftiofur (4%) and ceftriaxone (1%). Sixteen percent of the isolates displayed resistance to one antimicrobial, followed by 23% to two, 23% to three, 12% to four, 7% to five, 3% to six, 2% to seven and 2% to eight. Other studies demonstrate that E. coli recovered from foods are not necessarily resistant to multiple antimicrobials. Zhao et al. tested 404 fresh ground beef samples obtained at retail stores from New York, San Francisco, Philadelphia, Denver, Atlanta, Houston, and Chicago for the presence of Salmonella and E. coli (Zhao et al. 2002). Among the 102 generic E. coli isolates obtained, only three were resistant to multiple antibiotics.

There is still debate on the public health implications of the presence of antimicrobial-resistant E. coli in foods. Nevertheless, the numerous reports that have documented the presence of antimicrobial resistant E. coli in retail foods highlights the public health value of continuing efforts to educate consumers in proper food handling and preparation practices.

Conclusions: Antimicrobial resistant bacteria from animals, both commensal and pathogenic variants, can reach the general public via exposure to contaminated food products of animal origin if they are improperly cooked or otherwise mishandled. It has been theorized that these resistant bacteria
KEYNOTE LECTURES

have the potential to colonize humans and/or transfer their resistance determinants to resident constituents of the human microflora, including pathogens. Therefore, it is necessary to continue work to advance food processing technologies while emphasizing hygienic food handling at all stages of food production. There is also a continuing need for education programs aimed at improving food safety behaviors of consumers of all ages. Lastly, surveillance programs designed to detect emerging antimicrobial resistance phenotypes among foodborne pathogens in retail foods will continue to be a foundational tool for helping to ensure a safe food supply.

References:


