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Microbial Profile and Antibiotic Susceptibility of *Campylobacter* spp. and *Salmonella* spp. in Broilers Processed in Air-Chilled and Immersion-Chilled Environments

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ABSTRACT

Carcass chilling is considered a critical step for inhibiting bacterial growth during poultry processing. The objective of this study was to compare microbiological loads and the incidence of *Salmonella* spp. and *Campylobacter* spp. on broiler carcasses subjected to immersion chilling and air chilling. Additionally, the antibiotic resistance patterns of pathogen isolates were determined. The results of this study indicated that the incidence of *Salmonella* spp. and *Campylobacter* spp. tends to be significantly lower in air-chilled broilers, suggesting that cross-contamination may be more prevalent for immersion-chilled broilers. No significant differences were detected between chilling treatments for total aerobic populations or for generic *E. coli* or coliform counts. Psychrotrophic populations were significantly larger ($P < 0.05$) in immersion-chilled broilers than in their air-chilled counterparts. *Campylobacter* isolates from immersion-chilled broilers had a higher incidence of resistance to nalidixic acid (NAL) and related fluoroquinolones than isolates from air-chilled broilers did. Additionally, *Campylobacter* isolates from air-chilled broilers had a higher frequency of resistance to tetracycline than isolates from immersion-chilled broilers did. With regard to *Salmonella,* isolates from immersion-chilled broilers had a higher incidence of resistance to NAL than isolates from air-chilled samples did. No *Salmonella* isolates from immersion- or air-chilled broilers were resistant to the fluoroquinolones tested. The chilling method used during processing may influence the microbial profile of postchilled broilers.

The impact of foodborne illness on consumers and the food industry can be devastating. According to the Centers for Disease Control, foodborne diseases cause approximately 76 million illnesses in the United States annually, resulting in approximately 5,000 deaths and costing an estimated 9.2 billion dollars *(37).* Two foodborne pathogens often associated with poultry meat are *Salmonella* spp. and *Campylobacter* spp. *(5–7, 11, 19, 25, 27, 28, 38).* Of 5.2 million cases of bacterium-related foodborne illness in the United States, *Campylobacter* spp. account for 2.5 million (approximately 50%) *(37)* and are considered the leading cause of diarrhea in developed countries, causing 200 to 730 deaths annually *(1, 4, 16, 42, 46, 49, 50).* Similarly, *Salmonella* spp. (nontyphoidal) account for 1.4 million to 4 million cases (approximately 20%) annually *(37, 59),* resulting in 800 to 4,000 deaths per year *(16, 57).*

Specifically, *Salmonella* spp. and *Campylobacter* spp. represent human health risks when poultry is inadequately cooked or is cross-contaminated after cooking *(16, 48).* Cross-contamination can occur at each stage in the process of bringing the product to the consumer, beginning at the farm and continuing through processing *(6, 57).* Slaughtering and processing steps such as scalding, picking, and chilling may be a source of cross-contamination *(1, 6, 25, 33).* Additionally, meat can become contaminated with pathogens from intestinal contents, skin, or feathers *(38).* Epidemiological studies have shown a strong relationship between *Campylobacter* enteritis and the handling and consumption of raw or inadequately cooked poultry *(11, 19).* Reports from the ''Nationwide Broiler Chicken Microbial Baseline Data Collection Program'' (U.S. Department of Agriculture [USDA] Food Safety and Inspection Service) indicated that 88.2% of raw commercial broilers tested positive for *Campylobacter jejuni* and/or *Campylobacter coli (50, 56).* It was also estimated that between 20% *(56)* and 35% *(33)* of ready-to-market broilers tested positive for *Salmonella* spp., whereas only 3 to 4% of broilers entering the plant were *Salmonella*-positive. Thus, cross-contamination during normal processing and ways to alleviate it are important issues for poultry processors, government officials, and consumers.

During the processing of broilers, one of the most critical steps for inhibiting the growth of microorganisms is carcass chilling. Upon completion of the evisceration step, a carcass is warm after being submerged in the scalder tank, and a quick chilling step is necessary to lower its temperature to prevent bacterial growth. The USDA Food Safety and Inspection Service requires that the carcass temperature be reduced to 4.4°C (40°F) within 4 h after the evisceration step *(22, 55, 57).* Because of these regulatory limits, chilling is often included as a critical control point in hazard analysis critical control point plans for most broiler facilities *(57).*

The most common chilling methods are immersion

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chilling, primarily used in the United States, and air chilling, commonly used in Europe (80% of the market) and Canada (20% of the market) *(47).* During immersion chilling, the birds coming from the evisceration step are submerged in cold water. The cold water flows countercurrent to the direction in which the birds are being moved, creating a temperature gradient. The chill water contains residual chlorine levels of 20 to 50 ppm. An overflow system allows the exchange of approximately 1.79 liters of water for each bird entering the chiller *(22).* Because of the countercurrent water flow, the chicken exiting the chiller comes in contact with the coldest, cleanest water. In addition to reducing carcass temperature faster than other chilling methods do, the immersion-chilling process may rinse away some of the bacterial loads *(13).* However, during the immersion-chilling process, pathogen cross-contamination from one carcass to another may occur *(6),* and water retention is enhanced by the temperature gradient generated in the water chiller *(17).*

In contrast, in an air-chilling system, the lack of a communal water bath may reduce the risk of cross-contamination *(6).* Furthermore, psychrotrophic bacteria may be decreased *(30).* In air-chilling systems, water as a cooling medium is replaced by cold air drafts that are less likely than water to transport *Salmonella* spp. and *Campylobacter* spp. Air-chilled broilers are chilled individually on an assembly line, and contact between birds is minimized. Air application could be detrimental to some pathogens affected by aerobic environments, like *Campylobacter* spp., which are also very sensitive to drying conditions *(24).* Moreover, the dehydrating effect of air chilling may reduce overall water availability, which influences bacterial survival. Chicken-thaw fluid, blood, and other tissue fluids prolong survival times of microorganisms *(10),* and air-chilled broilers produce fewer residual fluids than their immersion-chilled counterparts do *(24).*

There is no current scientific basis for replacing immersion chilling with air chilling to improve food safety. In the United States, immersion chilling of poultry is the standard *(22).* Therefore, the broiler microbial baseline published by the USDA represents a national average of immersion-chilled products only *(56).* Because there is only one air-chilling plant in the United States, no current data are available on the quality or microbiological characteristics of commercially processed air-chilled poultry in the United States.

Another issue of concern with regard to poultry is the emergence of antibiotic-resistant organisms. The use of antibiotics in subtherapeutic doses as growth-promoting feed additives for animal production is widespread in the United States and throughout the world *(16, 43, 46, 53).* There is currently concern about the isolation of pathogens resistant to fluoroquinolones, which are strong antibiotics used to treat foodborne infections such as campylobacteriosis *(44).* According to recent studies *(41, 49),* the incidence of resistant bacteria has been increasing since the use of fluoroquinolones in poultry production was approved *(16, 31, 43, 45, 46).* On the other hand, stress conditions during processing and the presence of stressing agents including chlorine and sanitizers may result in the selection of microflora *(2, 3).* This selective pressure may enhance the survival of resistant strains or induce resistance, hence increasing virulence *(2, 3, 34).* Public concern led to the development of the National Antimicrobial Resistance Monitoring System (NARMS) in 1996 *(8),* and conclusive data forced the Food and Drug Administration to propose the banning of formerly approved fluoroquinolones used in poultry production in October 2000 *(16).*

The purpose of this study was to compare microbial profiles of ready-to-market immersion-chilled and air-chilled broilers by evaluating *Salmonella* spp. and *Campylobacter* spp. incidence, generic *E. coli* counts, coliforms, psychrotrophs, and total aerobic counts. Additionally, the antibiotic resistance patterns of the pathogenic isolates of *Salmonella* spp. and *Campylobacter* spp. obtained from immersion- and air-chilled broilers were determined.

MATERIALS AND METHODS

Sample size. A total of 300 market-age birds (~6 weeks) were collected. In each of 10 replications, 15 birds from an air-chilling facility and 15 birds from an immersion-chilling plant were sampled after chilling. Immersion-chilled birds were obtained from a facility in Missouri, while air-chilled broilers were processed in a facility in Nebraska. The estimation of the sample number was determined according to Hicks *(20),* with the standard error of previous estimations being used to determine the variability of the population. The facilities used in this project processed from 45,000 to 60,000 birds per day. It was necessary for the number of samples to be sufficient to detect the organism with the lowest incidence, in this case *Salmonella* spp., which has been reported to be present at levels below 25% *(33, 56).* From this information, it was statistically determined that 150 samples from each facility (300 total) was an appropriate sample number for comparative purposes.

Whole-carcass immersion-chilled broilers (no trimmed carcasses) were randomly sampled by plant personnel using USDA standards *(23, 57),* packaged on ice, stored at 4°C, and transported to the laboratory to be analyzed the next day. In the immersion-chilling facility, broilers were chilled in a three-stage countercurrent immersion chiller for a total time of 85 min. The first stage used water at 17.2°C (63°F), the second stage used water at 5.6 to 6.7°C (42 to 44°F), and the third stage used water at −1.1 to 0°C (30 to 32°F). The chiller also contained approximately 40 ppm chlorine. The plant was chosen because it used processing procedures that were equivalent to those used by the air-chilling facility tested with regard to conditions during stunning, scalding, and inside and outside washes. Air-chilled birds were treated for 120 min in an air-chilling room in two stages, with temperatures of −7.7 to −5.5°C (18 to 22°F) and −4.4 to −1.1°C (24 to 30°F), respectively. Samples were randomly obtained by our laboratory personnel in accordance with USDA standards, packaged on ice, transported to our laboratory, and held overnight at 4°C to mimic conditions of the immersion-chilled samples.

Carcass sampling. The whole-carcass rinse method recommended by the USDA *(57)* was used for microbial sampling of the birds. Carcasses were placed into sterile stomacher bags and rinsed with 400 ml of Butterfield's phosphate diluent for 1 min with a rocking motion to assure that all interior and exterior surfaces were rinsed. The rinsate was then transferred to a sterile bottle and divided into three parts for each analysis. All microbial analyses were based on methods described in the USDA Food

Safety and Inspection Service *Microbiology Laboratory Guidebook (55)* and in the *Food and Drug Administration Bacteriological Analytical Manual (23).* However, some procedures were adapted to the conditions of the laboratory used for the project.

Microbiological analysis. Total aerobic and psychrotroph populations were determined by using 10 ml of the original rinsate to prepare serial dilutions for each sample. Dilutions were prepared with 0.1% buffered peptone water (Difco Laboratories, Detroit, Mich.), and molten plate count agar (Difco) was used as the medium. For the recovery of total aerobic populations, plates were incubated at 37°C for 48 h, while plates for psychrotrophs were incubated for 10 days at 7°C.

Coliform and generic *E. coli* counts were determined by using violet red bile agar (Oxoid, Basingstoke, UK). Dilutions were mixed with regular violet red bile agar media, and when they solidified, an overlay of violet red bile agar supplemented with 0.4 μg of 4-methylumbellyferyl-β-D-glucuronide was added. Plates were incubated for 24 h at 37°C to determine total coliform and generic *E. coli* numbers by counting fluorescent colonies under UV light.

Fifty milliliters of the original rinsate was used for the detection of *Salmonella* spp. The sample was preenriched by adding 50 ml of buffered peptone water, and it was then incubated for 24 h at 37°C. Preenriched samples were put into two enrichment broths, 0.5 ml into Tetrathionate TT (Difco) broth tubes and 0.1 ml into Rappaport Vassiliadis (Difco) broth RV tubes. Tubes were incubated for 24 h at 41°C. Xylose lysine agar (Difco) supplemented with Tergitol 4 (XLT4) and brilliant green sulfa agar were used for selective plating. Enrichment solutions were streaked and incubated for 24 to 48 h at 37°C. Characteristic colonies were inoculated in slant tubes of lysine iron agar (Difco) and triple sugar iron agar (Difco) and incubated for 24 h at 37°C for biochemical characterization. Characteristic colonies, which appear black centered on XLT4 agar and translucent red on brilliant green sulfa agar, were selected for urease and indol testing. Finally, presumptive positive colonies were serologically confirmed with polyvalent serum A-V for *Salmonella* spp. (Difco). Confirmed cultures were sent to a diagnostics laboratory in Ames, Iowa, for serotyping.

The presence of *Campylobacter* spp. was determined by using 200 ml of rinse. Rinsate was sterile-filtered through cheesecloth into a sterile 250-ml centrifuge bottle. The filtrate was then centrifuged at 16,000 × *g* for 15 min. The supernatant was discarded and the pellet was resuspended in 10 ml of buffered peptone water. One milliliter of the inoculated peptone water was preenriched by the addition of 100 ml of Hunt enrichment broth (Oxoid) in a Whirl Pack bag. Air was removed from the bag, and a microaerophilic mixture of 5% O_2, 10% CO_2, and 75% N_2 gas was added to inflate the bag and produce a microaerophilic environment. The bag was then sealed and incubated at 37°C for 4 h in a shaker incubator. After 4 h, a solution of sterile cefoperazone was added to yield a final concentration of 30 mg/liter. The microaerophilic atmosphere was reestablished, and the bag was incubated for 20 h at 42°C. Modified *Campylobacter* charcoal differential agar (MCCDA; Oxoid) was used for selective plating. MCCDA plates were incubated at 42°C for 24 to 48 h in a microaerophilic environment by using anaerobic jars with pressure gauge valves. Characteristic colonies were tested for oxidase reaction, and further identification was achieved with a microscope. Brucella broth (Difco) supplemented with ferrous sulphate, potassium biphosphate, and sodium pyruvate (B-FBP; Sigma Chemical Co., St. Louis, Mo.) was inoculated for biochemical testing. B-FBP tubes were incubated at 42°C for 24 to 48 h in a microaerophilic atmosphere. A wet mount was examined under a phase-contrast microscope to identify characteristic spiral rods with screw motility under ×100 magnification. Several drops of B-FBP solution were added to semisolid glucose medium (Difco) and incubated under microaerophilic conditions at 42°C for 1 to 3 days to observe glucose fermentation. These tubes were also used to determine catalase reaction with hydrogen peroxide solution. Several drops of the same solution were spread over B-FBP agar plates with sterile cotton swabs. Nalidixic acid (BBL Microbiology Systems, Cockeysville, Md.) and cephalothin (BBL) disks were used for antibiotic susceptibility testing with further incubation for 24 to 48 h at 42°C. A sample was considered positive when it tested positive for oxidase and catalase and tested negative for glucose fermentation. *Campylobacter* spp. were considered susceptible to nalidixic acid but resistant to cephalothin. Further confirmation was carried out with the commercially available biochemical kit APICampy (Biomerieux, Marcy l'Etoile, France) and the latex agglutination test INDXCampy (jcl) (Integrated Diagnostics Inc., Baltimore, Md.). Isolates were sent to the Southern Plains Area USDA Agricultural Research Service diagnostics laboratory in Texas for final confirmation by ribotyping methods.

Antibiotic susceptibility. Isolates were saved in nutrient media supplemented with 10% glycerol. *Campylobacter* isolates were saved in B-FBP broth and *Salmonella* isolates were saved in tryptic soy broth (Difco), and all isolates were then stored at −70°C for 1 month prior to analysis. Frozen cultures were enriched in Bolton broth for *Campylobacter* isolates and in tryptic soy broth for *Salmonella* isolates. In addition, control strains from our pathogen bank were used for comparative purposes. *Campylobacter* tubes were incubated for 24 h in a microaerophilic environment at 42°C, while *Salmonella* isolates were incubated aerobically at 37°C for 24 h. Fresh broth cultures were swabbed into Mueller-Hinton base (Difco) with added lysed sheep's blood (Colorado Serum Company, Denver, Colo.) plates for determination of antimicrobial susceptibility. Antibiotic disks for the disk diffusion assay and E-strips (AB Biodisk, Piscataway, N.J.) for the E-test were aseptically placed in the plates to detect zones of growth inhibition. *Campylobacter* plates were incubated for 24 to 48 h at 42°C in a microaerophilic atmosphere, while *Salmonella* isolates were incubated aerobically at 37°C for 24 h.

The set of antibiotics to be used was established by selecting representative members of the different families of antibiotics. Nalidixic acid (quinolone) and the second-generation fluoroquinolones ciprofloxacin, levofloxacin, and grepafloxacin were used in the resistance profile analysis. In addition, enrofloxacin was used to observe resistance to veterinary fluoroquinolones. These antibiotics act by inhibiting DNA replication in bacteria. Other antibiotics included the β-lactam ampicillin and the cephalosporin cephalothin. Both act by inhibiting the formation of the cell wall. Other groups of antibiotics are active against protein synthesis in bacteria. These groups include tetracycline, which acts in the 30S ribosome during protein synthesis, and the macrolide erythromycin, which acts in the 50S ribosome.

Susceptibility was determined following the manufacturer's instructions by measuring the area of inhibition around the disks or directly determining the minimum inhibitory concentration from the E-strip scale. All of the isolates were tested for resistance to ciprofloxacin, grepafloxacin, levofloxacin, cephalothin, enrofloxacin, erythromycin, tetracycline, and ampicillin by using the E-test (AB Biodisk) or the disk diffusion test (BBL). Antimicrobial susceptibility testing of *Campylobacter* spp. is not standardized; the reference method is agar dilution *(18),* and in this case

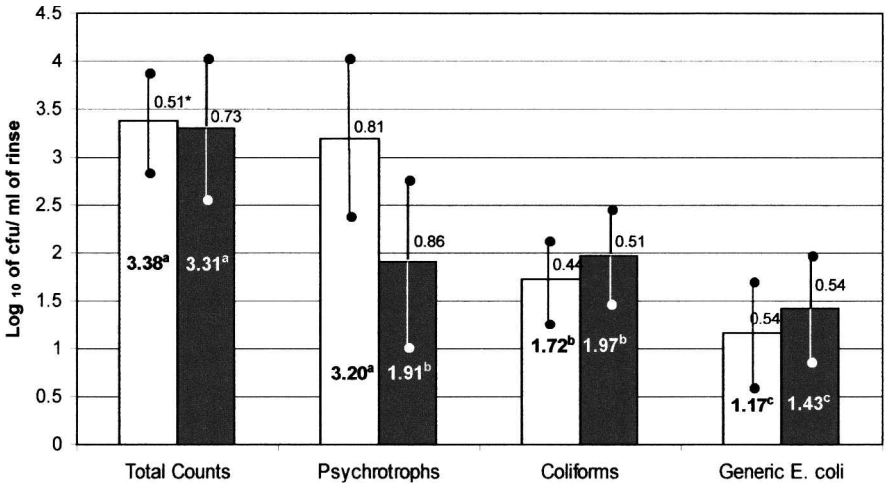

FIGURE 1. *Microbial profile for immersion-chilled (IC; □) versus air-chilled (AC; ■) broilers (* standard deviation). Data represent the average of 150 samples for each chilling method. Values with the same superscript are not significantly different.*

we used the disk diffusion method with antibiotic disks and E-tests, as used in several other studies *(18, 40, 49)*.

Statistical analysis. Bacterial counts were converted to $\log_{10}$ counts per milliliter of rinse and analyzed with the general linear model (SAS Institute, Inc., Cary, N.C.). Differences between the incidences of *Salmonella* spp. and *Campylobacter* spp. and between chilling regimens were determined with the chi-square analysis of SAS. The antibiotic resistance differences were also analyzed with the chi-square analysis of SAS.

RESULTS

Because pathogenic organisms such as *Campylobacter* spp. and *Salmonella* spp. may be attached firmly to the skin of poultry, some authors have suggested that skin excisions might be better than rinse methods for determining the incidence of pathogens *(33)*. However, preliminary trials indicated that the detection of pathogens was improved by the whole-carcass rinse method compared with the excision method, providing more consistency and representing an overall sample of the carcass (data not shown).

Microbial profile. No differences were observed in the overall aerobic plate counts for immersion-chilled broilers and air-chilled broilers. Overall psychrotrophic organisms were more numerous ($P < 0.05$) in immersion-chilled samples than in air-chilled samples. No differences were found for coliform and generic *E. coli* counts when air-chilled broilers and immersion-chilled broilers were compared (Fig. 1).

Pathogenic profile. The incidence of *Salmonella* spp. was lower for air-chilled samples than for immersion-chilled samples (Fig. 2). Of the strains isolated from immersion-chilled samples, 60% were *Salmonella* Kentucky strains, 20% were *Salmonella* Montevideo strains, and 20% were *Salmonella* Schwarzengrund strains. In contrast, of the *Salmonella* strains obtained from air-chilled broilers, 53.3% were *Salmonella* Enteritidis strains, 20% were *Salmonella* Oranienburg strains, and 26.7% were *Salmonella* Typhimurium strains according to the serological analysis performed in Iowa.

The incidence of *Campylobacter* spp. for air-chilled broilers was lower than that observed for immersion-chilled samples (Fig. 2). For the immersion-chilled samples, 50% of the strains isolated were *C. jejuni* strains and 25% were *C. coli* strains. The remaining isolates showed typical characteristics of *C. jejuni* but were not identified with the API test. Of the strains isolated from air-chilled broilers, 72.7% were *C. jejuni* strains and 18.2% were *C. coli* strains.

Antibiotic resistance. *Salmonella* isolates exhibited a variable profile for antibiotic susceptibility (Fig. 3). All of

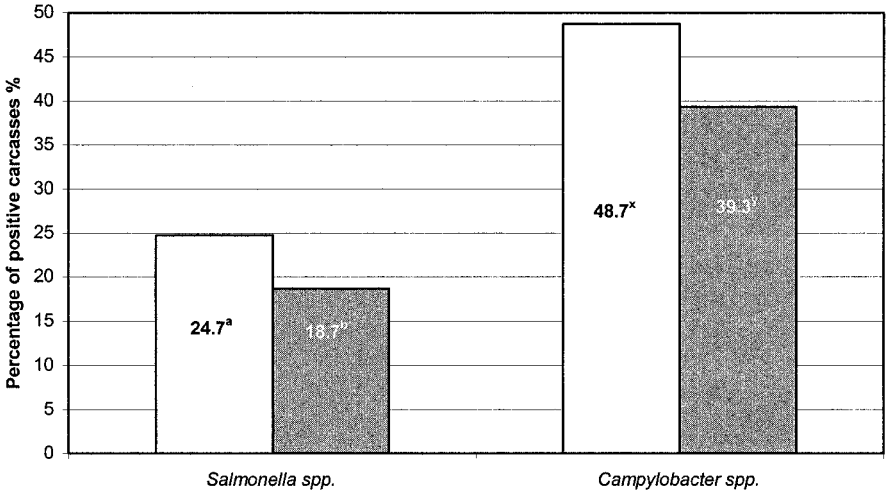

FIGURE 2. *Incidence of* Salmonella *spp. and* Campylobacter *spp in broilers processed in immersion-chilling (IC; □) and air-chilling (AC; ■) environments. Significant differences (*$P < 0.05$*) were observed when the two processes were compared. Data represent the average of 150 samples for each chilling method. Values with the same superscript are not significantly different.*

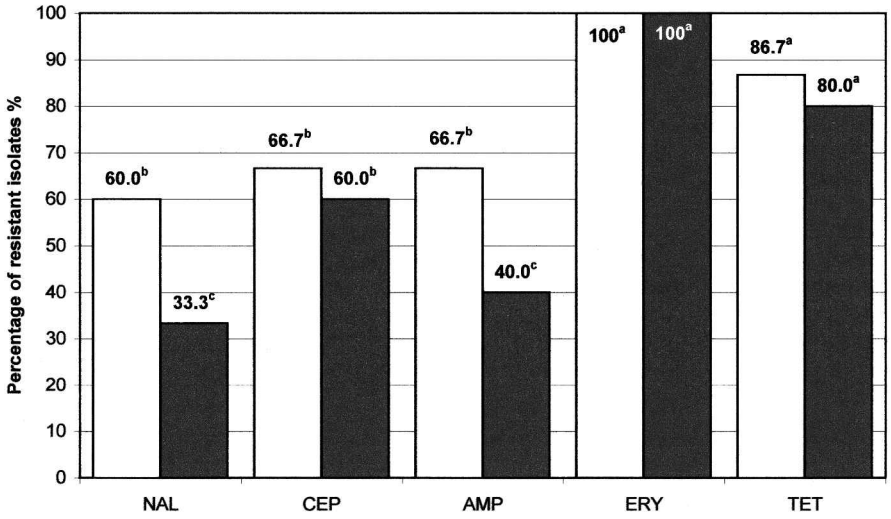

FIGURE 3. *Antimicrobial susceptibility profile of* Salmonella *spp. isolates obtained from whole-carcass rinses of broilers processed in immersion-chilling (IC; □) and air-chilling (AC; ■) facilities. Isolates were tested by agar diffusion methods for resistance to nalidixic acid (NAL), cephalothin (CEP), ampicillin (AMP), erythromycin (ERY), and tetracycline (TET). Isolates showed 0% resistance to fluoroquinolones (results not shown). Values with the same superscript are not significantly different.*

the *Salmonella* isolates from both types of broilers were resistant to at least one antibiotic. Although 60% of the *Salmonella* isolates from the immersion-chilled samples and 33% of the isolates from the air-chilled samples were resistant to nalidixic acid (NAL), none of them were resistant to the related fluoroquinolones. Like the isolates from the immersion-chilled samples, all of the isolates from the air-chilled samples were resistant to erythromycin.

Ninety-five percent of the *Campylobacter* spp. isolates tested were resistant to one or more of the antibiotics used (Fig. 4). *Campylobacter* spp. isolates from the air-chilling facility were less resistant to NAL and to the other fluoroquinolones. All of the air-chilled *Campylobacter* isolates were sensitive to enrofloxacin. However, resistance to tetracycline was stronger for air-chilled samples than for their immersion-chilled counterparts.

DISCUSSION

This study compared microbiological levels and pathogen incidences for broilers after either immersion chilling or air chilling in federally inspected commercial facilities. While immersion chilling is the norm for poultry processing in the United States, other meat industries, such as the beef and swine industry, use air chilling to reduce carcass temperatures. Previous studies have reported that continuous immersion chilling has reduced total microbial counts by 60 to 98% *(6, 26, 30, 36, 52).* In contrast, other scientists have reported that immersion chilling was related to an increase in total microbial counts for carcasses *(6, 32, 52).* The immersion-chilling process is improved by chlorination of the water and the use of countercurrent tanks and air-scrubbing systems to reduce the buildup of microorganisms in the chiller, especially coliforms and generic *E. coli (6, 12, 36).*

Early studies conducted by Knoop et al. *(30)* and Thomson et al. *(52)* contradicted each other with respect to the relationship between the chilling method and the resulting microbial loads. Knoop et al. found that air chilling of carcasses resulted in lower bacterial counts and an increased shelf life relative to immersion chilling, whereas Thomson et al. found that immersion in a commercial immersion chiller resulted in lower bacterial loads for carcasses after chilling than did air chilling. Our data indicate that without regard to the original loads of bacteria in broilers entering the plant, there are few differences in levels of generic *E. coli* organisms, coliforms, and aerobes (collectively referred to as indicator organisms) on ready-to-market carcasses from air-chilling and immersion-chilling facilities. Perhaps the difference between our study and those conducted earlier is that our study was based on two commercial operating facilities, whereas the earlier studies had to model one or both chilling methods. Moreover, the mi-

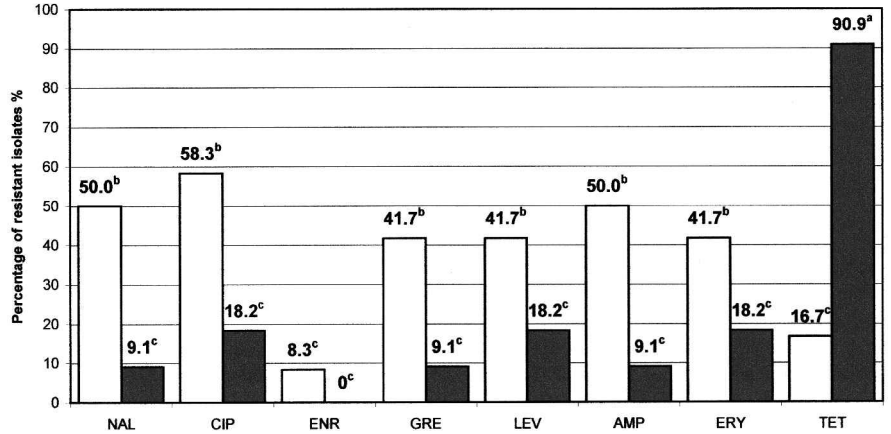

FIGURE 4. *Antimicrobial susceptibility profile of* Campylobacter *spp. isolates obtained from whole-carcass rinses of broilers processed in immersion-chilling (IC; □) and air-chilling (AC; ■) facilities. Isolates were tested by agar diffusion methods for resistance to nalidixic acid (NAL); the fluoroquinolones ciprofloxacin (CIP), enrofloxacin (ENR), grepafloxacin (GRE), and levofloxacin (LEV); ampicillin (AMP); erythromycin (ERY); and tetracycline (TET). Values with the same superscript are not significantly different.*

crobial counts and variability observed in our study are very similar to those presented in the national baseline *(56)* and other studies *(9, 13).* Because there was no established microbial baseline for air chilling in the United States, our study suggests that it may be acceptable to apply the current baseline data to the air-chilling facility with respect to the indicator organisms listed above.

In contrast to the results for indicator organisms, differences in the levels of psychrotrophic bacteria were detected between processing facilities. Because psychrotrophs account for most of the spoilage in poultry products, higher levels may have a negative impact on shelf life. The higher levels of psychrotrophic organisms observed in the immersion-chilled broilers in our study may be due to the selective conditions provided by the cold-water environment of the chiller. Other studies also reported higher levels of psychrotrophic organisms in immersion-chilled broilers than in air-chilled broilers *(6, 30).* Conversely, Thomson et al. *(52)* reported that psychrotrophs were more numerous on air-chilled broilers than on their immersion-chilled counterparts. Even though some bacteria may be removed during immersion chilling, psychrotrophs might be homogeneously distributed in the exterior pores of the skin and inside the carcass *(24).* Differences between our findings and those of others could be due to a number of factors, such as the cleanliness of the processing facilities, the manufacturing practices, the original loads of bacteria entering the plant from the farm, the equipment, and the general processing conditions.

Similar to our findings regarding psychrotrophs, data from our study indicate that the incidence of pathogens in ready-to-market immersion-chilled broilers was higher than that in their air-chilled counterparts. Our results suggest that there may be a greater chance of bacterial cross-contamination in the immersion tank from bird to bird or from contaminated water even when chlorine is present in the water. Organic matter rapidly inactivates chlorine, and organisms such as *Salmonella* spp. and *Campylobacter* spp. may be protected on the surface of the skin of poultry carcasses even if chlorinated water has been used *(28).* Several authors have suggested that immersion chilling is unhygienic, considering that pathogens such as *Salmonella* spp. and *C. jejuni,* which may be present in large numbers on relatively few carcasses before chilling, may be homogeneously distributed to other carcasses by cross-contamination with direct contact between broilers and the water used for chilling *(1, 6, 26).* Furthermore, in studies using marker organisms, it was found that even though the marker was inoculated in only a few carcasses before chilling, it was spread to other broilers after immersion-chilling *(6).* One report suggested that a buildup of microorganisms in the chiller tank occurs and that pathogenic bacteria may also be accumulated *(22).* In one study, *C. jejuni* was not isolated from samples of water obtained before processing but was almost always isolated from processing water after 5 h of continuous processing, indicating that this microorganism was introduced into the processing plant by the birds being processed *(1).* Moreover, Izat et al. *(25)* observed that *C. jejuni* was detected in the overflow from the carcass chill tank in all plants they tested, indicating that the microorganism contaminates and survives in the chiller water.

Although countercurrent water flow in the immersion-chilling tank appears to remove some of the microbial loads, perhaps it has a dilution effect on the actual levels of pathogenic organisms *(1, 38).* Oosterom et al. *(38)* observed that large numbers of *C. jejuni* were washed off of carcasses during immersion chilling. Despite the countercurrent water flow during immersion chilling, some pathogenic bacteria may attach firmly to the skin *(32),* and the mechanical force of the water may not be sufficient for their removal.

In contrast, with air-chilling systems it was observed that *C. jejuni* died in some instances, probably because of drying *(38).* During air chilling, air can be lethal to pathogens such as the microaerophillic *Campylobacter* spp. Furthermore, the dehydration effect on the surface of the skin may be detrimental to the survival of already-stressed organisms *(1).*

Cross-contamination can also occur during air chilling to a limited extent *(58).* Air chilling may reduce cross-contamination from one bird to another but may not remove pathogens already contaminating positive carcasses, and pathogen levels may remain constant even after the chilling process.

Salmonella spp. are usually found in small numbers on broiler carcasses exiting the processing plant, whereas *C. jejuni* is often found in large numbers *(6).* The usual incidence of *Salmonella* spp. in postchilled broilers ranges between 7 and 35% per carcass analyzed *(5, 26, 32, 56).* Our study indicated that levels of *Salmonella* spp. detected were in this range; however, more immersion-chilled carcasses than air-chilled carcasses tested positive. On the other hand, *Campylobacter* levels vary from 22.3 to 90% *(1, 25, 28, 38, 56).* In our study, the number of *Campylobacter*-positive samples was larger for the immersion-chilled broilers; however, the number of positive samples detected in the immersion-chilling and the air-chilling facilities was small compared with the national baseline *(56).* These differences may be attributed to the fact that the baseline is the result of the averaging of a representative number of processing facilities operating under different conditions. In our study, we tested only one facility for each process, and our results represent the pathogen loads in those facilities only.

Differences were also found between the profiles of *Salmonella* isolates obtained from immersion-chilled and air-chilled facilities. Both profiles differed from those presented in the NARMS 1999 report *(8).* The variability may be linked to the geographical area and the processing conditions in both facilities. NARMS results may be highly influenced by production in traditionally poultry-related states, accounting for the difference from our samples.

Campylobacter strains isolated from both processes were mainly *C. jejuni* strains, with very few *C. coli* strains, similar to results presented in the NARMS 1999 report *(8).* No significant differences were observed between chilling treatments.

In addition to examining the microbial profiles of the broilers, we evaluated the antibiotic resistance profiles of

the pathogen isolates. With regard to *Campylobacter* spp., we observed a stronger resistance to NAL for isolates from immersion-chilled samples than for isolates from air-chilled samples. Recent studies from several countries have reported that *Campylobacter* spp. are developing resistance to antibiotics. Isolates taken from humans *(8, 34)* and poultry *(34, 40, 43, 44, 46)* were resistant to NAL. These studies reported that large percentages of these isolates were resistant to structurally related fluoroquinolones *(46, 49, 54).* We observed similar patterns in our study regarding cross-resistance to ciprofloxacin.

Enrofloxacin is the veterinary equivalent to ciprofloxacin. Although enrofloxacin is not included in the NARMS screening for resistance, we determined resistance to enrofloxacin because it is the only veterinary fluoroquinolone approved for use in poultry *(15, 16).* Even though enrofloxacin is commonly used in animal agriculture, very few isolates from the carcasses we sampled were resistant to this drug. Moreover, *Campylobacter* isolates from immersion-chilled samples were most commonly resistant to ciprofloxacin, followed by NAL, while air-chilled isolates were most commonly resistant to tetracycline and then ciprofloxacin, erythromycin, and levofloxacin. Results for air-chilled isolates were similar to those presented in the NARMS report, in which resistances to tetracycline, NAL, and ciprofloxacin were the most common among clinical isolates *(8).*

Some susceptibility studies of *Campylobacter* spp. have compared resistance profiles throughout the years to address the effect of the introduction of fluoroquinolones into the poultry production process *(21, 34, 43, 44, 45, 46, 53, 54).* In addition, clinical isolates have been analyzed to determine the incidence of fluoroquinolone-resistant isolates in patients and the source of infection *(14, 18, 40, 43, 44).* In a number of countries, fluoroquinolone resistance rates for isolates from poultry products are similar to those for isolates from humans *(14).* However, other studies suggest that resistance profiles differ between veterinary isolates and human strains *(34).* To our knowledge, no study has reported differences in the antibiotic resistance profiles of veterinary *Campylobacter* spp. isolated from food animal processing environments having substantially different processing regimens.

As early as 1991, it was reported that the emergence of quinolone-resistant *Campylobacter* spp. coincided with the introduction of fluoroquinolones into veterinary medicine in The Netherlands *(14).* Similar results have been reported in Spain and Canada *(18, 40). Campylobacter* spp. have a natural ability for transformation, facilitating the uptake of naked DNA. Adaptation to stressful environments occurs quickly, and the mechanisms of acquisition need to be elucidated to reduce its emergence *(34, 51).* Since the link between the use of drugs in poultry production and the increased emergence of resistance to these drugs has been established *(14, 29),* the Food and Drug Administration is now collecting additional data to determine whether to limit or eliminate the use of these drugs *(16).*

Resistance to NAL was stronger for *Salmonella* isolates from immersion-chilled samples than for those from air-chilled samples. However, *Salmonella* isolates with positive resistance to NAL showed no resistance to the fluoroquinolones tested. These findings were similar to those of a study by Prats et al. *(40)* in which there was found to be no resistance to the fluoroquinolones among *Salmonella* spp. Another important finding was that all of the isolates were resistant to the macrolide erythromycin, commonly prescribed to treat foodborne infections *(14).* Concern has increased because of the isolation of multiresistant *Salmonella,* specifically, DT104 isolates showing resistance not only to the five antibiotics originally reported, but also to trimethoprim and ciprofloxacin *(53).* However, no fluoroquinolone-resistant *Salmonella* spp. were detected in our study.

The original microbial loads of the flocks entering the plant *(35, 39)* and the effects of the different chilling processes used may explain the differences observed for the incidence of pathogens and the antibiotic resistance capabilities between regimes. Immersion-chilled birds came from a wider range of farms in Missouri, whereas air-chilled birds were less diversely distributed. In addition, the heterogeneity of the strains present in the water tank may create a larger gene pool from which to draw. Low levels of antimicrobial agents, chlorine, and organic acids may reduce the numbers of susceptible organisms, allowing the prevalence of resistant strains *(3).* These stressing agents may activate mutational loci in several microorganisms, and the genetic mutations involved may increase resistance to these agents *(2, 3).* For example, the multiple-antibiotic-resistance *mar* operon present in *E. coli* and other pathogens provides increased resistance to antimicrobial agents including fluoroquinolones and oxidative stress agents including chlorine, sanitizers, and some organic acids *(2).* Hence, the use of antibiotics at the production level may not be the only factor contributing to the emergence of antibiotic-resistant strains.

Comparisons of ready-to-market poultry are important, but they do not reveal what is happening throughout the entire process. Differences between these processes warrant a closer look into the microbial profiles of carcasses from the farm through processing in air-chilling and immersion-chilling facilities. Ultimately, it is imperative to include farm management practices in the assessment of risk factors in the food chain. In addition, more in-depth investigations of antibiotic resistance capabilities, mechanisms for acquiring resistance, and genetic fingerprinting methods are needed to complement these findings.

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