Effect of Dry Air or Immersion Chilling on Recovery of Bacteria from Broiler Carcasses


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ABSTRACT

A study was conducted to investigate the effect of chilling method (air or immersion) on concentration and prevalence of Escherichia coli, coliforms, Campylobacter, and Salmonella recovered from broiler chicken carcasses. For each of four replications, 60 broilers were inoculated orally and intracloacally with 1 ml of a suspension containing Campylobacter at approximately $10^8$ cells per ml. After 1 day, broilers were inoculated with 1 ml of a suspension containing Salmonella at approximately $10^8$ cells per ml. Broilers were processed, and carcasses were cooled with dry air (3.5 m/s at -1.1°C for 150 min) or by immersion chilling in ice water (0.6°C for 50 min). Concentrations of E. coli, coliforms, Campylobacter, and Salmonella recovered from prechill carcasses averaged 3.5, 3.7, 3.4, and 1.4 log CFU/ml of rinse, respectively. Overall, both chilling methods significantly reduced bacterial concentrations on the carcasses, and no difference in concentrations of bacteria was observed between the two chilling methods ($P < 0.05$). Both chilling methods reduced E. coli and coliforms by 0.9 to 1.0 log CFU/ml. Air and immersion chilling reduced Campylobacter by 1.4 and 1.0 log CFU/ml and reduced Salmonella by 1.0 and 0.6 log CFU/ml, respectively. Chilling method had no effect on the prevalence of Campylobacter and Salmonella recovered from carcasses. These results demonstrate that air- and immersion-chilled carcasses without chemical intervention are microbiologically comparable, and a 90% reduction in concentrations of E. coli, coliforms, and Campylobacter can be obtained by chilling.

Increased consumption of poultry products has resulted in improvements in processing technologies, such as carcass chilling systems. Chilling systems typically use water, air, solid carbon dioxide, or liquid nitrogen as coolants (26, 51). Before 1978, immersion systems using water as a coolant were the most widely used chilling methods because of efficiency and low cost. However, these systems were criticized because immersion in a common bath was thought to promote cross-contamination. In the late 1970s, a ban on spin chilling or auger chilling was implemented in the European Union because of fear of cross-contamination from water absorption during immersion chilling (14, 49). This ban resulted in an extensive amount of research on immersion chilling and guidelines for chilling (overflow, counter-current, chlorine, and carcass-to-water ratio) (8, 26, 51). Research also focused on developing a suitable alternative for immersion chilling of poultry carcasses that would (i) overcome the objections of the European Economic Community ban; and (ii) satisfy the industry requirements for continuous product flow, efficient energy and water utilization with reasonable installation, and operation cost (49). Alternatives to the classical spin chiller are dry air or evaporative spray chilling, cryogenic chilling, or controlled continuous immersion chilling systems (49).

Air, immersion, and evaporative chilling systems are the three most common methods of cooling poultry used commercially (41). Several review articles on poultry chilling have been published (23, 26, 51, 53, 54). The main differences reported between the systems are water absorption levels and appearance of the end product. Systems may also differ in hygienic operation and cross-contamination, although scientific documentation of differences is limited.

Other researchers have reported that an immersion chilling system may reduce levels of Escherichia coli and coliforms recovered from carcasses by an average of 1.1 and 0.6 log units, respectively (6, 12, 15, 32, 33, 35). In most of the studies involving air chilling, no significant reductions in E. coli or coliforms on carcasses were reported (1, 15, 16, 32). For Campylobacter, lower numbers were found on carcasses after either immersion or air chilling, and average reductions of 1.2 and 0.8 log units, respectively, were found (6, 11, 25, 35, 37, 40, 44, 46). Although numbers were reduced after chilling, the overall prevalence of Campylobacter-positive carcasses after chilling was not affected by the method (air or immersion) of chilling (3, 5, 11, 22, 25, 30, 35, 39, 40, 44).

Studies have been conducted to evaluate the effect of chilling on numbers and prevalence of Salmonella. Both immersion and air chilling have a minimal effect on the prevalence of Salmonella on poultry carcasses (1, 15, 16). Other researchers have suggested that the washing effect during immersion chilling physically removes bacterial cells and thus reduces bacterial recovery, but this effect is likely offset by carcass cross-contamination (30, 39, 44, 50). Air chilling uses little (evaporative spray) or no water.
Cross-contamination can occur via aerosols but not to the same extent as via immersion chilling (3, 5, 13, 18, 31). Several authors have postulated that surface drying during air chilling reduces water activity, retards bacterial growth, and causes enough injury to pathogenic bacteria to reduce recovery (2, 37, 38, 42, 49).

In many of the publications that compare numbers of bacteria on chilled poultry carcasses, the effects of the chilling method are obscured by the initial (prechill) carcass bacterial load, chilling operation parameters, the use of antimicrobials, or differences in microbiological sampling techniques. The large number of variables has made it difficult to make a true comparison among chilling methods. The objective of this study was to determine the effect of chilling method (air and immersion) on *Escherichia coli*, *Campylobacter*, and *Salmonella* numbers and prevalence on broiler chicken carcasses.

**MATERIALS AND METHODS**

**Source of birds and inoculation.** For each of four experiments, 60 market-age (42 days) broiler chickens of mixed gender were obtained from the live bird holding area of a commercial processing plant. The birds were cooped, transported (about 15 min) to the Russell Research Center animal research facility, and placed on pine shavings in a pen (5 by 8 m) in a controlled-environment house. Birds were fed nonmedicated, corn- and soybean meal–based grower diet (3,200 kcal of metabolic energy per kg, 21% crude protein) for no more than 3 days until processing. On the same day they were transferred to the research facility, birds were challenged orally and intracloacally with 1 ml of a suspension containing approximately 10⁸ cells of *Campylobacter*. Twenty-four hours later, the same birds were given 1 ml of a suspension containing 10⁷ cells of *Salmonella* administered both orally and intracloacally. Feed was removed at least 4 h before inoculation and was replaced 4 h after inoculation. Broilers were processed at 44 to 45 days of age. The night before processing, feed but not water was removed for 8 to 10 h before cooping for a total feed withdrawal time of 10 to 12 h; water withdrawal time was 2 to 4 h. On the processing day, coated broilers were transported to the pilot processing plant where they were slaughtered and chilled.

*Salmonella* and *Campylobacter* cultures and inoculation. The inoculum cultures were prepared according to the procedure described by Bailey et al. (4). For *Salmonella*, three strains of nalidixic acid–resistant *Salmonella Typhimurium*, *Salmonella Montevideo*, and *Salmonella Enteritidis* were used to inoculate chickens. For preparation of the inoculum, the cultures were streaked onto brilliant green sulfa agar plates (BGS; Difco, Becton Dickinson, Sparks, Md.) containing 200 ppm of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.). Plates were incubated overnight at 37°C. A bacterial suspension was prepared in physiologic saline solution, and the optical density at 540 nm (Spec- tronic 20D+, Thermo Electron Corporation, Waltham, Mass.) was measured to determine the concentration of the inoculum. *Campylobacter jejuni* cultures were streaked on Campy-Cefex agar (47) and incubated at 42°C for 24 h under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂) in a GasPak jar with an activated CampyPak (BBL, Becton Dickinson) for 24 h, and the bacterial suspension was prepared as described above for *Salmo- nella*. Unless stated otherwise, these same plating procedures were used for recovery of *Salmonella* and *Campylobacter* from the carcass rinses.

**Processing.** For each of the four replications, 50 broiler chickens were stunned using a commercial stunner (model SF-7001, Simmons Engineering Co., Dallas, Ga.) that was set to deliver 16-V pulsed direct current at approximately 500 Hz for 18 s, followed by 16 V at 60 Hz alternating current for 9 s. Stunned birds were manually killed by cutting both the carotid artery and jugular vein on one side of the neck (unilateral cut). Birds were allowed to bleed for 120 s, scalped (model SS300CF, Cantrell Machine Co., Inc., Gainesville, Ga.) at 49.5°F (9.7°C) for 120 s, and defeathered with an in-line picker (model CPF-60, Cantrell Machine Co.) for 30 s. After picking, heads and feet were removed manually, and the carcasses were manually eviscerated and washed. Sixteen carcasses were randomly selected and identified by wing tags.

**Chilling treatments and microbiological sampling.** For the microbiological analyses, the same carcass was sampled by the whole carcass rinse (WCR) technique before and after chilling. Pre- and postchill carcasses were subjected to a low-volume WCR procedure (27). Carcasses were placed in a bag with 100 ml of 0.1% peptone solution and placed in an automated carcass shaking machine for 1 min. After shaking, carcasses were removed aseptically, and rinse samples were collected for bacterial culture. For the prechill carcasses, rinses in sterile cups were stored on ice for less than 45 min before microbiological analyses. After prechill WCR collection, carcasses were randomly assigned to either the immersion or air chilling treatment.

Twenty-four additional carcasses were added to the immersion chiller to obtain commercial chiller volumes, and 10 carcasses were added to the air chiller. Immersion-chilled carcasses were submerged in 133 liters of a mixture of ice and potable water (approximately 0.6°C) in a prototype tumble chiller. The paddles in the tumble chiller were operated at approximately 2 rpm for the duration of the 50-min chilling period. Air-chilled carcasses were cooled for 150 min with air temperature less than 0°C. The air in the chill room was distributed directly into the abdominal cavity of each carcass with flexible channels modified to provide a continuous flow of cold air (3.5 m/s) to each carcass.

For both treatments, the internal temperature of the breast of designated carcasses was continuously monitored with a recorder (temperature record system, COX Technologies Company, Belmont, N.C.). After immersion chilling, carcasses were hung in shackles and allowed to drip for 5 min before the postchill WCR sample was collected. WCR samples were collected from air-chilled carcasses immediately after the 150 min chilling period.

**Skin samples.** During the last two replications, a subset of four carcasses from each treatment was randomly selected after the postchill WCR. Approximately 20 g of breast skin was aseptically removed from each carcass, placed into a sterile sampling bag (Whirl-Pak sampling bags, Nasco, Fort Atkinson, Wis.), and stored on ice for less than 2 h before analysis.

**Microbiological analyses.** Serial dilutions of the WCR diluent were made in 0.1% peptone. *E. coli* and coliforms were enumerated by transferring 1 ml from serial dilutions onto Petrifilm *E. coli* /coliform count plates (3M Health Care, St. Paul, Minn.), which were incubated at 35°C for 24 h. Blue colonies with entrapped gas were counted as *E. coli*, and all blue and red colonies with entrapped gas were counted as coliforms.

For *Campylobacter* enumeration, 0.1 ml from each of a series of 1:10 dilutions was plated onto Campy-Cefex agar, and plates were incubated at 42°C for 48 to 72 h in a microaerophilic atmosphere. Presumptive *Campylobacter* colonies were examined microscopically for typical motility and cell morphology, and col-
TABLE 1. Concentrations of E. coli, coliforms, Campylobacter, and Salmonella recovered from broiler carcasses before and after either air or immersion chilling

<table>
<thead>
<tr>
<th>Treatment</th>
<th>E. coli (log CFU/ml)</th>
<th>Campylobacter (log CFU/ml)</th>
<th>Salmonella (log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Air (n = 32)</td>
<td>Immersion (n = 32)</td>
<td>Air (n = 32)</td>
</tr>
<tr>
<td>Prechilling</td>
<td>3.4 ± 0.1 x</td>
<td>3.5 ± 0.1 x</td>
<td>3.8 ± 0.1 x</td>
</tr>
<tr>
<td>Postchilling</td>
<td>2.4 ± 0.1 y</td>
<td>2.6 ± 0.1 y</td>
<td>2.8 ± 0.1 y</td>
</tr>
<tr>
<td>Reduction</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Within each bacterial group, means for air and immersion chilling without common letters x or y are significantly different (P < 0.05). Within each column and each bacterial group, means without common letters A or B are significantly different (P < 0.05).

The mean concentrations of E. coli, coliforms, Campylobacter, and Salmonella recovered from broiler carcasses before and after air or immersion chilling are given in Table 1. Before chilling, mean concentrations of E. coli, coliforms, Campylobacter, and Salmonella recovered from carcasses were 3.5, 3.7, 3.4, and 1.4 log CFU/ml, respectively. No difference was observed in the initial bacterial load (prechill) for carcasses (P > 0.05), indicating comparable bacterial concentrations before treatment. No significant differences were observed for concentrations of E. coli, coliforms, and Salmonella recovered from air- or immersion-chilled carcasses. However, a slight but significant difference was found for Campylobacter (P < 0.05), with lower concentrations on air-chilled carcasses than on immersion-chilled carcasses.

Overall, both chilling methods reduced the amount of bacteria recovered from carcasses, and the level of reduction was similar for both air- and immersion-chilled carcasses (P < 0.05). Sampling the same carcass before and after chilling provided a more sensitive comparison than simply analyzing the postchill bacterial concentrations for the two treatments. This comparison eliminated preexisting differences among individual carcasses and allowed a better comparison of chilling methods. Air and immersion chilling reduced the concentrations of E. coli and coliforms recovered from carcasses by 0.9 to 1.0 log units. A slightly higher reduction in Campylobacter was observed after air chilling (1.4 log CFU/ml) than after immersion chilling (1.0 log CFU/ml), but this difference was not significant. For Salmonella, reductions of 1.0 and 0.6 log CFU/ml were observed after air and immersion chilling, respectively, and the difference between chilling methods was not significant (Table 1).

The data from immersion chilling agree with previously reported findings (6, 11, 12, 15, 25, 32, 33, 35, 37, 40, 44, 46), but the significant bacterial reduction during air chilling was unexpected. Most of the published bacterial recovery data for air chilling are reported as cells per gram of macerated breast or neck skin (1, 15, 20, 32), whereas most data for immersion chilling are reported as CFU per milliliter of rinse obtained with the WCR technique. Allen et al. (2) conducted an air-chilling experiment in a controlled environment and reported that dry chilling did not affect the concentrations of bacteria recovered from breast skin but did effectively reduce the concentrations recovered from the body cavity. These authors attributed this finding to a more severe drying of the body cavity; however, they could also be related to surface differences between the skin and the cavity. Thomas and McMeekin (48) reported that the carcass skin surface before evisceration and chilling is covered by a liquid film, which consists of serum proteins, amino acids, and other suspended soluble compounds that originate from the underlying skin tissues or from processing water. Bacteria within such liquid films may be protected from cleaning and disinfection. Smith et al. (45) evaluated the microbiological characteristics of poultry car-
casses contaminated internally (carcass cavity) or externally (breast skin) with fecal material (1 g of cecal contents) and then washed in a commercial inside-outside bird washer. These authors found that after washing, carcasses with internal contamination had lower concentrations of E. coli (4.2 versus 4.9 log CFU/ml), coliforms (4.5 vs. 5.0 log CFU/ml), and Campylobacter (2.6 vs. 3.6 log CFU/ml) than did carcasses with external contamination. Lillard (29) evaluated carcasses after consecutive rinses of the skin and body cavity and suggested that bacteria present on the skin or inside the cavity detach in a similar manner. More information is required to evaluate the susceptibility of bacteria in the skin surface or inside the carcass cavity to processing procedures.

The research by Allen and coworkers (2) also may explain the difference between the results for air chilling in the present study. The WCR technique is more likely to detect bacterial reductions for the total carcass, whereas the skin maceration technique is effective for detecting changes on the sampled surface only. Gill and Badoni (19) reported that evaporative chilling reduced bacterial numbers for E. coli and coliforms by 0.42 and 0.11 log CFU/cm², respectively, when they used a skin excision technique, and by 0.73 and 0.32 log CFU/ml, respectively, when they used the WCR technique. Fluckey et al. (16) used the WCR sampling technique and reported that air chilling significantly reduced the numbers of coliforms and E. coli by 0.7 and 0.8 log units, respectively.

Chilling by air or immersion had no effect on the prevalence of Campylobacter-positive carcasses (100% both pre- and postchill). Based on direct enumeration, 31 of 64 prechill carcasses were positive for Salmonella. No significant difference was found between the prechill (18 of 32 carcasses for air versus 13 of 32 carcasses for immersion) and postchill (8 of 32 carcasses for air versus 7 of 32 carcasses for immersion) prevalence of Salmonella on air- and immersion-chilled carcasses. A significant reduction in prevalence from 56% prechill (18 of 32 carcasses) to 25% postchill (8 of 32 carcasses) was found after air chilling (P < 0.05). The prevalence of Salmonella-positive carcasses for pre- and postimmersion chilling was 40% (13 of 32 carcasses) and 22% (7 of 32 carcasses), respectively. A similar trend in Salmonella prevalence was reported by Northcutt et al. (35) for immersion chilling: 56% of carcasses (40 of 72) were positive for Salmonella before chilling, but only 15% (11 of 72) were positive after. The use of a preenrichment step in the present study increased Salmonella recovery to 100% before and after chilling.

Preenrichment of microbial samples allows recovery of bacteria that are injured by physical stressors such as extremes of temperature or dryness, resulting in a progressive loss of culturability on selective media even though the bacterial cells remain metabolically active (9, 55). The bacterial cell envelope is a common site of injury from chilling, freezing, or heating. A variety of changes can occur in the outer cell membrane after the injury, including morphological and structural changes, blebs and vesiculation, or damage or release of lipopolysaccharides (7). These changes can alter membrane permeability, causing the output flow of periplasmic enzymes and sensitivity to hydrophobic compounds, dyes, or surfactants. Such injury can be quickly repaired during enrichment, and the bacteria can recover viability (7, 52). Low numbers of bacteria (below the detection level) and the temporary loss of culturability of injured cells may explain why Salmonella enumeration was significantly reduced after chilling based on direct enumeration but no change in prevalence was observed when the preenrichment step was used.

Smith et al. (44) evaluated Salmonella prevalence and cross-contamination during immersion chilling in a pilot scale chiller. Split carcass halves were used, one half of each pair as the control and the other half as the treatment. These researchers found a 58% reduction in the number of Salmonella-positive carcass halves that were directly contaminated (24 of 24 reduced to 10 of 24) but an increase from 0 to 25% in the number of Salmonella-positive halves exposed to cross-contamination. This finding may explain contradictory results reported by other authors, where Salmonella prevalence increased after immersion chiller (17, 24, 28, 50) or did not change at all (10, 11, 24, 34).

Only a few researchers have addressed the effect of air chilling on Salmonella. Some authors have reported that air chilling of poultry had no effect in numbers or prevalence of Salmonella (1, 15, 16). Sanchez et al. (42) reported lower prevalence of Salmonella and Campylobacter on carcasses in a commercial air-chilling system compared with an immersion chilling system, but they did not include prechill Salmonella prevalence. In addition, these researchers compared broilers from different farms and geographical locations, which would likely vary in initial microbial contamination.

In the present study, air-chilled carcasses were dry and had apparently shrunken skin, which could have an effect on the recovery of bacteria after chilling. To test this hypothesis, subsets of carcasses were randomly selected and the concentrations from the same carcasses were compared using both the WCR and breast skin maceration techniques. The results for recovery of E. coli, coliforms, and Campylobacter from these carcasses are presented in Table 2. When skin maceration was used, chilling method had no significant effect on the recovery of E. coli, coliforms, and Campylobacter from these carcasses are presented in Table 2. When skin maceration was used, chilling method had no significant effect on the recovery of E. coli, coliforms, and Campylobacter from these carcasses are presented in Table 2.

Preenrichment step in the present study increased Salmonella recovery to 100% before and after chilling.
TABLE 2. Concentrations of E. coli, coliforms, and Campylobacter recovered from carcasses after air or immersion chilling using whole carcass rinse or breast skin maceration sampling method

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>Air chilling</th>
<th>Immersion chilling</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (mean ± SEM, n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole carcass rinse (log CFU/ml)</td>
<td>2.5 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>0.2932</td>
</tr>
<tr>
<td>Breast skin maceration (log CFU/g)</td>
<td>3.0 ± 0.3</td>
<td>2.7 ± 0.1</td>
<td>0.4001</td>
</tr>
<tr>
<td>P&lt;sup&gt;b&lt;/sup&gt; = 0.0761</td>
<td>0.8835</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliforms (mean ± SEM, n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>While carcass rinse (log CFU/ml)</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>0.6685</td>
</tr>
<tr>
<td>Breast skin maceration (log CFU/g)</td>
<td>3.1 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>0.6933</td>
</tr>
<tr>
<td>P&lt;sup&gt;b&lt;/sup&gt; = 0.1743</td>
<td>0.4777</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter (mean ± SEM, n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole carcass rinse (log CFU/ml)</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>0.9141</td>
</tr>
<tr>
<td>Breast skin maceration (log CFU/g)</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 0.1</td>
<td>0.9338</td>
</tr>
<tr>
<td>P&lt;sup&gt;b&lt;/sup&gt; = 0.3082</td>
<td>0.2098</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> ANOVA for the difference between the chilling methods.

<sup>b</sup> Paired t test of the difference between sampling methods (skin maceration and whole carcass rinse).

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The primary chilling of poultry carcasses—a review. Int. J. Refrig. 29:847–862.


