Research Note

Cetylpyridinium Chloride (CPC) Treatment on Poultry Skin
To Reduce Attached Salmonella

JEONG-WEON KIM and MICHAEL F. SLAVIK*

Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA

(MS# 95-140: Received 22 June 1995/Accepted 19 September 1995)

ABSTRACT

Cetylpyridinium chloride (1-hexadecylpyridinium chloride, CPC) was evaluated for its effectiveness in removing or killing salmonellae attached to poultry skin. Two different treatment methods were used: (i) spraying 0.1% CPC solution at 15°C or 50°C against inoculated skin surface for 1 min at 138 kPa, and (ii) immersing inoculated skin surface in 0.1% CPC solution at room temperature for either 1 min, 1 min plus 2 min holding without CPC, or 3 min. After rinsing, cells on the skins were enumerated by conventional plating as well as direct counting from scanning electron microscopy (SEM). Compared with controls, CPC spraying reduced the numbers of salmonellae by 0.9 to 1.7 log units (87 to 98%) assayed by the plating method (P < 0.05). SEM gave results similar to plating. Generally 50°C CPC spraying showed greater reduction than 15°C CPC spraying; however, the differences were not always significant. Water spraying at either temperature did not show any reduction compared to nonsprayed skins. In the immersion test, significant differences also were noticed among the control and the three other CPC-immersed groups (P < 0.05) as assayed by plating, ranging from 1.0 to 1.6 log units, which were similar to the CPC spraying results. However, no difference was noticed among the three CPC-immersed groups. Direct counting from SEM was not a suitable method for recovering cells in CPC immersion tests because dead cells were still attached to the skin while retaining their intact morphology. On the basis of the amount of CPC used, immersion appears to be more cost-effective than spraying CPC on poultry skin.

Key words: Cetylpyridinium chloride (CPC), Salmonella typhimurium, poultry skin

Cetylpyridinium chloride (CPC, 1-hexadecylpyridinium chloride) is a quaternary ammonium compound contained in certain commercial mouthwashes to prevent dental plaque (1, 2, 3, 5). As a cationic surfactant, the mechanism by which CPC kills bacteria involves the interaction of basic cetylpyridinium ions with the acid groups of bacteria to form weakly ionized compounds that subsequently inhibit bacterial metabolism (7). Preliminary tests in our laboratory showed more than a 6-log-unit reduction in salmonellae cells suspended in 10 ppm CPC solution for 1 min; therefore, CPC appeared to be a potential compound for use in controlling microbial pathogens during poultry processing.

Because there has never been a report on using CPC to reduce salmonellae on poultry skin, the purpose of this study was to evaluate CPC as a means of lowering the number of salmonellae on chicken skins during poultry processing.

MATERIALS AND METHODS

Salmonella typhimurium inoculum

Salmonella typhimurium (ATCC 14028) which had been maintained on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI) slants at 4°C was cultured in BHI broth at 37°C for 18 to 20 h and transferred every day for 2 to 3 days. Cells were centrifuged at 3,000 × g for 10 min at 4°C and resuspended in phosphate-buffered saline (PBS, pH 7.1). Cell density was adjusted to 1 to 2 × 10⁸ colony-forming units (CFU)/ml for inoculation.

Poultry skin preparation and inoculation

Six to ten prechilled chicken carcases were obtained from a processing plant in the morning of each trial day. Breast skins were cut from the carcasses and placed in skin holders designed in our lab (Figs. 1 and 2). The areas of exposed skin surface were 38 cm² and 10 cm² for spraying and immersion tests, respectively. Each skin piece was inoculated with S. typhimurium by gently dropping inoculum (0.1 ml/cm² of skin) on the skin surface using a micropipette, incubated at room temperature for 30 min, and rinsed with sterile distilled water (1.0 ml/cm² of skin) to remove loosely attached or unattached cells. Part of the inoculated skin samples were stomached and plated to determine the number of cells that were attached to skin before spraying or immersion treatments.

CPC spraying test

Each skin holder containing 3 pieces of inoculated skin (Fig. 1) was hung vertically and sprayed with 0.1% (wt/vol) of CPC solution (Sigma Chemical Co., St. Louis, MO) at 15°C or 50°C at 138 kPa for 1 min at a right angle. A flat cone nozzle with an 0.31-cm diameter was used for spraying and the distance between nozzles and skin samples was 38 cm. The whole holder was rinsed twice to remove residual CPC on each skin by gentle agitation in two buckets containing 20 1 each of tap water. Control skins were

* Author for correspondence. Tel: 501-575-4387; Fax: 501-575-3026.
CPC SPRAYING OR IMMERSION OF CHICKEN SKINS

(a)
*
*
38sq.cm
(b)
(c)
FIGURE 1. Skin holder used for spraying water or CPC solution onto chicken skin surface. (a) Flat bottom plate and the top plate having 5 holes, 38 cm² each. (b) Skin pieces were placed on the bottom plate. (c) Top plate was placed on top of the bottom plate and tightened. The inoculum was added in each well. The holder was hung vertically during spraying.

treated in the same way except plain water at 15 or 50°C was sprayed on instead of CPC solution. Spray-treated skin areas were aseptically cut out and used for enumerating salmonellae cells by microbiological plating and scanning electron microscopy. The whole experiment was repeated.

CPC immersion trial

Skin samples were divided into four groups (3 samples per group) and exposed to CPC solution for different periods. A 2.5-ml aliquot of 0.1% CPC solution was added in each well of the skin holder to cover the inoculated skin surfaces (10 cm², Fig. 2), and the skins were either (i) incubated for 1 min; the CPC was removed and the skin rinsed with 5 ml of water immediately; (ii) incubated for 1 min; the skin was held for 2 min before rinsing; or (iii) incubated for 3 min; the CPC was removed and the skin rinsed. For the control group, 2.5 ml of water was added instead of CPC solution and the skin was incubated for 3 min before rinsing. The purpose of second group was to observe the effect of residual CPC. Skins exposed to CPC were cut out and used for enumerating salmonellae cells by microbiological plating and scanning electron microscopy. The whole experiment was repeated.

Enumeration of salmonellae by microbiological plating

Skin samples treated with CPC solutions were placed in Whirl-Pak® bags (Fort Atkinson, WI) containing buffered peptone water (BPW) and stomached for 1 min using a Stomacher 400 Lab Blender (Seward Medical, London, UK). Sample solutions were serially diluted with 0.1% peptone water and surface plated on xylose lysine desoxycholate (XLD) (Difco) agar. Plates were incubated at 37°C for 1 day and counted as CFU/cm² of skin. To recover injured cells, samples were also plated on tryptic soy agar (TSA) (Difco); however, there were no statistical differences from XLD counts.

Direct counting by scanning electron microscopy (SEM)

Skin samples were fixed immediately after CPC treatments by immersing in Karnovsky’s fixative. They were dehydrated with graded ethanols (30, 50, 70, 80, and 95% and 3 changes of 100%) for 15 min in each bath and washed with hexamethyldisilazane (Electron Microscopy Sciences, Fort Washington, PA) three times for 5 min each. Samples were mounted on aluminum stubs and sputter-coated with gold and viewed by scanning electron microscope (ISI-60, International Scientific Instruments, Japan) at 30 kV. Attached cells were directly counted from the SEM screen at 5,000×. At least 20 randomly selected areas were counted per sample and the counts were converted to counts per cm² of skin.

Morphology of S. typhimurium in CPC solution

To examine the morphological changes of S. typhimurium in CPC solution, harvested cells were suspended in 0.1% CPC solution (10⁷ CFU/ml) for 1, 3, and 5 min, and deposited on membrane filters (Millipore type GS, 0.22-μm pores). The membranes were fixed and processed for SEM as described above. Also, suspended cells in each group were immediately diluted with BPW and plated on TSA to enumerate live cells.

Statistical analysis

The cell counts were all converted to log counts per cm² of skin and analyzed using an analysis of variance (ANOVA) and Fisher’s least significance difference (LSD) procedures to find the significance of difference among different CPC treatment groups.
RESULTS AND DISCUSSION

Compared with water-sprayed control skins, CPC-sprayed skins showed significantly lower counts of salmonellae cells ($P < 0.05$) at both temperatures, ranging from 0.9 to 1.7 log units (87 to 98% reduction) by the plating method (Table 1). SEM gave results similar to those from plating. Generally 50°C CPC spraying showed a greater reduction

TABLE 1. Log counts of salmonellae cells per cm$^2$ of chicken skin after the skins were sprayed or immersed in 0.1% CPC solution or water

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Condition</th>
<th>Trial 1</th>
<th>Plating</th>
<th>SEM</th>
<th>Trial 2</th>
<th>Plating</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spraying</td>
<td>Water 15°C</td>
<td>6.52 ± 0.52A$^a$</td>
<td>6.65 ± 0.18A</td>
<td></td>
<td>6.22 ± 0.16A</td>
<td>6.81 ± 0.12A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water 50°C</td>
<td>6.91 ± 0.36A</td>
<td>6.62 ± 0.17A</td>
<td></td>
<td>6.03 ± 0.16A</td>
<td>6.61 ± 0.15b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15°C</td>
<td>5.65 ± 0.69b</td>
<td>5.64 ± 0.34b</td>
<td></td>
<td>5.32 ± 0.07b</td>
<td>5.91 ± 1.04c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 50°C</td>
<td>5.21 ± 0.20b</td>
<td>5.04 ± 0.34c</td>
<td></td>
<td>4.95 ± 0.02c</td>
<td>5.91 ± 0.54c</td>
<td></td>
</tr>
<tr>
<td>Immersion</td>
<td>Water 3 min</td>
<td>6.88 ± 0.42A</td>
<td>6.99 ± 0.09A</td>
<td></td>
<td>7.39 ± 0.36A</td>
<td>NA$^c$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 1 min</td>
<td>5.60 ± 0.26b</td>
<td>7.01 ± 0.14A</td>
<td></td>
<td>6.42 ± 0.05B</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 1 + 2 min$^b$</td>
<td>5.72 ± 0.32B</td>
<td>6.93 ± 0.20A</td>
<td></td>
<td>6.21 ± 0.278C</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 3 min</td>
<td>5.36 ± 0.38B</td>
<td>6.98 ± 0.17 A</td>
<td></td>
<td>5.81 ± 0.23C</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values followed by different letters are significantly different in each treatment column ($P < 0.05$).

$^b$ After immersing for 1 min, CPC solution was removed from the skin; after waiting 2 min, the skin was rinsing with water.

$^c$ NA, not applicable. Because no difference was discernable by visual observation among the four groups, no enumeration was performed.
FIGURE 3. Scanning electron micrographs of Salmonella typhimurium cells suspended in (a) physiological saline and (b) 0.1% CPC solution for 5 min. Note the indentations on the cells.
than 15°C CPC spraying; however, the differences were not always significant. There were no differences between 15°C and 50°C water spraying. Interestingly, no difference was noticed between water-sprayed and nonsprayed skins (data not shown), suggesting that spraying water at 138 kPa did not have any influence on cells attached to the skin.

In immersion tests, significant differences \( (P < 0.05) \) in bacterial numbers were also found between the control and the three other CPC-immersed skin groups by the plating method, ranging from 1.0 to 1.6 log units (90 to 97.5% reduction) which is similar to the reduction level achieved by CPC spraying (Table 1). Although the differences were not always significant, longer immersion times showed a greater reduction in bacterial numbers than short exposures. The bactericidal effect of residual CPC was not clear in this study. Because separate studies in our lab had indicated the significance of retaining a CPC film on the skin surface before rinsing, a group of skin samples in this study was immersed in CPC solution for 1 min and held for 2 min without CPC before rinsing. However, no specific effect was observed compared with 3 min of immersion, probably due to the small volume of CPC solution applied to a unit area of skin (0.25 ml/cm² of skin) and the comparatively high number of cells attached to the skins (6 to 7 log cell number per cm²).

One interesting observation in the immersion tests was that no difference could be observed among the four groups by SEM (Table 1). Obviously, dead cells still remained attached to the skin surface after immersion in CPC solution and rinsing with water. Because it is difficult to differentiate live and dead cells in the screen of the SEM unless the dead cells have lost their intact morphology, direct enumeration of attached cells from SEM was not a suitable method for comparing the effects of CPC in the immersion tests. However, the SEM method showed that CPC does not detach cells from the chicken skin. Moreover, CPC was reported to enhance microbial adhesion to hydrophobic surfaces by diminishing surface charge and increasing cell surface hydrophobicity (6). The reason the SEM method showed a reduction in spraying trials might be that the spraying treatment supplied an excess of wetting agent (CPC) which replaced emulsified bacteria and consequently cleaned the skin surface (8).

The above observation led us to determine whether CPC causes any morphological damage to salmonellae cells in suspension. Compared with control cells suspended in physiological saline, cells suspended in CPC solution for 5 min showed minor indentations on the surface; however, cells suspended for 1 min or 3 min were difficult to distinguish from control cells (Fig. 3). This observation indicated that CPC may kill salmonellae cells by causing metabolic damage which has little effect on morphological structure.

Even though preliminary data in our laboratory showed that suspending salmonellae cells in 10 ppm (0.001%) CPC solution resulted in more than a 6-log-unit reduction in 1 min, once the cells are attached to poultry skin, the effectiveness dropped to less than 2 log units, although 100 times higher CPC concentration (0.1%) was used in this study. Since the immersion treatment used approximately 15 times less CPC solution than did spraying per unit area of skin, but still produced similar reductions, ranging from 1.0 to 1.7 log units, immersion appeared to be a more cost-effective method of reducing the number of bacteria on chicken skin.

Although the experimental conditions such as chemical concentration, contact time, and temperature were different among various chemical treatment studies on poultry, CPC appeared to be better than other treatments, because CPC produced a similar or greater reduction at a much lower concentration (0.1%) compared with some organic acids or trisodium phosphate used at the 1 to 10% level (4, 8). In addition, CPC did not cause any deleterious effects such as discoloration of the chicken skin or a chemical odor, which can be caused by most organic acids or other chemical treatments (9, 10). For further research, it is necessary to determine an optimum CPC treatment method for effective reduction of bacteria attached to poultry skin and future application of CPC in poultry processing.

ACKNOWLEDGMENTS

This research has been funded by Food Safety Grant from U.S. Department of Agriculture.

REFERENCES

ELISA FOR 3-ACETYLDEOXYNIVALENOL IN BARLEY

533


Erratum

The name and affiliation of YANBIN LI was inadvertently omitted from the article Cetylpyridinium Chloride (CPC) Treatment on Poultry Skin To Reduce Attached Salmonella. YANBIN LI, Department of Biological and Agricultural Engineering, University of Arkansas, should have been included with JEONG-WEON KIM and MICHAEL F. SLAVIK, Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA.