Journal of Food Protection, Vol. 63, No. 5, 2000, Pages 593–600

Antimicrobial Activity of Cetylpyridinium Chloride Washes against Pathogenic Bacteria on Beef Surfaces†

CATHERINE N. CUTTER,[1]* WARREN J. DORSA,[1]‡ ANDRONICA HANDIE,[2] SERGIO RODRIGUEZ-MORALES,[2] XIANG ZHOU,[2] PHILIP J. BREEN,[2] AND CESAR M. COMPADRE[2]

[1]U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, Nebraska 68933; and [2]Food Safety Group, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, Arkansas 72205, USA

MS 99-307: Received 13 October 1999/Accepted 17 December 1999

ABSTRACT

Cetylpyridinium chloride (CPC), a water-soluble, neutral pH, colorless compound, is widely used in oral hygiene products to inhibit bacteria responsible for plaque. Previously, researchers have demonstrated that CPC not only reduces *Salmonella* Typhimurium on poultry but also prevents cross-contamination. To determine the effectiveness of CPC against pathogens associated with lean and adipose beef surfaces, several spray-washing experiments (862 kPa, 15 s, 35°C) with 1% (wt/vol) CPC were conducted. On lean beef surfaces, CPC immediately reduced 5 to 6 $\log_{10}$ CFU/cm^2 of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium to virtually undetectable levels (0 $\log_{10}$ CFU/cm^2), as well as after 35 days of refrigerated (4°C), vacuum-packaged storage. On adipose beef surfaces, 5 $\log_{10}$ CFU/cm^2 *Salmonella* Typhimurium and *E. coli* O157:H7 were reduced immediately (>2.5 $\log_{10}$ CFU/cm^2) with 1% CPC; by day 35 the reduction was <1.3 $\log_{10}$ CFU/cm^2. Further plate overlay analyses indicated that the effectiveness of CPC against pathogens on adipose surfaces was not hampered by the presence of meat components or fatty acids. Additional chemical and microbiological analyses of 1% CPC-treated beef surfaces subjected to a secondary water wash (following contact times of 0, 5, 10, 15, or 30 min) or grinding did reduce pathogenic bacteria and CPC levels. However, residual CPC levels following any of the treatments were considered excessive for human consumption. Despite the residual levels, this study is the first to demonstrate the effect of CPC on pathogenic bacteria associated with beef surfaces immediately after treatment and also after long-term, refrigerated, vacuum-packaged storage.

The quaternary ammonium compound cetylpyridinium chloride (CPC) is a water-soluble, colorless compound that has been used for over 50 years in oral hygiene products including toothpaste, throat lozenges, and mouthwashes *(18)*. Research in oral bacteriology has indicated that 0.05 to 0.5% CPC found in mouthwashes reduces or inhibits bacterial gingivitis, biofilm, or plaque formation *(2, 8, 15, 19, 23)*. Because of its low surface tension, hydrophilic, and lipophilic properties, CPC works well in wetting and penetrating tissue *(12)*.

In general, quaternary ammonium compounds absorb into the bacterial cell surface, permeate and destroy the cell wall and cell membrane, and have a direct or indirect lethal effect on the cell. However, specific types of quaternary ammonium compounds may have different types of mechanisms *(17)*. In the specific case of CPC and its closely related analogues, it has been shown that they interact strongly with negatively charged surfaces, e.g., blastospores of *Candida albicans (20)*, and that their antibacterial activity is related to their hydrophobicity *(14, 16)*. Electron microscopy has shown that CPC analogues damage the bacterial membrane and produce leakage of cellular materials *(16)*. Apparently the degree of damage to the bacterial membrane is time and concentration dependent *(13)*.

In a series of published studies, it has been demonstrated that CPC is efficacious for reducing populations of *Salmonella* spp. on poultry carcasses *(3, 13, 22, 24)*. In 1996, Kim and Slavik *(13)* demonstrated a 1.7-$\log_{10}$ reduction of *Salmonella* Typhimurium after immersion of poultry in 0.1% CPC. In another study, a 30-s spray with 0.1% CPC on chicken skins reduced *Salmonella* Typhimurium up to 2.5 $\log_{10}$ *(22)*. In an experiment designed to investigate concentration and contact time, it was demonstrated that 0.4% CPC applied for 3 min exhibited a 4.9-$\log_{10}$ reduction of viable *Salmonella* Typhimurium. In this same study, CPC was effective in preventing bacterial contamination when 0.8% CPC was applied to poultry surfaces for 10 min and rinsed *(3)*. When compared to other antimicrobials, CPC affected a 1.9-$\log_{10}$ reduction of *Salmonella* spp. on chicken skins that was comparable to reductions associated with 10% trisodium phosphate (2.2 $\log_{10}$) or 2% lactic acid (2.2 $\log_{10}$) *(24)*. These studies clearly illustrate that CPC is an effective antimicrobial for reducing foodborne *Salmonella* spp. on poultry, as well as preventing cross-contamination.

To our knowledge, there is no published information

* Author for correspondence. Present address: Department of Food Science, 111 Borland Laboratory, The Pennsylvania State University, University Park, PA 16802. Tel: 814-865-8862; Fax: 814-863-6132; E-mail: cnc3@psu.edu.

† Names are necessary to report factually on available data; however the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

‡ Present address: John Morrell and Company, Inc., 805 E. Kemper Road, Springdale, OH 45246.

pertaining to the use of CPC for reducing foodborne bacteria on lean or adipose beef surfaces. We present a study to address the immediate and long-term effects of CPC against two bacterial pathogens attached to beef surfaces and to analyze lean, adipose, and ground beef samples for residual CPC.

MATERIALS AND METHODS

Bacterial cultures and feces. A streptomycin-resistant strain of *Escherichia coli* O157:H7 and a nalidixic acid resistant strain of *Salmonella* Typhimurium were obtained from the Roman H. Hruska U.S. Meat Animal Research Center (MARC) culture collection and maintained in 75% glycerol at −20°C *(7)*. *E. coli* O157:H7 and *Salmonella* Typhimurium were propagated for 18 h in trypticase soy broth (Difco, Detroit, Mich.) containing 500 μg/ml of streptomycin sulfate (Sigma, St. Louis, Mo.) or 250 μg/ml of nalidixic acid (Sigma), respectively, at 37°C for 18 h. Prior to inoculation in feces (see below), overnight cultures were diluted 1:100 in sterile physiological saline (pH 7.0) to obtain a viable cell population of approximately 6.0 $\log_{10}$ CFU/ml.

On each day of the experiments, fresh feces were obtained immediately after defecation from three cows fed a corn silage ration. One hundred grams of each feces sample were obtained and mixed together with 100 ml of sterile physiological saline containing select bacterial organisms for a final concentration of approximately 6 $\log_{10}$ CFU/g feces.

Experimental protocol—meat experiment 1. Prerigor beef carcass shortplates were obtained within 15 min postexsanguination from beef carcass sides processed at a local cow and bull slaughterhouse. Shortplates were removed from carcasses between the 5th and the 13th rib and about 25 cm from the vertebrae to within 10 cm of the midline. The cutaneous trunci muscle (consisting of lean tissue) covered the surfaces of the shortplates. Individual shortplates were placed in plastic bags, stored in insulated carriers to prevent rapid cooling, and transported to the Meat Animal Research Center and used within 2 h of slaughter *(7)*.

Eight adjacent areas were marked on each short plate with a sterile template (5 cm by 5 cm), a sterile cotton swab, and edible ink before inoculation with bovine feces. Marked shortplates were inoculated with the fecal slurry containing pathogens by paintbrush inoculation *(7)* and left undisturbed for 15 min. The final concentration of *E. coli* O157:H7 and *Salmonella* Typhimurium on meat surfaces was approximately 6.0 and 5.0 $\log_{10}$ CFU/cm^2, respectively.

All spray washes were conducted in the insertable pod of a biological safety hood *(7)*. CPC (Zeeland Chemicals, Zeeland, Mich.) was thoroughly mixed in 35°C tap water to obtain a final concentration of 1% (wt/vol). The operation parameters for the washer were as follows: spray nozzle oscillation speed, 60 cycles/min; exposure to spray, 15 s; line pressure, 862 kPa; flow rate, 4.8 liters/min; temperature of spray at nozzle (35/10, Spraying Systems Co., Wheaton, Ill.), 35 ± 2°C (model 40605 automatic 10 point temperature scanner, Davis Instruments, Inc., Baltimore, Md.).

After spray washing, shortplates were placed on sterile trays. For enumeration of remaining populations, samples were taken and processed within 1 h after spray washing (day 0). Additional samples were obtained after 2 days of aerobic storage at 4°C. On day 2, individual 25 cm^2 samples were aseptically excised from the shortplates and vacuum packaged (Hollymatic model LV10G, Countryside, Ill.) in a standard vacuum-packaging bag (3.2 mil nylon/copolymer bag with oxygen transmission rate at 23°C of 52 cc/m^2; Hollymatic, Inc.) and held at 4°C. Additional samples for bacterial enumeration (see below) were obtained at days 7, 21, 28, and 35. After excision of samples for enumeration, remaining pieces of untreated and treated surface tissues were used to assess surface pH values (flat electrode, Corning Instruments, Corning, N.Y.).

A fecal slurry containing no experimentally inoculated pathogens was applied to lean tissue of the shortplates, left untreated, or subjected to spray-washing treatments with water or 1% CPC as described above. Tissue samples were held at 4°C for 48 h, excised, vacuum-packaged, and stored until the specified day, at which time untreated and treated tissues were stored at −20°C until shipped. All samples were transported at 4°C to the University of Arkansas for Medical Sciences (Little Rock, Ark.) and kept at −20°C until chemical analyses were performed. Samples were thawed overnight at 4°C and brought to room temperature prior to analyses.

Experimental protocol—meat experiment 2. Prerigor adipose tissues were obtained from the loin and brisket area within 15 min postexsanguination from beef carcass sides processed at the Meat Animal Research Center. Tissues were cut to 7.5 cm by 7.5 cm prior to spray washing in order to fit on stainless steel boards used in the insertable hood. Prior to washing, adipose surfaces were paint brush-inoculated with bovine feces containing the pathogens as described above and allowed to remain undisturbed for 15 min. Using the spray-washing procedures described in meat experiment 1, adipose surfaces were left untreated or spray washed with water or 1% (wt/vol) CPC. After spray washing, tissues were placed on sterile trays. For enumeration of remaining populations, samples were taken and processed within 1 h after spray washing (day 0). Additional samples were obtained after 2 days of aerobic storage at 4°C. On day 2, individual 25-cm^2 samples were aseptically excised from the surface tissues and vacuum-packaged in a standard vacuum-packaging bag, and held at 4°C. Vacuum-packaged samples were refrigerated and enumerated (see below) at day 35.

A fecal slurry containing no pathogens was applied to adipose tissue, left untreated, or subjected to spray-washing treatments with water or 1% CPC as described above. Tissue samples were held at 4°C for 48 h, excised, vacuum-packaged, and stored until the specified day, at which time untreated and treated tissues were stored at −20°C until shipped. All samples were transported at 4°C to the University of Arkansas and kept at −70°C until chemical analyses were performed. Samples were thawed overnight at 4°C and brought to room temperature prior to analyses.

Experimental protocol—meat experiment 3. Prerigor lean tissues were obtained from the cutaneous trunci within 15 min postexsanguination from beef carcass sides processed at the Meat Animal Research Center. Tissues were cut to 15 cm by 15 cm prior to spray washing in order to fit on stainless steel boards used in the insertable hood. Lean surfaces were paint brush-inoculated with bovine feces containing the pathogens as described above and allowed to remain undisturbed for 15 min.

Using the spray-washing procedures described in meat experiment 1, inoculated prerigor lean beef surfaces were spray washed with 1% (wt/vol) CPC; held for 0, 5, 10, 15, or 30 min at 25°C; and subjected to another water wash (15 s, 862 kPa, 35°C). After spray washing, tissues were placed on sterile trays. For enumeration of remaining populations, samples were taken and processed within 1 h after spray washing (day 0). Additional samples were obtained after 2 days of aerobic storage at 4°C. On day 2, individual 25-cm^2 samples were aseptically excised from the surface tissues, vacuum-packaged in a standard vacuum-pack-

aging bag, and held at 4°C. Vacuum-packaged samples were refrigerated and enumerated (see below) at days 7 and 50.

Experimental protocol—meat experiment 4. In addition to analyses of surface tissue samples taken from meat experiments 1 through 3, analyses of residual CPC in ground beef samples also were conducted. Frozen, intact postrigor beef loins were sawed in half and thawed at 4°C for 4 days. Exposed lean and adipose surfaces were washed with 1% CPC in a 15-s spray wash, left undisturbed, and water-washed using the contact time (0, 5, 10, 15, or 30 min) and washing parameters described in meat experiment 3. After the combination washes, treated loins were stored at 4°C for 1 h to firm up before grinding. Approximately 80 cm^2 of the treated lean surfaces were excised and coarse ground (4.5 mm head on a model MG12, 0.25-hp grinder; Davpol Enterprises, Inc., New York, N.Y.) with ~800 g of non-CPC-treated postrigor lean tissue. Approximately 20 cm^2 of the treated adipose tissue were excised and ground with ~200 g of non-CPC-treated postrigor adipose tissue. In doing this, the CPC-treated tissue was diluted 1:10 with nontreated tissue. The lean and fat were then coarse ground together once more, placed in a plastic bag, and thoroughly mixed by hand kneading for 2 min to ensure even distribution of the CPC throughout the final product. The fat content of the resulting ground beef was determined to be approximately 18 to 22% by proximate analyses. The resulting ground product was frozen at −20°C until shipped. All samples were transported at 4°C to the University of Arkansas for Medical Sciences and kept at −70°C until chemical analyses were performed. Samples were thawed overnight at 4°C and brought to room temperature prior to analyses.

Ground beef processed from loins treated with water or CPC and allowed contact for 0 and 5 min were saved and frozen at −20°C until needed. Prior to the experiment, frozen ground beef was thawed overnight at 4°C. An overnight culture of *E. coli* O157:H7 or *Salmonella* Typhimurium was diluted 1:1,000 in buffered peptone water and the diluted culture added to approximately 200 g of thawed ground beef to obtain approximately 3 to 4 $\log_{10}$ CFU/g. Four 50-g samples of inoculated ground beef were vacuum-packaged and held at 4°C. Remaining bacterial populations were determined by sampling 25 g of each sample at days 0 and 11 (see bacterial enumeration procedures below).

Bacterial enumeration. Following excision of 25-cm^2 (meat experiments 1, 2, 3) or removal of 25-g samples (meat experiment 4) on the specified days, excised or ground beef was pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, Ohio) in a Sterefil Stomacher bag (Spiral Biotech, Bethesda, Md.) with 25 ml of buffered peptone water (pH 7.0; BBL, Cockeysville, Md.) containing 0.1% Tween 20 (Fisher, St. Louis, Mo.) Each stomachate was serially diluted in buffered peptone water and either spiral plated (model D spiral plater; Spiral Biotech) in duplicate or spread plated in quadruplicate on respective agar. For the detection of *E. coli* O157:H7 or *Salmonella* Typhimurium, stomachates were spiral plated in duplicate onto sorbitol McConkey agar (Difco) containing 500 μg/ml of streptomycin (Sigma) or Rambach agar (Merck, Darmstadt, Germany) containing 250 μg/ml of nalidixic acid (Sigma), respectively. Trypticase soy agar (Difco) was used for enumeration of mesophilic aerobic plate counts (APC). All plates were enumerated manually or with the CASBA IV image analyzer (Spiral Biotech) after incubation for 48 h at respective temperatures. The lowest level of detection of organisms was 1.30 $\log_{10}$ CFU/cm^2 using spiral plating procedures; samples that were spread plated in quadruplicate were used to detect total number of CFU/cm^2.

On day 35 of meat experiment 1, 1 ml of each CPC-treated stomachate was enriched in 9 ml of EC broth (Bioproducts, Seattle, Wash.) for *E. coli* O157:H7, and selenite cystine broth (Difco) or tetrathionate broth (Accumedia, Baltimore, Md.) for *Salmonella* Typhimurium and incubated at 37°C (EC broth, selenite cystine) or 42°C (tetrathionate) for 24 h. After growth, samples were subcultured by streaking a loopful of broth onto duplicate plates of sorbitol McConkey agar or Rambach agar, respectively, containing appropriate antibiotic and incubated at 37°C for 24 h.

Plate overlay assays. The antimicrobial activity of 1% CPC under various conditions was determined in a series of plate overlay assays. In the first experiment, CPC was added to water to a final concentration of 1%. Twenty microliters of 1% CPC were spotted onto lawns of *E. coli* O157:H7 or *Salmonella* Typhimurium made as follows. Briefly, tryptic soy agar plates were overlaid with 8 ml of semisoft tryptic soy agar (0.5% wt/vol agar) seeded with 80 μl of an overnight broth culture of *E. coli* O157:H7 or *Salmonella* Typhimurium. The seed density was approximately 6 $\log_{10}$ CFU/ml of overlay. Duplicate plates were scored (±) for zones of inhibition after 24 h of incubation at respective temperature, and scores were recorded *(20)*.

In the second experiment, a final concentration of 1% CPC was added to solutions of 0, 2, 1, 0.5, 0.05, 0.025, 0.013, 0.006, and 0.003% Tween 20 and allowed to remain undisturbed for 15 min. Twenty microliters of each of the CPC-treated solutions were spotted directly onto a lawn of *E. coli* O157:H7 or *Salmonella* Typhimurium, as described in the first plate overlay experiment.

In the third experiment, 1% CPC was added to swabbings from lean and adipose tissues as follows. Lean and adipose surface tissues were obtained from prerigor beef carcasses, vacuum-packaged, stored at 4°C for 24 h, and frozen at −20°C until needed. On the day of the experiment, tissues were thawed at 4°C and allowed to equilibrate to room temperature. Individual sterile sponges (NASCO, Ft. Atkinson, Wis.) were premoistened in 25 ml of sterile physiological saline and the liquid expressed prior to swabbing. Using a premoistened sponge and a stainless steel template, samples from each tissue type were taken as follows: 100 cm^2 of the lean or adipose surfaces were swabbed 10 times in a horizontal direction, the sponge flipped, and swabbed 10 times in a vertical direction. The sponge was returned to the NASCO bags, stomached for 2 min (model 400 stomacher, Tekmar), and the remaining liquid expressed from the sponge (approximately 15 ml). The expressed liquid was transferred to a 50-ml conical centrifuge tube and stored at 4°C until needed *(4)*. CPC was added to each of the swabbings for a final concentration of 1%, remained undisturbed for 15 min, and 20 μl spotted onto lawns of *E. coli* O157:H7 or *Salmonella* Typhimurium as described in the first plate overlay experiment.

In the fourth experiment, approximately 50 ml of purge was obtained from postrigor beef trim, frozen at −20°C until needed, and thawed overnight at 4°C prior to the experiment. One-milliliter aliquots of the thawed purge were dispensed into microcentrifuge tubes and allowed to equilibrate to room temperature (25°C). CPC was added to the purge to a final concentration of 1% and remained undisturbed for 0, 5, 10, 15, and 30 min. After the specified time, 20 μl was spotted onto a lawn of *E. coli* O157:H7 or *Salmonella* Typhimurium as described in the first plate overlay experiment.

Analytical methods for CPC residue determination. CPC residues in all beef samples were determined using a high-performance liquid chromatography assay developed at the University of Arkansas for Medical Sciences *(11)*. For the analysis of beef samples, about 25 g were extracted with 70 ml of 95% ethanol at 60°C for 1 h with mixing in an orbital shaker at 200 rpm. After

TABLE 1. *Meat experiment 1*[a]

Organism	Treatment	Day of vacuum-packaged, 4°C storage					
		0	2	7	21	28	35
E. coli O157:H7	Untreated	6.4 A	5.10 A	5.0 A	4.2 A	4.1 A	3.4 A
E. coli O157:H7	Water	3.9 B	3.2 B	2.8 B	1.7 B	1.6 B	1.8 B
E. coli O157:H7	1% CPC	ND C	ND C	ND C	ND C	0.1 C	ND C
Salmonella Typhimurium	Untreated	4.9 A	4.4 A	4.0 A	4.0 A	3.4 A	3.0 A
Salmonella Typhimurium	Water	2.9 B	2.8 B	1.9 B	1.8	1.2 B	1.1 B
Salmonella Typhimurium	1% CPC	ND C	ND C	ND C	ND B	0.1 B	ND B
APC	Untreated	6.4 A	5.9 A	6.1 A	6.8 A	8.5 A	8.5 A
APC	Water	4.1 B	4.0 B	4.1 B	5.0 B	5.9 B	6.5 B
APC	1% CPC	0.6 C	0.3 C	0.6 C	1.5 C	1.4 C	1.7 C

[a] Remaining bacterial populations ($\log_{10}$ CFU/cm^2) of *E. coli* O157:H7, *Salmonella* Typhimuium, and APC from beef shortplates left untreated or immediately following spray washes (15 s, 862 kPa, 35 + 2°C) with water or 1% CPC and after long-term, vacuum-packaged, 4°C storage. Different letters denote significant differences between treatments within columns for a given organism ($P \leq 0.05$). ND, not detected; cell counts were below the detectable level (0 $\log_{10}$ CFU/cm^2).

cooling to room temperature the extract was transferred to a volumetric flask and diluted with 95% ethanol to a final volume of 100 ml.

CPC residues in all beef samples were measured in a Waters (Milford, Mass.) high-pressure liquid chromatography system consisting of a 600E multisolvent delivery system, 490E programmable multi-wavelength detector, 746 data module, and 600E system controller. The analyses were conducted using an Alltima cyano column (250 by 4.6, 5 μm) and guard column (7.5 by 4.6, 5 μm) (Alltech Associates, Deerfield, Ill.). The following parameters were used in the analyses: mobile phase was methanol: 0.008 M tetramethylammonium hydroxide pentahydrate/acetic acid buffer with pH 3.6 (37/63) at a flow rate of 2 ml/min; molarity of acetic acid in the buffer was 0.14 M; ultraviolet absorbance detection was at 260 nm; dodecylpyridinium chloride was used as an internal standard for the assays. The method was validated by constructing a seven-point calibration curve over the concentration range of 3 to 200 μg/ml with linearity established by: $y = 0.03108x - 0.03234$, where x is the concentration and y is the peak/area ratio between CPC/dodecylpyridinium chloride. Additional parameters for analyses include: goodness of fit (r^2) = 0.9998; interassay precision and accuracy determined by analyzing replicate ($n = 5$) blank extracts containing CPC at 3.57, 91.3, and 183 μg/ml and with the addition of 26.9 μg/ml DPC as internal standard; and error at less than 5% for the medium and high concentrations. The intra-assay error for the lower concentration, 3.57 μg/ml, was 9.5% while coefficient of variation was less than 7% for all the concentrations used. The recoveries obtained following the application of 0.6 or 3.0 mg of CPC on the surface of CPC-free cubed beef samples and extracting with ethanol were 101.6 ± 6.64 or 102.2 ± 6.63% ($n = 5$), respectively.

Calculations and statistical analyses of population data. The lowest level of detection of APC or pathogen was 1.3 $\log_{10}$ CFU/cm^2 using spiral plating procedures; samples that were spread plated in quadruplicate were used to detect total number of CFU/cm^2. After enumeration, bacterial populations from duplicate plates were averaged and converted to $\log_{10}$ CFU/cm^2. Least squared means of bacterial populations ($\log_{10}$ CFU/cm^2) from each treatment were calculated from six samples for experiments 1 and 2 and three samples for experiment 3. Statistical analyses were performed using Analysis of Variance procedure of SAS (SAS for Windows, release version 6.12, SAS Institute, Inc., Cary, N.C.). Inoculum counts were used as a covariant to normalize data between treatment replications. Statistical significance was defined as $P \leq 0.05$, unless otherwise noted.

RESULTS

Preliminary experiments were conducted to determine the concentration of CPC to be used for the duration of the study. Lean surfaces were experimentally inoculated with a fecal slurry containing the pathogens; treated with 0.4, 0.8, and 1.6% CPC or water; and stored under refrigerated, vacuum-packaged conditions for up to 35 days. Preliminary data indicated that *E. coli* O157:H7 and *Salmonella* Typhimurium were reduced immediately to undetectable levels when treated with 1.6% CPC, and virtually no growth (0 $\log_{10}$ CFU/cm^2) was detected after 35 days of refrigerated, vacuum-packaged storage. Similar results were observed with 0.8% CPC that exhibited virtually no growth (0 $\log_{10}$ CFU/cm^2) at days 0 or 35. In another experiment, lean beef tissue was treated with 0.8, 1, 1.2, 1.4, or 1.6% CPC, and APCs were determined immediately after treatment. APCs remaining after treatments with 1, 1.2, 1.4, and 1.6% CPC were not statistically different from each other. Based on this preliminary information, the remaining spray-washing and plate overlay experiments were conducted using 1% CPC.

Meat experiment 1. Spray treatments with a 1% concentration of CPC effectively reduced bacterial populations of *E. coli* O157:H7, *Salmonella* Typhimurium, and APC (Table 1) on lean beef tissue immediately after treatments and after long-term refrigerated, vacuum-packaged storage. Specifically, spray washing with 1% CPC immediately reduced *E. coli* O157:H7 from 6.4 $\log_{10}$ CFU/cm^2 to undetectable levels and *Salmonella* Typhimurium from 4.9 $\log_{10}$ CFU/cm^2 to undetectable levels. After 35 days of refrigerated, vacuum-packaged storage, 1% CPC-treated samples did not exhibit significant growth of *E. coli* O157:H7 or *Salmonella* Typhimurium, such that selective enrichment of stomachates from CPC-treated samples obtained on day 35 did not reveal the presence of either pathogen. In addition to the observed reductions in pathogen populations, 1%

TABLE 2. *Meat experiment 1*[a]

Treatment	Day of vacuum-packaged, 4°C storage					
	0	2	7	21	28	35
Untreated	6.82	6.59	6.10	5.78	5.74	5.73
Water	6.64	6.26	5.90	5.78	5.91	5.76
1% CPC	6.53	6.28	5.97	5.94	5.97	5.83

[a] The pH of beef shortplates and samples left untreated or immediately following spray washes (15 s, 862 kPa, 35 + 2°C) with water or 1% CPC and after long-term, vacuum-packaged, 4°C storage. No significant differences ($P \leq 0.05$) were observed between treatments within columns.

CPC immediately reduced levels of APC from 6.4 to 0.60 $\log_{10}$ CFU/cm^2. After 35 days of refrigerated, vacuum-packaged storage, CPC-treated surfaces exhibited a remaining APC of 1.7 $\log_{10}$ CFU/cm^2, as compared to untreated surfaces with APC of 8.5 $\log_{10}$ CFU/cm^2. This information clearly suggests that 1% CPC can effectively reduce pathogens immediately after treatment as well as suppress bacterial populations on lean beef surfaces during long-term refrigerated storage.

In addition to bacterial enumeration, analyses of pH and residual CPC from the treated lean beef samples were conducted. A solution of 1% CPC exhibited a pH of 6.4 and water exhibited a pH of 6.6. When applied to beef lean surfaces, the pH readings of CPC-treated samples were not statistically different than untreated or water-treated tissues on any of the days examined (Table 2). The results of the residue determination indicated that CPC-treated lean surfaces exhibited CPC residue levels of: 0.405 mg/cm^2 immediately after treatment, 0.425 mg/cm^2 after 21 days of storage; and 0.340 mg/cm^2 after 35 days of storage. No residual CPC was detected in untreated or water-treated samples.

Meat experiment 2. Spray treatments with a 1% concentration of CPC reduced bacterial populations of *E. coli* O157:H7, *Salmonella* Typhimurium, and APC (Table 3) on adipose beef tissue immediately after treatments and after long-term refrigerated, vacuum-packaged storage. Populations of *E. coli* O157:H7 and *Salmonella* Typhimurium immediately following CPC treatments were reduced significantly by 4.9 and 3.8 $\log_{10}$ CFU/cm^2, respectively, and remained lower than populations from untreated or water-treated tissues at day 2. However, by day 35, populations of *E. coli* O157:H7 and *Salmonella* Typhimurium from CPC-treated tissues were not different than populations from water-treated tissues. While APC from CPC-treated tissues (3.8 $\log_{10}$ CFU/cm^2) were not statistically different from APC from water-treated tissues (4.2 $\log_{10}$ CFU/cm^2) at day 0, populations were significantly lower at days 2 and 35.

Analysis of residual CPC from treated adipose beef samples indicated that these surfaces exhibited residual levels of 0.295 mg/cm^2 after CPC treatment and 7 days storage. No residual CPC was detected in untreated or water-treated samples. Despite the efficacy of CPC against foodborne pathogens, residual chemical analyses on treated lean and adipose beef surfaces indicated an unacceptable CPC level for human consumption. Therefore, another experiment was devised to determine if an application of CPC, followed by a contact time up to 30 min and a secondary water wash, could inhibit pathogen populations on lean beef surfaces while also reducing residual compound.

Meat experiment 3. In the next experiment, a 15-s spray with CPC was applied to experimentally inoculated lean beef tissues; allowed contact for 5, 10, 15, and 30 min; and then subjected to a secondary, 15-s water wash. On day 0, remaining populations of *E. coli* O157:H7, *Salmonella* Typhimurium, and APC from tissues subjected to a CPC wash, but no water wash (C) and treatments that allowed contact for 5 (C5), 10 (C10), or 15 (C15) minutes were not statistically different from each other (Table 4). However, tissues that were contacted for 30 min (C30) prior to the secondary water wash exhibited higher bacterial populations in all instances. Populations of *E. coli* O157:H7 and *Salmonella* Typhimurium were not statistically different

TABLE 3. *Meat experiment 2*[a]

Organism	Treatment	Day of vacuum-packaged, 4°C storage		
		0	2	35
E. coli O157:H7	Untreated	5.8 A	5.0 A	4.4 A
E. coli O157:H7	Water	3.9 B	4.0 B	3.7 AB
E. coli O157:H7	1% CPC	0.9 C	2.5 C	3.0 B
Salmonella Typhimurium	Untreated	5.4 A	4.9 A	3.9 A
Salmonella Typhimurium	Water	3.4 B	3.8 A	4.3 A
Salmonella Typhimurium	1% CPC	1.6 C	2.4 B	3.8 A
APC	Untreated	6.5 A	6.3 A	6.5 A
APC	Water	4.2 B	5.2 B	6.2 A
APC	1% CPC	3.8 B	4.3 C	5.5 B

[a] Remaining bacterial populations ($\log_{10}$ CFU/cm^2) of *E. coli* O157:H7, *Salmonella* Typhimurium, and APC from beef adipose tissue left untreated or immediately following spray washes (15 s, 862 kPa, 35 + 2°C) with water or 1% CPC and after long-term, vacuum-packaged, 4°C storage. Different letters denote significant differences between treatments within columns for a given organism ($P \leq 0.05$).

TABLE 4. *Meat experiment 3*[a]

Organism	Treatment	Day of vacuum-packaged, 4°C storage			
		0	2	7	50
E. coli O157:H7	C = 1% CPC applied, no water wash	1.3 A	1.1 A	0.32 A	ND A
E. coli O157:H7	C5 = 1% CPC applied, contact 5 min, water washed	1.6 A	1.1 A	0.6 A	ND A
E. coli O157:H7	C10 = 1% CPC applied, contact 10 min, water washed	1.5 A	0.8 A	0.8 A	ND A
E. coli O157:H7	C15 = 1% CPC applied, contact 15 min, water washed	1.6 A	0.8 A	0.2 A	ND A
E. coli O157:H7	C30 = 1% CPC applied, contact 30 min, water washed	2.7 B	0.9 A	1.6 B	ND A
Salmonella Typhimurium	C = 1% CPC applied, no water wash	1.5 A	1.1 A	0.5 A	ND A
Salmonella Typhimurium	C5 = 1% CPC applied, contact 5 min, water washed	1.4 A	1.6 A	1.0 A	ND A
Salmonella Typhimurium	C10 = 1% CPC applied, contact 10 min, water washed	1.3 A	1.1 A	0.8 A	ND A
Salmonella Typhimurium	C15 = 1% CPC applied, contact 15 min, water washed	1.5 A	0.8 A	0.8 A	ND A
Salmonella Typhimurium	C30 = 1% CPC applied, contact 30 min, water washed	2.7 B	1.2 A	1.8 B	0.1 A
APC	C = 1% CPC applied, no water wash	2.4 A	2.8 A	3.0 A	3.6 A
APC	C5 = 1% CPC applied, contact 5 min, water washed	2.4 A	2.9 A	3.9 B	3.9 A
APC	C10 = 1% CPC applied, contact 10 min, water washed	2.3 A	2.8 A	3.6 B	3.9 A
APC	C15 = 1% CPC applied, contact 15 min, water washed	2.6 A	3.1 A	3.7 B	3.6 A
APC	C30 = 1% CPC applied, contact 30 min, water washed	3.5 B	3.8 B	3.8 B	3.9 A

[a] Remaining bacterial populations ($\log_{10}$ CFU/cm^2) of *E. coli* O157:H7 from prerigor beef lean tissue treated with 1% CPC (15 s, 862 kPa, 35 ± 2°C), left undisturbed or sprayed with water after 5, 10, 15, or 30 min (15 s, 125 psi, 35 ± 2°C), and after long-term, vacuum-packaged, 4°C storage. Different letters denote significant differences between treatments within columns for a given organism ($P \leq 0.05$). ND, not detected; cell counts were below the detectable level (0 $\log_{10}$ CFU/cm^2).

and virtually undetectable by day 35. By day 35 of the experiment, remaining APCs were not statistically different for any of the different contact times and ranged from 3.6 to 3.9 $\log_{10}$ CFU/cm^2.

Residual CPC determination indicated the C5-, C10-, C15-, and C30-treated and water-washed lean surfaces exhibited average residue levels of 0.139, 0.183, 0.125, and 0.152 mg/cm^2, respectively, after 50 days of refrigerated, vacuum-packaged storage. The treated but unwashed control (C) had a residue level of 0.204 mg/cm^2.

Because the surfaces of beef carcasses can be incorporated into ground beef during the fabrication process, we conducted an additional experiment to determine if residual CPC could be diluted by processing the adipose and lean treated beef tissues with nontreated tissues in a ground product. The results from this experiment demonstrated that residual CPC levels from C5-, C10-, C15-, and C30-treated, diluted 1/10 with untreated lean and adipose surfaces and subsequently ground product were 103.2, 80.9, 95.7, and 84.0 mg/kg, respectively. The ground beef from the treated but unwashed control (C) had a residue level of 157.9 mg/kg.

Meat experiment 4. To determine if residual CPC activity could suppress bacterial populations in ground beef, CPC-treated ground beef samples with residual concentrations of 0, 47, 111, and 136 ppm were inoculated with 3 to 4 $\log_{10}$ CFU/g of *E. coli* O157:H7 and *Salmonella* Typhimurium, and bacterial populations were monitored at days 0 and 11 under vacuum-packaged, refrigerated storage. The results indicated that residual CPC, even up to 136 ppm did not affect any reductions in either pathogen in ground beef (Table 5).

Plate overlay assays. In initial studies, 1% CPC effectively inhibited *E. coli* O157:H7 and *Salmonella* Typhimurium in plate overlay assays. While CPC did inhibit pathogens on adipose surfaces (meat experiment 2), the reductions were not as great as was observed on lean surfaces

TABLE 5. *Meat experiment 4*[a]

Organism	Concentration of residual CPC (ppm/g)	Day 0	Day 4	Day 11
E. coli O157:H7	0	4.3	4.3	4.2
E. coli O157:H7	47	3.9	4.2	4.4
E. coli O157:H7	111	4.7	4.9	3.9
E. coli O157:H7	136	4.0	4.1	4.4
Salmonella Typhimurium	0	3.9	3.9	3.6
Salmonella Typhimurium	47	3.7	3.7	3.4
Salmonella Typhimurium	111	3.9	4.0	3.9
Salmonella Typhimurium	136	4.3	3.7	3.6

[a] Bacterial populations ($\log_{10}$ CFU/g) of *E. coli* O157:H7 in ground beef containing varying levels of residual CPC after vacuum-packaged, 4°C storage.

(meat experiment 1). To determine whether fatty acids interfered with CPC activity, two experiments were conducted. Different concentrations of Tween 20 (0, 2, 1, 0.5, 0.05, 0.025, 0.013, 0.006, 0.003%) added to 1% CPC did not affect antimicrobial activity of CPC in plate overlay assays. Additionally, the presence of meat components obtained from purge or taken from carcass swabbings of lean or adipose tissues did not appear to affect CPC activity because zones of inhibition were detected equally in all cases.

DISCUSSION

For the last 50 years, CPC has been used in oral hygiene products ranging from mouthwashes to throat lozenges. Recent studies have clearly demonstrated that CPC is an effective poultry decontaminant *(3, 13, 22, 24)*. To determine the effectiveness of CPC against pathogens associated with beef surfaces, a series of experiments was devised and presented. We have demonstrated that spray treatments with 1% CPC reduce populations of mesophilic APC, *E. coli* O157:H7, and *Salmonella* Typhimurium from lean and adipose beef surfaces immediately after spray treatments and after extended, refrigerated, vacuum-packaged storage. Previous studies have determined that spray treatments with organic acids or trisodium phosphate also are effective for the immediate and longterm reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium on beef surfaces *(6, 7)*. In these studies, populations of APC grew over the course of refrigerated storage such that populations were no different from controls. In the present study, spray treatments with 1% CPC reduced APC substantially on both lean and adipose surfaces, as compared to tissues left untreated or water-treated. More importantly, APC and pathogen populations remained suppressed following CPC treatments and 35 days of refrigerated, vacuum-packaged storage. Additional experiments indicated that CPC does not appear to be inactivated by beef lean or adipose components. Based on this limited information, we can offer no explanation as to why immediate reductions and long-term suppression of APC and pathogen populations were not as great on beef adipose surfaces as compared to lean beef surfaces. However, previous studies have demonstrated that other antimicrobial compounds behave differently on adipose versus lean surfaces *(5)*.

In this study, a single, 15-s spray at 862 kPa delivered approximately 2 liters of 1% CPC to the beef surfaces. While there are different technologies available to apply antimicrobials to carcasses, most large plants utilize a two-step spray-washing system. After evisceration, carcasses undergo a high-pressure water wash (2,069 to 2,758 kPa) applied for 15 to 45 s, followed by a low-pressure (138 to 345 kPa) antimicrobial rinse or wash for 15 to 30 s. Depending upon droplet size, the 15-s, secondary antimicrobial rinse could deliver approximately 0.9 to 1 liter/carcass *(9)*. Based on this information, the authors calculated that CPC applied in a secondary antimicrobial rinse would cost approximately \$0.17/carcass or <\$0.01 per pound of processed beef.

Contact times up to 15 min followed by a secondary water wash immediately suppressed APC and pathogen populations as well as reduced overall CPC concentration on treated surfaces. Subsequent grinding of CPC-treated surfaces with nontreated beef tissues also resulted in a dilution of CPC concentration in the final product. However, residual CPC in ground beef was found not to be effective for inhibiting pathogens after contamination. It appears that the direct application of 1% CPC for up to 15 min followed by a water wash is effective for reducing APC, *E. coli* O157:H7, and *Salmonella* Typhimurium on beef surfaces, as well as in ground beef.

The CPC residue levels for equal areas of lean and adipose surfaces were very similar in this study. Long-term refrigerated and vacuum-packaged storage of the samples did not produce a significant difference in the residue levels. As indicated in meat experiments 3, CPC residues also appear to be unaffected by the time between CPC treatment or the application of a secondary water wash. The presence of residual CPC observed even after washing is probably sufficient to inhibit remaining bacterial populations over the 50 days of refrigerated, vacuum-packaged storage.

Although no regulatory action exists in regard to CPC in foods, the average levels determined in ground beef after washing (91.0 mg/kg) seem to be in excess of what is acceptable or safe. For example, according to established criteria, the allowed daily intakes of food additives would be 1% of the amount found not to produce deleterious effects when the additive is chronically administered *(1)*. For CPC it has been reported that 6.25 mg/kg body weight per day did not produce any adverse effects in rats when orally administered for up to 90 days *(10)*. Thus, according to this criterion for an average adult (70 kg body weight), the allowed daily intake would be 4.4 mg per day for an adult, and for a 4- to 5-year-old child (20 kg) it would be 1.25 mg per day. In contrast, a 114-g hamburger containing the CPC residues found in this study would yield about 10 mg of CPC, clearly surpassing the allowed daily intake.

Further research on the application of CPC to inhibit foodborne pathogens might address better delivery systems of CPC or technologies to reduce the levels of residual CPC in beef products without affecting antimicrobial activity. Despite the limitations presented in this study, CPC has the potential to be used as a carcass decontamination agent to improve the microbiological safety of beef products.

ACKNOWLEDGMENTS

The authors are thankful for the technical support of Mrs. Jane Long and Mrs. Julie Dyer. The authors also thank Mr. Jeff Waechter for conducting the proximate analyses. This research was supported in part by grants from the Arkansas Science and Technology Authority and U.S. Department of Agriculture/NRICGP grant 9802739.

REFERENCES

1. Anonymous. 1996. Toxicity profile of cetylpyridinium chloride, British Industrial Biological Research Association, Carshalton (England). Government Reports, Announcements & Index (GRA&I), Issue 19.
2. Barnes, G. P., D. W. Roberts, R. V. Katz, and E. D. Woolridge, Jr. 1976. Effects of two cetylpyridinium chloride-containing mouthwashes on bacterial plaque. J. Periodontol. 47:419–422.
3. Breen, P. J., H. Salari, and C. M. Compadre. 1997. Elimination of

Salmonella contamination from poultry tissues by cetylpyridinium chloride solutions. J. Food Prot. 60:1019–1021.
4. Cutter, C. N. 1999. The effectiveness of triclosan-incorporated plastic against bacteria on meat surfaces. J. Food Prot. 62:474–479.
5. Cutter, C. N., and G. R. Siragusa. 1994. Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. J. Food Prot. 57:97–103.
6. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua,* and *Clostridium sporogenes.* J. Food Prot. 60:619–624.
7. Dorsa, W. J., C. N. Cutter, G. R. Siragusa, and M. Koohmaraie. 1996. Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. J. Food Prot. 59:127–135.
8. Frost, M. R., and M. P. W. Harris. 1994. An in vitro study to assess the efficacy of antiplaque agents in mouthwash formulations. Microbios 79:101–108.
9. Gangel, M. 1999. Personal communication.
10. Gunner, S. W. 1991. Novel sweeteners, regulatory issues and implications, p. 302–312. *In* D. E. Walters, F. T. Orthoefer, and G. E. Dubois (ed.), Sweetners: discovery, molecular design and chemoreception. American Chemical Society, Symp. Ser. Washington, D.C.
11. Handie, A. S. M. 1999. Development, validation, and application of analytical methods for the determination of cetylpyridinium chloride in biological matrices. M.S. thesis. University of Arkansas for Medical Sciences, Little Rock.
12. Huyck, C. L. 1944. Cetylpyridinium chloride. Am. J. Pharm. 116: 51–59.
13. Kim, J. W., and M. F. Slavik. 1996. Cetylpyridinium chloride (CPC) treatment on poultry skin to reduce attached *Salmonella.* J. Food Prot. 59:322–326.
14. Kourai, H., H. Takechi, T. Horie, N. Uchiwa, K. Takeiche, and I. Shibasaki. 1985. The antimicrobial characteristics of quaternary ammonium salts. Part X. Antimicrobial characteristics and mode of action of *N*-alkylpyridinium iodides against *Escherichia coli* K12. J. Antibact. Antifung. Agents 1:3–10.
15. Kozlovsky, A., A. Sintov, M. Moldovan, and H. Tal. 1994. Inhibition of plaque formation by local application of a degradable controlled release system containing cetylpyridinium chloride. J. Clin. Periodontol. 21:32–37.
16. Maeda, T., S. Goto, Y. Manabe, K. Okazaki, H. Nagamune, and H. Kourai. 1996. Bactericidal action of *N*-alkylcyanopyridinium bromides against *Escherichia coli* K12 W2110. Biocontrol Sci. 1:41–49.
17. Maeda, T., J. Okazaki, H. Nagune, Y. Manabe, and H. Kourai. 1998. Bactericidal action of 4,4′-(α,ω-polymethylenedithio)bis-(1-alkylpyridinium iodides). Biol. Pharm. Bull. 21:1057–1061.
18. Mastler, P. 1996. Effect of cetylpyridinium chloride (1-hexadecylpryidinium) on reducing *Salmonella* Typhimurium attached to prechill chicken carcasses. M.S. thesis. University of Arkansas, Fayetteville.
19. Renton-Harper, P., M. Addy, J. Moran, F. M. Doherty, R. G. Newcombe. 1996. A comparison of chlorhexidine, cetylpyridinium chloride, triclosan, and C31G mouthrinse products for plaque inhibition. J. Periodontol. 67:486–489.
20. Schep, J., D. S. Jones, and M. Sheperd. 1995. Primary interactions of three quaternary ammonium compounds with blastospore of *Candida albicans* (MEN strain). Pharm. Res. 12:649–651.
21. Siragusa, G. R., and C. N. Cutter. 1993. Brochocin-C, a new bacteriocin produced by *Brochothrix campestris.* Appl. Environ. Microbiol. 59:2326–2328.
22. Wang, W.-C., Y. Li, M. F. Slavik, and H. Xiong. 1997. Trisodium phosphate and cetylpyridinium chloride spraying on chicken skin to reduce attached *Salmonella* Typhimurium. J. Food Prot. 8:992–994.
23. Wilson, M., H. Patel, and J. Fletcher. 1996. Susceptibility of biofilms of *Streptococcus sanguis* to chlorhexidine gluconate and cetylpyridinium chloride. Oral Microbiol. Immunol. 11:188–192.
24. Xiong, H., Y. Li, M. F. Slavik, and J. T. Walker. 1998. Spraying chicken skin with selected chemicals to reduce attached *Salmonella typhimurium.* J. Food Prot. 61:272–275.