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Disinfectant and Antimicrobial Susceptibility Profiles of *Salmonella* Strains from Feedlot Water-Sprinkled Cattle: Hides and Feces

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Abstract

The disinfectant and antimicrobial susceptibility profiles of 145 *Salmonella* strains obtained from feedlot water-sprinkled cattle were determined. A low prevalence of antimicrobial resistance (AMR) was observed, resistance was primarily observed to streptomycin (29.7%) and sulfamethoxazole (8.3%). All strains were susceptible to the 8 fluoroquinolones tested. The most common AMR traits were streptomycin resistance in serovar Kentucky, sulfamethoxazole resistance in Muenster and Cerro and amoxicillin/clavulanic acid-ceftiofur resistance in Meleagridis. *Salmonella* were more resistant to the disinfectants P-I, DC&R and Tek-Trol and disinfectant component THN than to other disinfectants. All strains were susceptible to triclosan, and all strains were resistant to chlorhexidine. Nearly 1/3 of the strains had low level resistance to benzalkonium chloride. The benzyl ammonium chlorides (BACs) C12, C14 and C16 were the most active ingredient in the disinfectant DC&R. C14BAC and C16BAC were shown to be more active as a disinfectant than was C12BAC. Didecyldimethyl ammonium chloride (C10AC) was the most active ingredient in the disinfectant P-128, and the most active ammonium chloride in this work and in all of our previous studies. No cross-resistance was observed. All manufacturer recommended application levels were above the observed MICs. However, observed MICs for DC&R and Tek-Trol were close to the suggested application rates and a small error in the disinfectant dilution could easily render these disinfects non-functional. The continued use of THN and formaldehyde in DC&R is questionable because these components are not effective, and their inclusion results only in additional unwanted chemicals in the environment.

Keywords

Antimicrobial, Disinfectant, Feedlot cattle, *Salmonella*, Susceptibility, Water-sprinkled cattle

Introduction

Nontyphoidal *Salmonella* serotypes are among the most prominent food safety problems [1], and *Salmonella enterica* is considered the major foodborne pathogen in the United States [2] and the world [3]. The Centers for Disease Control and Prevention (CDC) has estimated that each year, *Salmonella* causes one million foodborne illnesses in the United States alone, with 19,000 hospitalizations and 380 deaths [4]. An analysis of the risk of *Salmonella* illness per serving of beef, lamb, pork, and poultry suggests there is a similar risk for the four different meats [5]. From the period of July 2005 to June 2007 a group of ten *Salmonella* serovars were isolated from commercial ground beef: Montevideo, Anatum, Muenster, Mbandaka, Agona, Cerro, Meleagridis, Typhimurium, Dublin and Kentucky [6]. These ten serovars have been associated with previous outbreaks of foodborne illness: Montevideo [7, 8], Anatum [9], Muenster [10], Mbandaka [7], Agona [11], Cerro [12, 13], Meleagridis [14], Typhimurium [15-17], Dublin [18, 19] and Kentucky [20, 21].

Bacteria may resist antimicrobials by using a number of mechanisms, including (i) alteration of the antimicrobial agent, (ii) mutation of the target site, (iii) decreased accessibility to the target through decreased uptake or increased efflux, and (iv) implementation of alternative metabolic pathways not affected by the drug or by acquisition of drug-sensitive enzymes [22]. In the case of disinfectants, the low permeability of the cell wall or decreased accessibility to the target due to active efflux mechanisms can reduce the efficacy of the disinfectant [23]. Genes that confer resistance to disinfectants may be linked to antimicrobial resistance (AMR) genes due to their proximity on mobile genetic elements such as plasmids, transposons or integrons. In these cases, acquisition of the genetic elements may confer resistance to unrelated antimicrobial agents and to other chemical disinfectants.

The strategies to control pathogens must be comprehensive, following food products from the farm to the table [24]. Bacteria in the food chain continuum are controlled by strategies that include the use of biocides as antiseptics and disinfectants (see our paper on interactions of organic acids with *Salmonella* [25]). Disinfectants are chemicals that inhibit or kill a broad-spectrum of microorganisms [26], often contain a variety of active ingredients, and are routinely used in animal production, veterinary medicine, the food processing industry, human medicine, restaurants, and consumers' homes [27]. Bacteria may be exposed to disinfectant concentrations lower than that required to deliver a lethal insult [28]. Lower levels of disinfectants can influence the formation of biofilms and antimicrobial resistance (AMR) [29]. Increased multidrug-resistant pathogens are a concern in both human and veterinary medicine [30, 31], and reports suggest there is increasing bacterial resistance to biocides [32, 33]. Biocide use also has resulted in cross-resistance to antimicrobials [34-38].

Since there is limited information available on disinfectant susceptibility in food pathogens, our laboratory has evaluated the susceptibility of various foodborne pathogens to a large variety of disinfectants. This study characterized the antimicrobial and disinfectant susceptibility profiles among the *Salmonella enterica* strains isolated from water-sprinkled cattle at a commercial feedlot. The objectives were to evaluate the AMR and disinfectant susceptibility of these cattle hide and feces strains, and to establish a base understanding of the effects of 21 disinfectants/disinfectant components on *Salmonella* and if there is cross-resistance between the antibiotics and disinfectants.

Materials and Methods

Salmonella enterica serovars

Salmonella enterica serovars Kentucky (20), Meleagridis (7) and Muenster (19) were isolated from the feces and serovars Anatum (1), Cerro (9), Gaminara (1), Kentucky (45), Meleagridis (13) and Muenster (30) were isolated from the hides of Charolais-crossbred heifers at a commercial feedlot in Lockney, TX [39]. *Salmonella* strains were stored in glycerol and TSB at –80 °C until used. All cultures were grown at 37 °C.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) for antimicrobials were determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) [40, 41]. MICs were determined as the lowest concentration of a compound that showed no visible growth of the organism [42]. Antimicrobial MICs were obtained using the National Antimicrobial Resistance Monitoring System (NARMS) Gram-negative plates (CMV1AGNF) and the Sensititre fluoroquinolone plates (CMV1DW) during 2006 and the isolates were stored at –80 °C until the disinfectant susceptibilities were conducted. Demineralized water (5 mL), cation-adjusted Mueller-Hinton broth (MHB) with TES (Tris, EDTA, and NaCl, pH 8) (11 mL) and dose heads (#E3010) (Remel Inc., Lenexa, KS, USA) were all obtained through Thermo Fisher Scientific (Hanover Park, IL, USA). MICs of the following 16 antimicrobials, amikacin (AMI), ampicillin (AMP), amoxicillin/clavulanic acid (AUG), cefoxitin (FOX), ceftiofur (XNL), ceftriaxone (AXO), cephalothin (CEP), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SMX), tetracycline (TET) and trimethoprim/sulfamethoxazole (SXT), and 8 fluoroquinolones, ciprofloxacin (CIP), danofloxacin (DANO), difloxacin (DIF), enrofloxacin (ENRO), gatifloxacin (GAT), levofloxacin (LEVO), marbofloxacin (MARB) and orbifloxacin (ORB) were determined using the Sensititre susceptibility system according to the manufacturer's instructions (Trek Diagnostic Systems Inc., Independence, OH, USA). For those drugs that lacked specific interpretive criteria, CLSI breakpoints for Enterobacteriaceae were used [43]. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls for antimicrobial susceptibility testing.

Disinfectant susceptibility testing

Fifteen disinfectants and six disinfectant components were tested in this study: their abbreviations, recommendations for use, and sources are listed in Table 1. Dimethyl sulfoxide (DMSO) was used to solubilize some disinfectants and was obtained from MilliporeSigma (St. Louis, MO, USA). Reverse osmosis water ($^{RO}H_2O$) was produced on site by a reverse osmosis system obtained from MilliporeSigma (Bedford, MA, USA). The following disinfectants exist as mixtures of multiple components: DC&R is comprised of the following active ingredients: THN 19.2%; (C12BAC-67%, C14BAC-25%, C16BAC-7% and (C8, C10, C18)-1% benzyldimethylammonium chlorides (BACs)) 3.08%; and formaldehyde 2.28%. The active ingredients of the three disinfectants FSS, F25 and FS512 are the following: (C12BAC-5%, C14BAC-60%, C16BAC-30% and C18BAC-5%) 0.105% and (C12BAC-68%, C14BAC-32%) 0.105%. The active ingredients of Tek-Trol are *o*-phenylphenol 12%, *o*-benzyl-*p*-chlorophenol 10% and *p*-tert-amylphenol 4%. The active ingredients of P-128 are C10AC 5.07% and (C12BAC-40%, C14BAC-50% and C16BAC-10%) 3.38%. Since DC&R, Tek-Trol, FSS, F25, FS512 and P-128 are

Table 1: Disinfectants and disinfectant components.

Disinfectant	Abbreviation	Recommended use	Source
Benzalkonium chloride	BKC	Veterinary clinics, human hospitals	Sigma-Aldrich (Milwaukee, WI)
Betadine solution, 10% povidone-iodine	P-I	Veterinary clinics, human hospitals	Medicine Chest Pharmacy (Bryan, TX)
Cetylpyridinium bromide hydrate	CPB	Soaps	Sigma-Aldrich (Milwaukee, WI)
Chlorhexidine diacetate (nolvasan solution)	Chlor	Veterinary clinics, human hospitals, farms	Producers Cooperative Association (Bryan, TX)
DC&R	DC&R	Veterinary clinics, farms	Producers Cooperative Association (Bryan, TX)
Ethylhexadecyldimethylammonium bromide	CDEAB	Soaps	Sigma-Aldrich (St. Louis, MO)
Food Service Sanitizer	FSS	Restaurants, food processing plants	DadePaper (Loxley, AL)
F-25 Sanitizer	F25	Restaurants, food processing plants, dairies	DadePaper (Capital Heights, MD)
Final Step 512 Sanitizer	FS512	Restaurants, food processing plants, dairies	DadePaper (Loxley, AL)
Hexadecylpyridinium chloride	CPC	Mouth washes, toothpastes, lozenges and nasal sprays (prevents dental plaque and reduces gingivitis)	Sigma-Aldrich (St. Louis, MO)
Hexadecyltrimethylammonium bromide	CTAB	Soaps	Sigma-Aldrich (St. Louis, MO)
OdoBan	OdoBan	Deodorizer, disinfectant, and sanitizer in the home, hospitals, restaurants, and schools	Sam's Club (College Station, TX)
P-128	P-128	Veterinary clinics, human hospitals, farms, food processing plants, dairies	Burns Veterinary Supply, Inc. (Farmers Branch, TX)
Tek-Trol	Tek-Trol	Veterinary clinics, human hospitals, farms, schools, poultry production, poultry transportation vehicles	Producers Cooperative Association (Bryan, TX)
Triclosan (Irgasan)	Triclosan	Human hospitals, personal health care products and soaps	Sigma-Aldrich (Milwaukee, WI)
Disinfectant components			
Didecyldimethylammonium chloride	C10AC (DDAC)[a]		Lonza Inc. (Fairlawn, NJ)
Benzyldimethyldodecylammonium chloride	C12BAC		Sigma-Aldrich (Milwaukee, WI)
Benzyldimethyltetradecylammonium chloride	C14BAC		Sigma-Aldrich (Milwaukee, WI)
Benzyldimethylhexadecylammonium chloride	C16BAC		Sigma-Aldrich (Milwaukee, WI)
J.T. Baker 37% formaldehyde solution	Formaldehyde		VWR International, Inc. (Marietta, GA)
Tris(hydroxylmethyl)nitromethane	THN		Sigma-Aldrich (Milwaukee, WI)

[a] DDAC is the common abbreviation for didecyldimethylammonium chloride, but we use C10AC to reflect the carbon chain length and that no benzyl groups are attached to this ammonium chloride.

The following concentrations of disinfectants and components were tested: BKC, 0.25–256 µg/mL; CDEAB,.25–256 µg/mL; Chlor, 0.06–64 µg/mL; CPB, 0.25–256 µg/mL; CPC, 0.25–256 µg/mL; CTAB, 0.25–256 µg/mL; C10AC, 0.06–64 µg/mL; C12BAC, 0.25–256 µg/mL; C14BAC, 0.125–128 µg/mL; C16BAC, 0.125–128 µg/mL; DC&R, 1–1,024 µg/mL; formaldehyde, 2–2,048 µg/mL; FSS, 0.125–128 µg/mL; FS512, 0.125–128 µg/mL; F25, 0.125–128 µg/mL; OdoBan, 0.25–256 µg/mL; P-I, 32–32,768 µg/mL; P-128, 0.06–64 µg/mL; Tek-Trol, 0.5–512 µg/mL; THN, 4–4, 096 µg/mL; and triclosan, 0.004–4 µg/mL.

mixtures of several disinfectant components, the MICs for these disinfectants were determined on the composite mixtures. We used the susceptible/resistant criteria of Heath and Rock [44] for triclosan, bacteria with MICs < 0.5 µg/mL were susceptible, and MICs > 2 µg/mL were resistant. For chlorhexidine, a cell morphology study of the effect of chlorhexidine on both Gram-positive and Gram-negative bacteria had very similar results for both types of bacteria [45]. Therefore, the breakpoint defined for staphylococci resistance by Leelaporn et al. [46] was used, such that bacteria with MICs ≥ 1 µg/mL chlorhexidine were resistant. The criteria for resistance for benzalkonium chloride was that used for Gram-negative bacteria by Sidhu et al. [47], bacteria with MICs < 30 µg/mL were susceptible, with MICs from 30 to 50 µg/mL had low-level resistance, and bacteria with MICs > 50 µg/mL were resistant.

The disinfectants and disinfectant components were diluted with $^{RO}H_2O$ to make working solutions and then filter sterilized using a 0.2 µm × 25 mm syringe filter (No. 431224, Corning Inc., Corning, NY, USA). DMSO was added to some disinfectants allowing solutions to be produced that were more concentrated than when only $^{RO}H_2O$ was used as the diluent. DMSO was added to triclosan (% DMSO

= 1.1%), C14BAC (1%), C16BAC (3%), THN (5%), CPB (7.5%) and CTAB (10%) to aid chemical solubility. The amount of DMSO in the final assays did not exceed 5%. The method used for disinfectant susceptibility determination was similar to that used for disinfectant susceptibility testing of vancomycin-resistant *Enterococcus faecium* (VRE) from community wastewater [27], beta hemolytic *E. coli* from neonatal swine [36], *P. aeruginosa* from veterinary isolates [48], *E. coli* O157:H7 from cattle carcasses, feces, and hides, and ground beef [49], non-O157 STECs [50], and *Salmonella* from turkeys [51]. *E. coli* ATCC 25922 was used as control for disinfectant susceptibility testing, and the concentrations of disinfectants tested can be found in Table 1.

Results

Antimicrobial resistance

Table 2 shows the AMR profiles among 145 *Salmonella* strains obtained from water-sprinkled cattle by providing the MIC_{50}, MIC_{90}, the antimicrobial range tested, the number of organisms resistant and the breakpoints for the antimicrobials tested. Overall, a low prevalence of AMR was observed in the 145 *Salmonella* strains; resistance was primarily observed to streptomycin (29.7%) and sulfamethoxazole (8.3%). Two strains were resistant to cefoxitin and one strain was resistant to amoxicillin/clavulanic acid and ceftiofur. Table 3 shows the AMR profiles among the 6 different *Salmonella* serovars isolated from water-sprinkled cattle. Strains of serovar Cerro from hides had resistance to streptomycin (11.1%) and sulfamethoxazole (44.4%). Strains of serovar Kentucky from hides and feces had resistance to streptomycin of 71.1 and 90.0%, respectively. Strains of serovar Muenster from hides and feces had resistance to sulfamethoxazole of 13.3 and 10.5%, respectively. Also, a single strain of serovar Meleagridis from cattle hide was resistant to both amoxicillin/clavulanic acid and ceftiofur. All 145 *Salmonella* strains were susceptible (data not shown) to the fluoroquinolone antibiotics, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, gatifloxacin, levofloxacin, marbofloxacin and orbifloxacin. A Muenster strain had the highest fluoroquinolone antibiotic MIC recorded, a difloxacin MIC of 0.5 µg/mL.

Salmonella serovar resistance traits

The most common resistance traits found among the *Salmonella* serovars are shown in Table 3. There was no resistance traits found in the one Anatum strain or the one Gaminara strain from cattle hides or in the 7 strains of Meleagridis from cattle feces. But the resistance trait of amoxicillin/clavulanic acid-ceftiofur and cefoxitin was each observed in 1 of 13 Meleagridis strains from hides. The resistance trait of sulfamethoxazole was found in 2 of 9 Muenster strains from feces. Sulfamethoxazole was also the trait found in 4 of 30 Muenster strains and 4 of 9 Cerro strains from hides. Serovar Kentucky had the most prevalence of resistance with the trait of streptomycin in 32 of 45 strains (71%) from hides and 18

Table 2: Antimicrobial resistance profiles among 145 *Salmonella* strains from water-sprinkled cattle.

Antimicrobial	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range of MICs (µg/mL)	No.(%) Resistant	Breakpoint
Aminoglycosides					
Amikacin	1	2	≤ 0.5 – 4	0 (0)	≥64
Gentamicin	≤ 0.25	0.5	≤ 0.25 – 4	0 (0)	≥16
Kanamycin	≤ 8	≤ 8	≤ 8 – 32	0 (0)	≥64
Streptomycin	≤ 32	64	≤ 32 – >64	43 (29.7)	≥64
β-Lactams					
Amoxicillin/Clavulanic acid	≤ 1/0.5	≤ 1/0.5	≤ 1/0.5 – 32/16	1 (0.7)	≥32/16
Ampicillin	≤ 1	≤ 1	≤ 1 – 8	0 (0)	≥32
Ceftiofur	1	1	≤ 0.12 – >8	1 (0.7)	≥8
Ceftriaxone	≤ 0.25	≤ 0.25	≤ 0.25 – 4	0 (0)	≥64
Cefoxitin	4	8	2 – >16	2 (1.4)	≥32
Cephalothin	4	4	≤ 2 – 16	0 (0)	≥32
Fluoroquinolones and quinolones					
Ciprofloxacin	0.03	0.03	≤ 0.015 – 0.12	0 (0)	≥1
Nalidixic acid	4	4	4 – 16	0 (0)	≥32
Phenicols					
Chloramphenicol	8	8	4 – 16	0 (0)	≥32
Sulphonamides and potentiated sulphonamides					
Sulfamethoxazole	64	256	≤ 16 – >512	12 (8.3)	≥512
Trimethoprim/Sulfamethoxazole	≤ 0.12/2.38	≤ 0.12/2.38	≤ 0.12/2.38 – 0.5/9.5	0 (0)	≥4/76
Tetracyclines					
Tetracycline	≤ 4	≤ 4	≤ 4 – 8	0 (0)	≥16

of 20 strains (90%) from feces. The antimicrobial resistance profiles of all serovars are shown in detail in the Supplemental Material (SM). The following SM tables show the individual AMR profiles of the animal hide strains in *Salmonella* serovars Anatum (Table S1), Cerro (Table S2), Gaminara (Table S3), Kentucky (Table S4), Meleagridis (Table S5) and Muenster (Table S6), and the following SM tables show the individual AMR profiles of the animal feces strains in *Salmonella* serovars Kentucky (Table S7), Meleagridis (Table S8) and Muenster (Table S9).

Disinfectant susceptibility

Table 4 shows the overall MIC distribution profiles of the 145 *Salmonella enterica* strains for the disinfectants and disinfectant components tested. All 145 *Salmonella* strains were susceptible to triclosan [44]. All *Salmonella* strains were resistant to chlorhexidine [46]. We see that C10AC, chlorhexidine and triclosan have the lowest *Salmonella* MIC values. Out of the 145 strains tested, 40 (27.6%) had low-level resistance (MIC = 32 µg/mL) and 1 (0.69%) was resistant to benzalkonium chloride [47]. The highest MICs observed were for providone-iodine, these being 2,048 (2 strains), 4,096 (117 strains) and 8,192 µg/mL (26 strains). The next lower level of observed MICs was for DC&R, Tek-Trol and THN from 64–512 µg/mL. The next level of MICs were primarily observed between 8 and 64 µg/mL and included many of the disinfectants and disinfectant components including BKC, FSS, F25, FS512, OdoBan, CPB, CPC, CDEAB, CTAB, C12BAC, C14BAC, C16BAC and formaldehyde. The following SM tables show the disinfectant susceptibility profiles of the individual *Salmonella* hide strains in serovar Anatum (Table S10), Cerro (Table S11), Gaminara (Table S12), Kentucky (Table S13), Meleagridis (Table S14) and Muenster (Table S15), and the following SM tables show the disinfectant susceptibility profiles of the individual *Salmonella* feces strains in serovar Kentucky (Table S16), Meleagridis (Table S17) and Muenster (Table S18).

Table 3: The percentage of antimicrobial resistance among the *Salmonella* serovars isolated from water-sprinkled cattle.

Bacterial serovar	Total No. of strains	Source	Antibiotics[a]	Most common resistance trait	No. strains (% Resistant)
Anatum	1	Hide	All		0 (0.0)
Cerro	9	Hides	Streptomycin		1 (11.1)
			Sulfamethoxazole	X	4 (44.4)
Gaminara	1	Hide	All		0 (0.0)
Kentucky	45	Hides	Streptomycin	X	32 (71.1)
			Cefoxitin		1 (2.2)
			Sulfamethoxazole		1 (2.2)
Kentucky	20	Feces	Streptomycin	X	18 (90.0)
			Cefoxitin		1 (5.0)
			Sulfamethoxazole		1 (5.0)
Meleagridis	13	Hides	Amoxicilin/ Clavulanic acid	X	1 (7.7)
			Ceftiofur	X	1 (7.7)
Meleagridis	7	Feces	All		0 (0.0)
Muenster	30	Hides	Sulfamethoxazole	X	4 (13.3)
Muenster	19	Feces	Sulfamethoxazole	X	2 (10.5)

[a] Antimicrobials evaluated were the aminoglycosides: amikacin, gentamicin, kanamycin, streptomycin; β-lactams: amoxicillin/clavulanic acid, ampicillin, ceftiofur, ceftriaxone, cefoxitin, cephalothin; fluoroquinolones and quinolones: ciprofloxacin, nalidixic acid; Phenicols: chloramphenicol; sulphonamides and potentiated sulphonamides: sulfamethoxazole, trimethoprim/sulfamethoxazole; and tetracycline.

Table 5 shows the percentage of chlorhexidine resistance among the individual *Salmonella* serovars. In this table and the following tables the molar MIC values (MIC_M) for concentrations of disinfectants are also provided, which allows the direct comparison between disinfectants with different molecular weights. The predominant MIC_Ms observed for chlorhexidine were 3.96 to 15.83 nmol/mL. Table 6 shows the susceptibility values for benzalkonium chloride among the individual *Salmonella* serovars. The predominant MIC_Ms observed ranged from 28.18 to 112.72 nmol/mL. Table 7 shows the susceptibility values for C10AC among the individual *Salmonella* serovars. The predominant C10AC MIC_Ms for the different serovars ranged from 5.52 to 22.09 nmol/mL. Table S19 shows the distribution of susceptibility values for C12BAC among the individual *Salmonella* serovars. The predominant C12BAC MIC_Ms for the serovars were 47.06 and 94.12 nmol/mL. Table S20 shows the susceptibility values for C14BAC among the individual *Salmonella* serovars. The predominant C14BAC MIC_Ms for the serovars were 21.74 and 43.47 nmol/mL. Table S21 shows the distribution of susceptibility values for C16BAC among the individual *Salmonella* serovars. The predominant C16BAC MIC_Ms for the serovars were 20.20 and 40.40 nmol/mL.

Calculation of DC&R component MICs

The MIC of the individual active components of DC&R can be calculated by multiplying the DC&R MICs by the component of interest percentage and dividing by the sum of all the active component percentages in DC&R [51]. For example, to calculate the MIC of the BAC component (where the BAC component is primarily a composite of C12BAC, C14BAC and C16BAC) in DC&R would be the following: for a DC&R MIC = 64 µg/mL (Table 4) the $BAC_{DC\&R}$ component level = 64 µg/mL × 3.08/24.56 = 8.03 µg/mL. Using the same type of calculation, the $BAC_{DC\&R}$ components at the DC&R MICs of 128 and 256 µg/mL (Table 4) are calculated to be a $BAC_{DC\&R}$ component level of 16.05 and 32.1 µg/mL, respectively. In a similar manner the $THN_{DC\&R}$ component level of the DC&R MICs can be calculated to be a $THN_{DC\&R}$ component level of 50.03, 100.07 and 200.13 µg/mL for the DC&R MICs of 64, 128 and 256 µg/mL, respectively (Table 4), and the calculated formaldehyde (Form) portion of the DC&R MICs results in the $Form_{DC\&R}$ component levels of 5.94, 11.88 and 23.77 µg/mL for the DC&R MICs of 64, 128 and 256 µg/mL, respectively.

Calculation of P-128 component MICs

The MIC of the individual components of P-128 can be calculated similarly to the DC&R components above

Table 4: Distribution of disinfectant and disinfectant component susceptibility profiles of 145 *Salmonella enterica* strains from water-sprinkled cattle.

	MIC (μg/mL)																	MIC50	MIC90
Disinfectant[a]	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1,024	2,048	4,096	8,192	(μg/mL)	(μg/mL)
DC&R										16[b]	127	2						128	128
Tek-Trol											45	99	1					256	256
Chlor					9	123	13											4	4
Triclosan	19	105	21															0.25	0.5
P-128					2	127	16											4	8
BKC							6	98	40	1								16	32
P-I															2	117	26	4,096	8,192
FSS							81	63	1									8	16
F25							81	64										8	16
FS512							93	52										8	16
OdoBan						1	50	92	2									16	16
CPB							1	110	34									16	32
CPC							3	127	15									16	32
CDEAB								15	127	3								32	32
CTAB								8	126	11								32	32
C10AC[c]					27	109	8	1										4	4
C12BAC[c]								66	78	1								32	32
C14BAC[c]							57	87	1									16	16
C14BAC[c]							34	109	2									16	16
THN[c]											17	119	9					256	256
Formaldehyde[c]							2	79	57	7								16	32

[a] Disinfectant and disinfectant component abbreviations: BKC, benzalkonium chloride; Chlor, chlorhexidine; CPB, cetylpyridinium bromide hydrate; CPC, hexadecylpyridinium chloride monohydrate; CDEAB, ethylhexadecyldimethylammonium bromide; CTAB, hexadecyltrimethylammonium bromide; FS512, Final Step 512 Sanitizer; FSS, Food Service Sanitizer; F25, F-25 Sanitizer; P-I, providone-iodine; C10AC, didecyldimethylammonium chloride; C12BAC, benzyldimethyldodecylammonium chloride; C14BAC, benzyldimethyltetradecylammonium chloride; C16BAC, benzyldimethylhexadecylammonium chloride; and THN, tris(hydroxylmethyl)nitromethane.
[b] Number of strains at this MIC.
[c] These entries are disinfectant components.

Table 5: The percentage of chlorhexidine resistance among the *Salmonella* serovars isolated from water-sprinkled cattle.

			Chlorhexidine MICs (No. strains (% Resistant[a]))			
Salmonella serovars	No. of strains	Source	MICs MIC_Ms	2 μg/mL 3.96 nmol/mL	4 μg/mL 7.91 nmol/mL	8 μg/mL 15.83 nmol/mL
Anatum	1	Hide		–[b]	1 (100.0)	–
Cerro	9	Hides		5 (56.0)	4 (44.0)	–
Gaminara	1	Hide		–	1 (100.0)	–
Kentucky	45	Hides		2 (4.4)	38 (84.4)	5 (11.1)
Kentucky	20	Feces		–	17 (85.0)	3 (15.0)
Meleagridis	13	Hides		1 (7.7)	12 (92.3)	–
Meleagridis	7	Feces		–	7 (100.0)	–
Muenster	30	Hides		1 (3.3)	27 (90.0)	2 (6.7)
Muenster	19	Feces		–	16 (84.2)	3 (15.8)

[a] The breakpoint defined for chlorhexidine resistance in staphylococci by Leelaporn et al. [46] was used, such that MICs ≥ 1 μg/mL were resistant.
[b] No observed strains.

by multiplying the P-128 MIC by the active component of interest percentage and dividing by the sum of all the active component percentages for P-128 [51]. The BAC_{P-128} component MICs can be calculated. The BAC_{P-128} component MICs for the P-128 MICs of 2, 4 and 8 μg/mL (Table 4) were 0.8, 1.6 and 3.2 μg/mL, respectively. Using a similar calculation, the *Salmonella* strains $C10AC_{P-128}$ component MICs were 1.2, 2.4 and 4.8 μg/mL, respectively.

Discussion

Antimicrobial resistance

A low prevalence of AMR was observed in the 145 *Salmonella* strains. Resistance was observed only to streptomycin and sulfamethoxazole and to various β-lactams, amoxicillin/clavulanic acid, ceftiofur and cefoxitin. One Cerro strain from hide was resistant to streptomycin and sulfamethoxazole. One Kentucky strain from hides and one from feces was resistant to streptomycin and sulfamethoxazole. Also, one Meleagridis strain from hide was resistant to amoxicillin/clavulanic acid and cefoxitin. The rest of the observed resistance in *Salmonella*

Table 6: Benzalkonium chloride susceptibility among the *Salmonella* serovars isolated from water-sprinkled cattle.

				Benzalkonium chloride MICs (No. strains)			
***Salmonella* serovars**	**No. of strains**	**Source**	**MICs MIC_Ms**	**8 µg/mL 28.18 nmol/mL**	**16 µg/mL 56.36 nmol/mL**	**32 µg/mL 112.72 nmol/mL**	**64 µg/mL 225.45 nmol/mL**
Anatum	1	Hide		–a	1	–	–
Cerro	9	Hides		–	7	2	–
Gaminara	1	Hide		–	1	–	–
Kentucky	45	Hides		3	42	–	–
Kentucky	20	Feces		3	16	1	–
Meleagridis	13	Hides		–	5	7	1
Meleagridis	7	Feces		–	3	4	–
Muenster	30	Hides		–	14	16	–
Muenster	19	Feces		–	9	10	–

[a] No observed strains.

Table 7: C10AC susceptibility among the *Salmonella* serovars isolated from water-sprinkled cattle.

				C10AC MICs (No. strains)			
***Salmonella* serovars**	**No. of strains**	**Source**	**MICs MIC_Ms**	**2 µg/mL 5.52 nmol/mL**	**4 µg/mL 11.05 nmol/mL**	**8 µg/mL 22.09 nmol/mL**	**16 µg/mL 44.19 nmol/mL**
Anatum	1	Hide		–[a]	1	–	–
Cerro	9	Hides		5	3	1	–
Gaminara	1	Hide		–	1	–	–
Kentucky	45	Hides		14	30	1	–
Kentucky	20	Feces		7	12	1	–
Meleagridis	13	Hides		–	12	1	–
Meleagridis	7	Feces		1	4	1	1
Muenster	30	Hides		–	28	2	–
Muenster	19	Feces		–	18	1	–

[a] No observed strains.

strains was due to single antibiotics. All strains were susceptible to the 8 fluoroquinolone antibiotics tested. The *Salmonella* tested here tended to have slightly higher MICs for difloxacin than for the other fluoroquinolone antibiotics. That is a similar result as what we observed when evaluating the disinfectant and antibiotic susceptibilities of *Salmonella* serovars from turkeys in commercial plants [51].

Antimicrobial resistance among individual *Salmonella* serovars

The serovar Kentucky, either from hides or feces had the highest prevalence of AMR in this study. The prevalence of resistance observed in Kentucky was followed by the prevalence of resistance observed in serovar Cerro and then Muenster. The most common AMR trait observed was streptomycin resistance in Kentucky, sulfamethoxazole resistance in Muenster and Cerro and amoxicillin/clavulanic acid-ceftiofur resistance in serovar Meleagridis.

Disinfectant susceptibility

The *Salmonella* strains were more resistant to the disinfectants P-I, DC&R and Tek-Trol and the disinfectant component THN than to the other disinfectants or disinfectant components tested, chlorhexidine, triclosan, P-128, BKC, FSS, F25, FS512, OdoBan, CPB, CPC, CDEAB, CTAB, C10AC, C12BAC, C14BAC, C16BAC and formaldehyde. This is a similar result as observed with ten different *Salmonella* serovars isolated from turkeys [51]. However, the BACs demonstrated much higher MIC_Ms than did C10AC. Suggesting, the benzyl compounds are less active at inhibiting *Salmonella* than is C10AC. We found the formaldehyde activity against *Salmonella* to be similar to that of the benzyl ammonium compounds and formulations containing benzyl amines, as well as the pyridinium bromides. But lower levels of formaldehyde or the benzyl compounds were not effective. This is in agreement with a study of a *S. enterica* serovar Enteritidis field isolate [52]. All 145 *Salmonella* strains were susceptible to triclosan. Triclosan acts by inhibiting a highly conserved enzyme enol-ACP reductase of bacterial fatty-acid biosynthesis [44]. Previously, cross-resistance between antibacterial agents and triclosan in *Salmonella* Typhimurium strains was observed [53]. But we did not see cross-resistance between antibiotics and triclosan in *Salmonella* strains from turkeys [51], nor did we see cross-resistance occurring in this study. Of the different bacteria that we have studied to date, both VRE [27] and *P. aeruginosa* [48] were the only bacteria shown to be resistant to triclosan. The highest measured MICs were for P-I. The manufacturer of P-I recommends an application rate of 100,000 µg/mL solution to be applied directly on wound surfaces. The recommended application solution would be in about a 12- to 95-fold excess over that required for disinfection of the *Salmonella* serovars tested here.

All *Salmonella* strains were resistant to chlorhexidine. The highest chlorhexidine MIC values were observed in serovars Kentucky and Muenster from both hide and feces samples. The chlorhexidine MIC values observed here were similar to those observed for *Salmonella* from turkeys [51]. However, higher chlorhexidine MIC values were previously observed for *Salmonella* (2–64 µg/mL) isolated from broilers, cattle, and pig feces [54]. The highest MIC level observed here was in the range of levels previously observed in clinical *Salmonella* Typhimurium (8–16 µg/mL) isolates [55]. But the MIC values for chlorhexidine were lower than many disinfectants, which was in agreement with a study of a *S. enterica* serovar Enteritidis isolate [52].

DC&R is made up of a number of active components, and an interesting relationship exists for the contribution of these components compared to the observed MICs. The MICs of each individual component was calculated and the main active component in DC&R was determined. The calculated $THN_{DC\&R}$ MICs and $Form_{DC\&R}$ MICs were lower than required for disinfection. But the calculated $BAC_{DC\&R}$ MICs were identical to those required for disinfection of the *Salmonella* strains. Therefore, the active principle component in DC&R against *Salmonella* is the BAC component. This result is similar to those previously reported for DC&R activity against other bacteria [27, 48-51], and suggests that THN and Form are not useful in DC&R for disinfection of pathogenic VRE [27], *P. aeruginosa* [48], *E. coli* O157:H7 [49], non-O157 STECs [50] and *Salmonella* isolated from turkeys [51] or here from cattle. The manufactures application rates for DC&R and Tek-Trol are 1,919 and 1,016 µg/ml, respectively, and the observed MICs for DC&R and Tek-Trol were below these application rates. However, observed MICs were very close to the suggested application rates, thus a small change in the disinfectant dilution, either by mistake in dilution preparation or with inadvertent liquid being present in the application area, could easily render these disinfectants non-functional.

Similarly, the individual concentrations of active components in P-128 *Salmonella* MICs were calculated. The P-128 active components are comprised of the BACs and C10AC. Here the calculated $BAC_{P\text{-}128}$ component concentrations are below the levels required for disinfection of the *Salmonella* serovars. The $C10AC_{P\text{-}128}$ component concentration is slightly lower than shown for all *Salmonella* strains; however, most of the strains would be disinfected using the calculated $C10AC_{P\text{-}128}$ component concentrations. Therefore, the P-128 activity against *Salmonella* can be attributed to the C10AC component, which was shown to be the primary active component in P-128 in all previous studies [27, 48-51]. C10AC is referred to in the literature as DDAC and is a common component in many disinfectants. DDAC (C10AC) significantly impaired reproductive health in mice [56, 57]. The BAC component in P-128 is not required for disinfection activity of P-128 in *Salmonella* from cattle and turkeys [51] as well as VRE [27], *P. aeruginosa* [48], *E. coli* O157:H7 [49] and non-O157 STECs [50].

The disinfectants FSS, F25, FS512 and OdoBan are largely made up of BAC components, and the observed results of these disinfectants are very similar to those for the individual BACs. The *Salmonella* MICs for BKC, CPB, CPC, CDEAB and CTAB also are similar to those for C12BAC. But to appropriately compare the BACs or other disinfectants, one must transform their MICs into molar MICs (MIC_Ms). Therefore, C14BAC and C16BAC are more active here as a disinfectant than C12BAC in *Salmonella* from cattle, turkeys [51], and in VRE [27], *P. aeruginosa* [48], *E. coli* O157:H7 [49] and non-O157 STECS [50]. Of this group of disinfectants or components, benzalkonium chloride shows the highest MIC_Ms in *Salmonella*.

Conclusion

A low prevalence of AMR was found in *Salmonella* from feedlot water-sprinkled cattle. Therefore, feedlot water-sprinkled cattle here were not a source of multidrug resistant *Salmonella*. Most common observed AMR traits were streptomycin in Kentucky, sulfamethoxazole in Muenster and Cerro and amoxicillin/clavulonic acid-ceftiofur in serovar Meleagridis. All *Salmonella* strains were susceptible to triclosan. The BACs or benzyl ammonium chlorides C12, C14 and C16 were the most active ingredient in the disinfectant DC&R. The BACs C14BAC and C16BAC were more active than C12BAC. Didecyldimethyl ammonium chloride (C10AC) was the most active ingredient in P-128, and C10AC has been the most active ammonium chloride in all our previous studies. No cross-resistance was observed. All manufacturer recommended application levels for disinfectants were above the observed MICs. However, MICs for DC&R and Tek-Trol were close to the suggested application rates and a small change in the disinfectant dilution could easily render these disinfectants non-functional. The use of THN and formaldehyde in DC&R is questionable because these components in DC&R are not effective against *Salmonella*, and their inclusion results in unwanted chemicals in the environment and may increase the production of resistance in bacteria.

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References

1. Bäumler AJ. 2004. Foodborne *Salmonella* infections. In: Beier RC, Pillai SD, Phillips TD, Ziprin RL (eds) Preharvest and postharvest food safety: contemporary issues and future directions. Blackwell publishing: Ames, IA, USA, pp 3–12.
2. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, et al. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 17(1): 7–15. https://doi.org/10.3201/eid1701.P11101
3. WHO. 2016. *Salmonella*. Available at: http://www.who.int/topics/salmonella/en/ (Accessed on: 14 September 2016).
4. CDC. 2016. *Salmonella*. Available at: https://www.cdc.gov/salmonella/ (Accessed on: 13 September 2016).
5. Hsi DJ, Ebel ED, Williams MS, Golden NJ, Schlosser WD. 2015. Comparing foodborne illness risks among meat commodities in the United States. *Food Control* 54: 353–359. https://doi.org/10.1016/j.foodcont.2015.02.018
6. Bosilevac JM, Guerini MN, Kalchayanand N, Koohmaraie M. 2009. Prevalence and characterization of Salmonellae in commercial ground beef in the United States. *Appl Environ Microbiol* 75(7): 1892–1900. https://doi.org/10.1128/AEM.02530-08
7. CDC. 2013. Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Mbandaka infections linked to tahini sesame paste (final update). Available at: http://www.cdc.gov/salmonella/montevideo-tahini-05-13/signs-symptoms.html (Accessed on: 14 September 2016).
8. CDC. 2016. Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg infections linked to Wonderful Pistachios (final update). Available at: http://www.cdc.gov/salmonella/montevideo-03-16/ (Accessed on: 14 September 2016).
9. Pakalniskiene J, Falkenhorst G, Lisby M, Madsen SB, Olsen KEP, et al. 2009. A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, Novermber 2006. *Epidemiol Infect* 137(3): 396–401. https://doi.org/10.1017/S0950268808000484
10. CDC. 2016. Eight multistate outbreaks of human *Salmonella* infections linked to live poultry in backyard flocks (Final Update). Available at: http://www.cdc.gov/salmonella/live-poultry-05-16/ (Accessed on: 13 September 2016).
11. CDC. 2011. Multistate outbreak of human *Salmonella* Agona infections linked to whole, fresh imported papayas (final update). Available at: http://www.cdc.gov/salmonella/2011/papayas-8-29-2011.html (Accessed on: 14 September 2016).
12. CDC. 1985. Epidemiologic notes and reports salmonellosis associated with carne seca – New Mexico. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00000628.htm (Accessed on: 12 September 2016).
13. Tewari D, Sandt CH, Miller DM, Jayarao BM, M'ikanatha M. 2012. Prevalence of *Salmonella* Cerro in laboratory-based submissions of cattle and comparison with human infections in Pennsylvania, 2005–2010. *Foodborne Path Dis* 9(10): 928–933. https://doi.org/10.1089/fpd.2012.1142
14. Clark M. 1996. Multistate multiple sprouts 1996. Available at: http://outbreakdatabase.com/details/multistate-multiple-sprouts-1996/? (Accessed on: 14 September 2016).
15. CDC. 2012. Multistate outbreak of *Salmonella* Typhimurium infections linked to ground beef (final update). Available at: http://www.cdc.gov/salmonella/2011/ground-beef-2-1-2012.html (Accessed on: 15 September 2016).
16. Food Safety News. 2013. Salmonella Typhimurium outbreak leads to ground beef recall. Available at: http://www.foodsafetynews.com/2013/01/two-state-salmonella-typhimurium-outbreak-leads-to-beef-recall (Accessed on: 15 September 2016).
17. CDC. 2013. Multistate outbreak of *Salmonella* Typhimurium infections linked to ground beef (final update). Available at: http://www.cdc.gov/salmonella/typhimurium-01-13/ (Accessed on: 15 September 2016).
18. Maguire H, Cowden J, Jacob M, Rowe B, Roberts D, et al. 1992. An outbreak of *Salmonella dublin* infection in England and Wales associated with a soft unpasteurized cows' milk cheese. *Epidemiol Infect* 109(3): 389–396. https://doi.org/10.1017/S0950268800050378
19. Vaillant V, Haeghebaert S, Desenclos JC, Bouvet P, Grimont F, et al. 1996. Outbreak of *Salmonella* Dublin infection in France, November-December 1995. *Euro surveillance* 1(2): 9–10.
20. Food Safety News. 2016. Patient count increasing in Kentucky Salmonella outbreak. Available at: http://www.foodsafetynews.com/2016/02/patient-count-increasing-in-kentucky-salmonella-outbreak (Accessed on: 15 September 2016).
21. CDC. 2016. Multistate outbreak of *Salmonella* infections linked to alfalfa sprouts from one contaminated seed lot (final update). Available at: http://www.cdc.gov/salmonella/muenchen-02-16/index.html (Accessed on: 15 September 2016).
22. McDermott PF, Walker RD, White DG. 2003. Antimicrobials: modes of action and mechanisms of resistance. *Int J Toxicol* 22(2): 135–143. https://doi.org/10.1080/10915810305089
23. Fraise AP. 2002. Biocide abuse and antimicrobial resistance—a cause for concern? *J Antimicrob Chemother* 49(1): 11–12. https://doi.org/10.1093/jac/49.1.11
24. Wachsmuth IK, Sparling PH, Barrett TJ, Potter ME. 1997. Enterohemorrhagic *Escherichia coli* in the United States. *FEMS Immunol Med Microbiol* 18(4): 233–239. https://doi.org/10.1016/S0928-8244(97)00053-9
25. Beier RC, Callaway TR, Andrews K, Poole TL, Crippen TL, et al. 2017. Interactions of organic acids with *Salmonella* strains from feedlot water-sprinkled cattle. *J Food Chem Nanotechnol* 3(2): 60-66. https://doi.org/10.17756/jfcn.2017-038
26. White DG, McDermott PF. 2001. Biocides, drug resistance and microbial evolution. *Curr Opin Microbiol* 4(3): 313–317. https://doi.org/10.1016/S1369-5274(00)00209-5
27. Beier RC, Duke SE, Ziprin RL, Harvey RB, Hume ME, et al. 2008. Antibiotic and disinfectant susceptibility profiles of vancomycin-resistant *Enterococcus faecium* (VRE) isolated from community wastewater in Texas. *Bull Environ Contam Toxicol* 80(3): 188–194. https://doi.org/10.1007/s00128-007-9342-0
28. Chapman JS. 2003. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int Biodeterior Biodegradation* 51(4): 271–276. https://doi.org/10.1016/S0964-8305(03)00044-1
29. Capita R, Riesco-Peláez F, Alonso-Hernando A, Alonso-Calleja C. 2014. Exposure of *Escherichia coli* ATCC 12806 to sublethal concentrations of food-grade biocides influences its ability to form biofilm, resistance to antimicrobials, and ultrastructure. *Appl Environ Microbiol* 80(4): 1268–1280. https://doi.org/10.1128/AEM.02283-13
30. Cohen SH, Morita MM, Bardford MA. 1991. A seven-year experience with methicillin-resistant *Staphylococcus aureus*. *Am J Med* 91(3B): 224S–237S. https://doi.org/10.1016/0002-9343(91)90374-7
31. Hartmann FA, Trostle SS, Klohnen AAO. 1997. Isolation of methicillin-resistant *Staphylococcus aureus* from a postoperative wound infection in a horse. *J Am Vet Med Assoc* 211(5): 590–592.
32. McDonnell G, Russell AD. 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 12(1): 147–179.
33. Russell AD. 2002. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol* 92(Suppl): 121S–135S. https://doi.org/10.1046/j.1365-2672.92.5s1.12.x
34. Maris P. 1991. Resistance of 700 gram-negative bacterial strains to antiseptics and antibiotics. *Ann Rech Vet* 22(1): 11–23.

35. Al-Jailawi MH, Ameen RS, Al-Jeboori MR. 2013. Effect of disinfectants on antibiotics susceptibility of *Pseudomonas aeruginosa*. *J Appl Biotechnol* 1(1): 54–63. https://doi.org/10.5296/jab.v1i1.4038

36. Beier RC, Bischoff KM, Ziprin RL, Poole TL, Nisbet DJ. 2005. Chlorhexidine susceptibility, virulence factors, and antibiotic resistance of beta-hemolytic *Escherichia coli* isolated from neonatal swine with diarrhea. *Bull Environ Contam Toxicol* 75(5): 835–844. https://doi.org/10.1007/s00128-005-0826-5

37. Braoudaki M, Hilton AC. 2004. Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *J Clin Microbiol* 42(1): 73–78.

38. Sidhu MS, Heir E, Leegaard T, Wiger K, Holck A. 2002. Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn552 among clinical staphylococci. *Antimicrob Agents Chemother* 46(9): 2797–2803. https://doi.org/10.1128/AAC.46.9.2797-2803.2002

39. Morrow JL, Mitloehner FM, Johnson AK, Galyean ML, Dailey JW, et al. 2005. Effect of water sprinkling on incidence of zoonotic pathogens in feedlot cattle. *J Anim Sci* 83(8): 1959–1966.

40. Clinical and Laboratory Standards Institute (CLSI). 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-Ninth edition. Approved standard, M7-A9. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

41. Clinical and Laboratory Standards Institute (CLSI). 2014. Performance standards for antimicrobial susceptibility testing. 15th informational supplement. M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

42. Andrews JM. 2001. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* 48(Suppl 1): 5–16.

43. Rubin J, Walker RD, Blickenstaff K, Bodeis-Jones S, Zhao S. 2008. Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of *Pseudomonas aeruginosa* isolated from canine infections. *Vet Microbiol* 131(1–2): 164–172. https://doi.org/10.1016/j.vetmic.2008.02.018

44. Heath RJ, Rock CO. 2000. A triclosan-resistant bacterial enzyme. *Nature* 406(6792): 145–146. https://doi.org/10.1038/35018162

45. Cheung H-Y, Wong MM-K, Cheung S-H, Liang LY, Lam Y-W, et al. 2012. Differential actions of chlorhexidine on the cell wall of *Bacillus subtilis* and *Escherichia coli*. *PLoS One* 7(5): e36659. https://doi.org/10.1371/journal.pone.0036659

46. Leelaporn A, Paulsen IT, Tennent JM, Littlejohn TG, Skurray RA. 1994. Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. *J Med Microbiol* 40(3): 214–220. https://doi.org/10.1099/00222615-40-3-214

47. Sidhu MS, Sørum H, Holck A. 2002. Resistance to quaternary ammonium compounds in food-related bacteria. *Microb Drug Resist* 8(4): 393–399. https://doi.org/10.1089/10766290260469679

48. Beier RC, Foley SL, Davidson MK, White DG, McDermott PF, et al. 2014. Characterization of antibiotic and disinfectant susceptibility profiles among *Pseudomonas aeruginosa* veterinary isolates recovered during 1994–2003. *J Appl Microbiol* 118(2): 326–342. https://doi.org/10.1111/jam.12707

49. Beier RC, Poole TL, Brichta-Harhay DM, Anderson RC, Bischoff KM, et al. 2013. Disinfectant and antibiotic susceptibility profiles of *Escherichia coli* O157:H7 strains from cattle carcasses, feces, and hides and ground beef from the United States. *J Food Protect* 76(1): 6–17. https://doi.org/10.4315/0362-028X.JFP-12-253

50. Beier RC, Franz E, Bono JL, Mandrell RE, Fratamico PM, et al. 2016. Disinfectant and antimicrobial susceptibility profiles of the big six non-O157 Shiga toxin-producing *Escherichia coli* strains from food animals and humans. *J Food Protect* 79(8): 1355–1370. https://doi.org/10.4315/0362-028X.JFP-15-600

51. Beier RC, Anderson PN, Hume ME, Poole TL, Duke SE, et al. 2011. Characterization of *Salmonella enterica* isolates from turkeys in commercial processing plants for resistance to antibiotics, disinfectants, and a growth promoter. *Foodborne Pathog Dis* 8(5): 593–600. https://doi.org/10.1089/fpd.2010.0702

52. Martínez-Martínez S, Yubero-Delgado S, Rodríguez-Ferri E-F, Frandoloso R, Álvarez-Estrada Á, et al. 2016. *In vitro* efficacy of several disinfectants against *Salmonella enterica* serovars Enteritidis and *Escherichia coli* strains from poultry. *Cienc. Rural* 48(8): 1438–1442. https://doi.org/10.1590/0103-8478cr20151288

53. Braoudaki M, Hilton AC. 2004. Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *J Clin Microbiol* 42(1): 73–78. https://doi.org/10.1128/JCM.42.1.73-78.2004

54. Aarestrup FM, Hasman H. 2004. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. *Vet Microbiol* 100(1–2): 83–89. https://doi.org/10.1016/j.vetmic.2004.01.013

55. Block SS. 2001. Disinfection, Sterilization, and Preservation, 5th edition, Lippincott Williams and Wikins, Philadelphia, PA, USA p 325

56. Maher B. 2008. Cage-cleaning chemicals cause birth defects and fertility problems in mice. *Nature* 453: 964. https://doi.org/10.1038/453964a

57. Melin VE, Potineni H, Hunt P, Griswold J, Siems B, et al. 2014. Exposure to common quaternary ammonium disinfectants decreases fertility in mice. *Reprod Toxicol* 50: 163–170. https://doi.org/10.1016/j.reprotox.2014.07.071