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Research Note

Chlorine Inactivation of Nonresistant and Antibiotic-Resistant Strains of *Salmonella* Typhimurium Isolated from Chicken Carcasses†

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ABSTRACT

A study was conducted to test the hypothesis that strains of *Salmonella* Typhimurium that are resistant to antibiotics are more resistant to chlorine in chilled water than strains of *Salmonella* Typhimurium that are not resistant to antibiotics. To test this hypothesis, strains ($n = 16$) of *Salmonella* Typhimurium with four antibiotic resistance profiles were tested for their inactivation kinetics in chlorinated (30 ppm, pH 6) water at 4°C. The four antibiotic resistance profiles were (i) none; (ii) tetracycline-sulfisoxazole (T-Su); (iii) tetracycline-ampicillin-amoxicillin-cefoxitin-ceftiofur-sulfisoxazole (T-A-Am-C-Ce-Su); and (iv) tetracycline-ampicillin-amoxicillin-cefoxitin-ceftiofur-sulfisoxazole-kanamycin (T-A-Am-C-Ce-Su-K). Inactivation of *Salmonella* Typhimurium in chlorinated water displayed nonlinear kinetics with a concave downward curve that fit well ($R^2 = 0.964$) to the power law model, with a shape parameter of 1.37. The time for a single log reduction (D-value) of *Salmonella* Typhimurium from an initial concentration of 5.36 log/ml did not differ ($P > 0.05$) among the four antibiotic resistance groups and ranged from 3.8 to 4.3 min for $n = 4$ strains per group. Thus, the hypothesis was rejected, and it was concluded that expression of an antibiotic resistance phenotype does not confer cross-protection in *Salmonella* Typhimurium to chlorine inactivation in chilled water.

The poultry industry is taking a comprehensive approach to protect consumers from exposure to human disease–causing bacteria that might contaminate poultry during any step of the farm-to-table chain *(12)*. Central to this control strategy is the application of antimicrobial compounds throughout the farm-to-table continuum to reduce the prevalence of human pathogens on poultry. However, a potential unintended consequence of this approach is that the use of an antimicrobial compound might cause expression of genes in human pathogens that result in cross-protection to other antimicrobials *(4, 10)* that might be applied at a subsequent step in the farm-to-table chain, thus rendering the strategy of antimicrobial application ineffective or less effective.

Antibiotics are used on the farm to treat subclinical and clinical infections of poultry and, in the past, were used to promote growth and feed efficiency *(13)*. Use of antibiotics in live poultry that harbor human pathogens could induce expression of defense mechanisms in the pathogen that make them more resistant to disinfectants, such as chlorine, that are applied later during poultry processing *(7, 11)*. Specifically, previous exposure to antibiotics could reduce expression of outer membrane proteins (i.e., porins) and increase expression of efflux pumps, resulting in decreased uptake and increased export, respectively, of disinfectant molecules *(11)*. Thus, the objective of the current study with *Salmonella* Typhimurium was to test the hypothesis that strains resistant to antibiotics are more resistant to chlorine in chilled water than strains that are not resistant to antibiotics. Serotype Typhimurium was selected because it is one of the predominant serotypes of *Salmonella* that infects poultry and causes clinical cases of human foodborne illness *(2, 3)*.

MATERIALS AND METHODS

***Salmonella*.** Sixteen isolates of *Salmonella* Typhimurium were selected from a culture collection obtained in a previous study *(8)*. Selection was based on their pattern of resistance to 15 antibiotics. Four isolates were susceptible to all 15 antibiotics tested (none), four isolates were resistant to two antibiotics (tetracycline and sulfisoxazole; T-Su), four isolates were resistant to six antibiotics (tetracycline, ampicillin, amoxicillin, cefoxitin, ceftiofur, and sulfisoxazole; T-A-Am-C-Ce-Su), and four isolates were resistant to seven antibiotics (tetracycline, ampicillin, amoxicillin, cefoxitin, ceftiofur, sulfisoxazole, and kanamycin; T-A-Am-C-Ce-Su-K). All 16 isolates displayed different patterns

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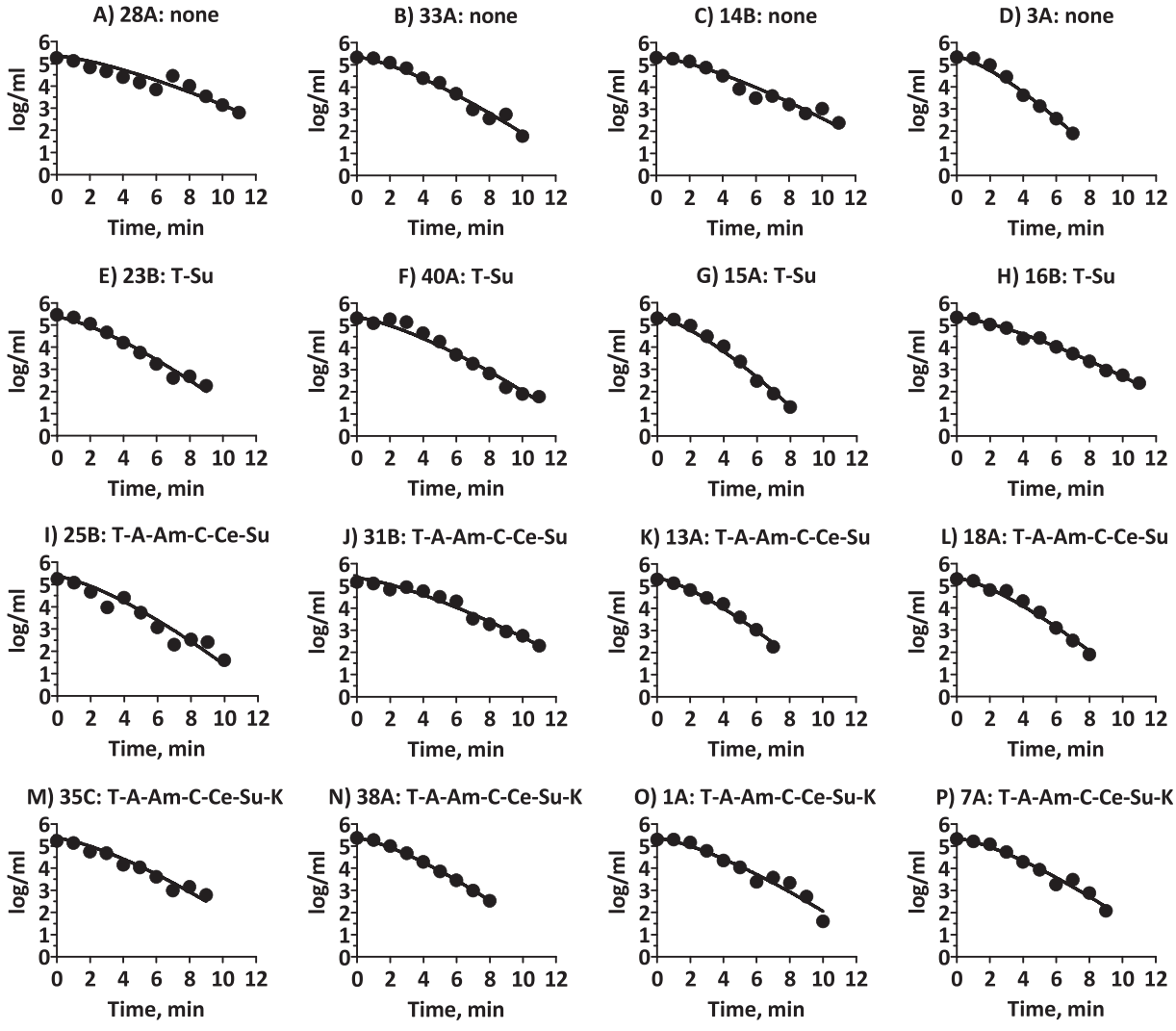

FIGURE 1. *Survival of nonresistant and antibiotic-resistant strains of* Salmonella *Typhimurium in chlorinated (30 ppm, pH 6) and chilled (4°C) water. Symbols are means, and lines are best-fit lines.*

when analyzed by pulsed-field gel electrophoresis *(6)* and, thus, represented different strains. Stock cultures of the strains were maintained at −70°C in brain heart infusion broth (BBL, Difco, BD, Sparks, MD) that contained 15% (vol/vol) glycerol (Sigma, St. Louis, MO).

Cultures for inoculation. Five microliters of the stock cultures was inoculated into 5 ml of brain heart infusion broth, which was then incubated overnight at 30°C and 150 rpm to obtain stationary-phase cells for inoculation of chlorinated water. One hundred microliters of a 10^{-1} dilution of the overnight culture in buffered peptone water (BPW; Difco, BD) was inoculated into 100 ml of chlorinated water to initiate the inactivation experiments. The average initial concentration of *Salmonella* in the chlorinated water was 5.36 log/ml.

Chlorinated water. BPW (2 ml) was added to 98 ml of sterilized spring water (Eastern Springs Water Company, Preston, MD) in a 250-ml Erlenmeyer flask, followed by addition of, on average, 32 μl (range: 26 to 41 μl) of sodium hypochlorite solution (Intercostal Trading Inc., Secretary, MD) and, on average, 71 μl (range: 65 to 77 μl) of 1 M acetic acid to produce a chlorinated water solution with 30 ppm of residual chlorine and with an average pH of 6 (range: 5.6 to 6.2). Residual chlorine levels and pH were measured using a chlorine test kit (model PCT-DR, LaMotte, Chestertown, MD) and pH meter (pH Spear, Oakton Instruments, Vernon Hill, IL), respectively. The BPW (0.21 mg/ml) was included in the chlorinated water to provide organic material to reduce the activity of the chlorine and slow the death of *Salmonella* Typhimurium so that the inactivation kinetics could be measured by spiral plating.

Viable counts. At 1-min intervals (from 0 to 11 min after inoculation), viable counts of *Salmonella* Typhimurium in the chlorinated water (4°C) were determined by spiral plating (Whitely Automated Spiral Plater, Microbiology International, Frederick, MD) appropriate serial dilutions (1:10) in BPW onto brain heart infusion agar (Difco, BD). Dilution of the samples into BPW was found to neutralize the chlorine and halt the inactivation of *Salmonella* Typhimurium, as reported previously for tryptic soy broth with yeast extract *(14)*. Agar plates were incubated at 35°C for 18 to 24 h before automated counting of colonies (Protocol Automated Colony Counter, Microbiology International). Four replicate trials were conducted per strain of *Salmonella* Typhimurium, for a total of 64 trials (16 strains × 4 replicates).

Curve fitting. Viable counts (log per milliliter) were combined among the four trials per strain and graphed as a

TABLE 1. *Chlorine inactivation kinetics of nonresistant and antibiotic-resistant strains of* Salmonella *Typhimurium*[a]

Antibiotic resistance	Strain	*D* (min)[b]	R^2	Sy.x	*n*
None	28A	5.60 ± 0.24	0.865	0.281	12
None	33A	4.05 ± 0.09	0.976	0.190	11
None	14B	4.75 ± 0.14	0.945	0.244	12
None	3A	2.84 ± 0.05	0.988	0.140	8
T-Su	23B	3.72 ± 0.09	0.974	0.194	10
T-Su	40A	4.16 ± 0.09	0.975	0.217	12
T-Su	15A	2.89 ± 0.05	0.989	0.158	9
T-Su	16B	4.88 ± 0.05	0.994	0.079	12
T-A-Am-C-Ce-Su	25B	3.66 ± 0.14	0.914	0.362	11
T-A-Am-C-Ce-Su	31B	4.88 ± 0.11	0.973	0.167	12
T-A-Am-C-Ce-Su	13A	3.20 ± 0.05	0.990	0.106	8
T-A-Am-C-Ce-Su	18A	3.34 ± 0.07	0.983	0.158	9
T-A-Am-C-Ce-Su-K	35C	4.18 ± 0.14	0.940	0.218	10
T-A-Am-C-Ce-Su-K	38A	3.75 ± 0.02	0.998	0.039	9
T-A-Am-C-Ce-Su-K	1A	4.18 ± 0.13	0.953	0.254	11
T-A-Am-C-Ce-Su-K	7A	3.94 ± 0.10	0.974	0.176	10

[a] R^2, coefficient of determination; sy.x, standard deviation of the residuals; *n,* number of data points in the curve fit; T, tetracycline; Su, sulfisoxazole; A, ampicillin; Am, amoxicillin; C, cefoxitin; Ce, ceftiofur; K, kanamycin.
[b] Time for a 1-log reduction; values are mean ± standard error.

function of time (minutes). The data were fitted (version 5.0, Prism, GraphPad Software Inc., San Diego, CA) to a power law model *(9)* using the mean values at each sampling time:

$$N(t) = N_0 - \left(\left(\frac{t}{D} \right)^p \right)$$

where $N(t)$ is the concentration of *Salmonella* Typhimurium (log/ml) at time (t) in min, N_0 is the initial concentration of *Salmonella* Typhimurium (log/ml), D is the time (min) required for a 1-log reduction of *Salmonella* Typhimurium, and p is the shape parameter (unitless). When $p = 1$, the curve fit is a straight line; when $p < 1$, the curve fit is a concave upward line; and when $p > 1$, the curve fit is a concave downward line. N_0 was fixed at 5.36 log/ml (i.e., mean value among initial curve fits), and p was fixed at 1.37 (i.e., mean value among initial curve fits) during curve fitting to facilitate comparison of D-values among antibiotic-resistant groups.

Statistical analysis. One-way analysis of variance was used to compare D-values from the final curve fits as a function of the antibiotic resistance pattern: (i) none; (ii) T-Su; (iii) T-A-Am-C-Ce-Su; or (iv) T-A-Am-C-Ce-Su-K. There were four replicate values (one per strain) for D-values per antibiotic resistance group. Dunnet's multiple comparison test was used to make three comparisons among mean values of D as follows: (i) none versus T-Su; (ii) none versus T-A-Am-C-Ce-Su; and (iii) none versus T-A-Am-C-Ce-Su-K. The effect of the pattern of antibiotic resistance on values of D was considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Sodium hypochlorite at 20 to 50 ppm is a common disinfectant added to water used for processing broiler chickens in the United States. An organic acid, such as acetic acid, is often added to the process water to lower the pH and increase the disinfection potency of the chlorine. As the processing shifts progress, organic material from the chicken carcasses builds up in process waters and reaches a steady state. Chlorine is believed to reduce cross-contamination of chicken carcasses with *Salmonella* and other pathogens by killing pathogens released into the process water; it has been found to have minimal effects on pathogens attached to the chicken because of its rapid inactivation by the organic material associated with the carcass *(1, 5)*.

Because we did not have access to process water from a commercial plant, simulated process water was created by adding sodium hypochlorite, acetic acid, and peptone (i.e., organic material) to sterilized water. This provided a controlled environment in which to test the hypothesis that *Salmonella* Typhimurium pathogens that are resistant to antibiotics are more resistant to chlorine in chilled water than *Salmonella* Typhimurium pathogens that are not resistant to antibiotics.

In our previous study *(8), Salmonella* pathogens were isolated from broiler chicken carcasses collected before and after immersion chilling in a commercial processing plant. The main serotypes of *Salmonella* isolated were Kentucky (59.5%) and Typhimurium (17.8%). A majority of the isolates (53.1%) were resistant to three or more antibiotics. The primary antibiotic resistance profiles for the isolates of *Salmonella* Typhimurium were (i) T-Su (21.1%); (ii) T-A-Am-C-Ce-Su (31.6%); and (iii) T-A-Am-C-Ce-Su-K (23.7%). These were the antibiotic resistance profiles tested in the present study.

The survival of *Salmonella* Typhimurium in chlorinated water in the present study was found to display nonlinear inactivation kinetics, with a concave downward curve that fit well ($R^2 = 0.964$) to the power law model with a shape parameter of 1.37 (Fig. 1). The time for a single log reduction (D-value) of *Salmonella* Typhimurium from an initial average concentration of 5.36 log/ml did not differ ($P > 0.05$) among the four antibiotic resistance groups: (i) none = 4.3 ± 1.2 (mean ± SD) min; (ii) T-Su = 3.9 ± 0.8 min; (iii) T-A-Am-C-Ce-Su = 3.8 ± 0.8 min; and (iv) T-A-Am-C-Ce-Su-K = 4 ± 0.2 min, with $n = 4$ strains

per antibiotic resistance group (Table 1). These results are similar to those of Yang et al. *(15),* who reported *D*-values of 3.4 and 5.8 min for survival of *Salmonella* Typhimurium in chilled, chlorinated (30 ppm) water collected at 0 and 8 h of processing, respectively, in a commercial chicken processing plant.

Thus, the hypothesis was rejected, and it was concluded that expression of an antibiotic resistance phenotype does not confer cross-protection in *Salmonella* Typhimurium to chlorine inactivation in chilled water. These results agree with those of our previous study *(8),* in which we did not observe an increase in persistence of antibiotic-resistant *Salmonella* during immersion chilling in a commercial processing plant.

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REFERENCES

1. Campbell, D. F., R. W. Johnston, G. S. Campbell, D. McClain, and J. F. Macaluso. 1983. The microbiology of raw, eviscerated chickens: a ten year comparison. *Poult. Sci.* 62:437–444.
2. Foley, S. L., and A. M. Lynne. 2008. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. *J. Anim. Sci.* 86:E173–E187.
3. Jones, T. F., L. A. Ingram, P. R. Cieslak, D. J. Vugia, M. Tobin-D'Angelo, S. Hurd, C. Medus, A. Cronquist, and F. J. Angulo. 2008. Salmonellosis outcomes differ substantially by serotype. *J. Infect. Dis.* 198:109–114.
4. Kunonga, N. I., R. J. Sobieski, and S. S. Crupper. 2000. Prevalence of the multiple antibiotic resistance operon (marRAB) in the genus *Salmonella*. *FEMS Microbiol. Lett.* 187:155–160.
5. Lillard, H. S. 1993. Bactericidal effect of chlorine on attached *Salmonellae* with and without sonification. *J. Food Prot.* 56:716–717.
6. Mohamed, T. 2010. Characterization of antibiotic resistant *Salmonella* Typhimurium and *Salmonella* Kentucky isolated from pre- and post-chill whole broiler chickens. Ph.D. dissertation. University of Maryland Eastern Shore, Princess Anne.
7. Moken, M. C., L. M. McMurry, and S. B. Levy. 1997. Selection of multiple-antibiotic-resistant (mar) mutants of *Escherichia coli* by using the disinfectant pine oil: roles of the *mar* and *acrAB* loci. *Antimicrob. Agents Chemother.* 41:2770–2772.
8. Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and antimicrobial resistance of *Salmonella* recovered from processed poultry. *J. Food Prot.* 70: 2466–2472.
9. Piyasena, P., S. Liou, and R. C. McKellar. 1998. Predictive modelling of inactivation of *Listeria* spp. in bovine milk during high-temperature short-time pasteurization. *Int. J. Food Microbiol.* 39: 167–173.
10. Potenski, C. J., M. Gandhi, and K. R. Matthews. 2003. Exposure of *Salmonella* Enteritidis to chlorine or food preservatives increases susceptibility to antibiotics. *FEMS Microbiol. Lett.* 220:181–186.
11. Randall, L. P., S. W. Cooles, A. R. Sayers, and M. J. Woodward. 2001. Association between cyclohexane resistance in *Salmonella* of different serovars and increased resistance to multiple antibiotics, disinfectants and dyes. *J. Med. Microbiol.* 50:919–924.
12. Russell, S. M. 2012. Controlling *Salmonella* in poultry production and processing. CRC Press, Boca Raton, FL.
13. Vandeplas, S., D. R. Dubois, Y. Beckers, P. Thonart, and A. Thewis. 2010. *Salmonella* in chicken: current and developing strategies to reduce contamination at farm level. *J. Food Prot.* 73:774–785.
14. Virto, R., D. Sanz, I. Alvarez, S. Condon, and J. Raso. 2004. Relationship between inactivation kinetics of a *Listeria monocytogenes* suspension by chlorine and its chlorine demand. *J. Appl. Microbiol.* 97:1281–1288.
15. Yang, H., Y. Li, and M. G. Johnson. 2001. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *J. Food Prot.* 64:770–776.