Thermal Inactivation of Susceptible and Multiantimicrobial-Resistant Salmonella Strains Grown in the Absence or Presence of Glucose

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The heat resistance of susceptible and multiantimicrobial-resistant Salmonella strains grown to stationary phase in glucose-free tryptic soy broth supplemented with 0.6% yeast extract (TSBYE-G; nonadapted), in regular (0.25% glucose) TSBYE, or in TSBYE-G with 1.00% added glucose (TSBYE+G; acid adapted) was determined at 55, 57, 59, and 61°C. Cultures were heated in sterile 0.1% buffered peptone water (50 μl) in heat-sealed capillary tubes immersed in a thermostatically controlled circulating-water bath. Decimal reduction times (D values) were calculated from survival curves having $r^2$ values of >0.90 as a means of comparing thermal tolerance among variables. $D_{90}$ values increased ($P < 0.05$) from 0.50 to 0.58 to 0.66 min for TSBYE-G, TSBYE, and TSBYE+G cultures, respectively. $D_{60}$ values of antimicrobial-susceptible Salmonella strains increased ($P < 0.05$) from 0.14 to 0.19 as the glucose concentration increased from 0.00 to 1.00%, respectively, while $D_{60}$ values of multiantimicrobial-resistant Salmonella strains did not differ ($P > 0.05$) between TSBYE-G and TSBYE+G cultures. When averaged across glucose levels and temperatures, there were no differences ($P > 0.05$) between the $D$ values of susceptible and multiantimicrobial-resistant inocula. Collectively, $D$ values ranged from 4.23 to 5.39, 1.47 to 1.81, 0.50 to 0.66, and 0.16 to 0.20 min for Salmonella strains inactivated at 55, 57, 59, and 61°C, respectively. $z_D$ values were 1.20, 1.48, and 1.49°C for Salmonella strains grown in TSBYE+G, TSBYE, and TSBYE-G, respectively, while the corresponding activation energies of inactivation were 497, 493, and 494 kJ/mol. Study results suggested a cross-protective effect of acid adaptation on thermal inactivation but no association between antimicrobial susceptibility and the ability of salmonellae to survive heat stress.

Exposure to heat has long been recognized as a primary method for preserving foods, typically resulting in the production of either a pasteurized or a commercially sterile food product, or the more classic cooking of food in preparation for consumption (7). Characteristic survival models describe the effects of heat stress on bacterial populations in terms of decimal reduction time ($D$ value); that is, thermal inactivation is generally known to increase exponentially with an increase in temperature (14).

Various factors may influence the heat resistance of microorganisms. Inherently, different types of microorganisms subjected to identical heat stress conditions react differently, as some are able to withstand higher temperatures for extended periods of time (4, 33). Heat resistance also is affected by various environmental influences during growth and formation of cells or spores prior to heat stress, such as, among others, metabolic phase, growth temperature, and nutrient availability (33). It has been reported that heat shocking of Escherichia coli O157:H7, Salmonella enterica serovar Typhimurium, Listeria monocytogenes, and Aeromonas hydrophila cells during exponential growth resulted in increased thermal resistance (11, 20, 22, 37). Additionally, protective mechanisms allowing for an adaptive response to low-pH exposure have been reported among several microorganisms, including salmonellae (12).

Among Salmonella serotypes, S. enterica serovar Typhimurium has received the most attention regarding its acid-adaptive capabilities (13, 26). S. enterica serovar Typhimurium possesses two growth phase-dependent, low-pH-inducible systems, each capable of mounting an acid tolerance response (ATR; 12, 13). In addition to the protection provided against subsequent low-pH exposure, acid-adapted S. enterica serovar Typhimurium may exhibit cross-protection against heat, osmotic, and oxidative stresses (13, 24, 27, 42). This stress-induced cross-protection phenomenon also has been reported among other bacteria, including E. coli O157:H7 and L. monocytogenes (6, 9). Lastly, environmental factors during heat exposure, such as carbohydrate availability, water activity and salt concentration, pH, and the presence of organic or inorganic compounds, in addition to conditions during cell recovery, influence the ability of microorganisms to survive or repair damage associated with heat stress (33). For example, Junjea et al. (19) reported increased resistance in salmonellae subjected to thermal stress in ground meat compared to those subjected to thermal stress in chicken broth.

In recent years, the recognition or emergence of antimicrobial-resistant bacterial pathogens has resulted in worldwide concern focused on the potential for increased morbidity and mortality resulting from failing antimicrobial treatments (39). In the United States, the reported prevalence of Salmonella strains resistant to a minimum of one antimicrobial increased from 16% in 1980 to 37% in 1996, the year of the first recog-

* Corresponding author. Mailing address: Center for Red Meat Safety, Department of Animal Sciences, Colorado State University, Ft. Collins, CO 80523-1171. Phone: (970) 491-7703, Fax: (970) 491-0278. E-mail: John.Sofos@colorstate.edu.
nized outbreak involving a multiantimicrobial-resistant *Salmonella* strain (*S. enterica* serovar Typhimurium DT104; P. Dabney, C. Bopp, F. Tenover, L. Tollefson, F. Angulo, and The NAMS Working Group, abstr. 97th Gen. Meet. Am. Soc. Microbiol., abstr. C-136, p. 144, 1997; 29). More recently, antimicrobial susceptibility to 17 antimicrobials used by the National Antimicrobial Resistance Monitoring System has been determined for *Salmonella* strains isolated from ground meats (i.e., chicken, turkey, pork, and beef) sampled at retail supermarkets in the northeastern United States (38, 41). Of 45 isolates, 84.4% (n = 38) and 53.3% (n = 24) were resistant to at least one and three of the antimicrobials, respectively, while 15.6% (n = 7) were simultaneously resistant to at least nine (41). In addition to ground meats, multiantimicrobial-resistant *Salmonella* strains have been isolated from numerous other domestic and imported foods, including sprouts, spices, frozen seafood, freshwater fish, ice cream, meat, bone meal, herbs, cheese, and lettuce (23). In a study evaluating the antimicrobial susceptibility of 502 *Salmonella* strains isolated from various foods and associated samples between 1999 and 2000, 49.2% (n = 247) were resistant to at least one of the antimicrobials tested, and of those, 31.2% (n = 77) were multiantimicrobial resistant (23).

The presence of viable susceptible and antimicrobial-resistant bacterial pathogens at the retail level demonstrates the importance of safe product handling and proper preparation during cooking. Information regarding the relationship between antimicrobial susceptibility and the ability to resist heat stress is lacking. Therefore, the objective of this study was to determine if heat resistance differences exist between inocula of susceptible and multiantimicrobial-resistant *Salmonella* prepared under acid tolerance-inducing and noninducing conditions.

### MATERIALS AND METHODS

**Salmonella strains.** The *Salmonella* strains used in this study (Table 1) were previously isolated, and their susceptibility to 13 antimicrobial agents was determined (2). Briefly, all of the strains, with the exception of *S. enterica* serovar Typhimurium DT104 ATCC 700408, were isolated from animal hides following stunning and exsanguination, but before hide opening and subsequent hide removal processes, in four commercial beef-packing plants. Following isolate confirmation (API 20E; bioMérieux, Hazelwood, Mo.), antimicrobial susceptibility was determined by the disk diffusion method in accordance with experimental standards and procedures established by the NCCLS (3, 31, 32). It is worth noting that in 2002, NCCLS guidelines were republished. Antimicrobial drug selection, agent concentration, zone-of-inhibition categorization, and utilization of quality control organisms were based, where appropriate, on the emergence of drug-resistant *S. enterica* serovar Typhimurium DT104 (1, 10, 40), previously published recommendations for routine testing by veterinary diagnostic laboratories (32), and the antimicrobial-containing disk (BBL Semi-Disk) manufacturer’s (Becton Dickinson, Sparks, Md.) recommendations. Antimicrobial susceptibility to the following agents (concentration[s]) was determined: amoxicillin and clavulanic acid (20 and 10 μg/ml), ampicillin (10 μg/ml), amoxicillin-clavulanic acid (30 μg/ml), chloramphenicol (30 μg/ml), ciprofloxacin (5 μg/ml), enrofloxacin (5 μg/ml), gentamicin (10 μg/ml), levofloxacin (5 μg/ml), streptomycin (10 μg/ml), sulfisoxazole (250 μg/ml), tetracycline (30 μg/ml), and the combination of trimethoprim and sulfamethoxazole (1.25 and 23.75 μg/ml) (2). Serotype confirmation and subsequent phase typing of *S. enterica* serovar Typhimurium were performed at the National Veterinary Services Laboratory, U.S. Department of Agriculture, Ames, Iowa (2).

**Culture preparation.** The culture media used in this study included glucose-free tryptic soy broth (pH 7.3) supplemented with 0.6% yeast extract (TSBYE-G), commercial TSBYE containing 0.25% glucose (TSBYE), and glucose-free TSBYE supplemented with 1.00% glucose (TSBYE+G; Diño Laboratories, Becton Dickinson). Working stock cultures were subcultured twice, each time for 18 h at 37°C in 9 ml of TSBYE, at which time *Salmonella* strains were individually grown for 18 h at 37°C in 9 ml of TSBYE–G, TSBYE, and TSBYE+G to produce stationary-phase cells exhibiting nonadapted and induced acid tolerance characteristics (5, 42). Following overnight incubation (37°C), a homogeneous 0.2-ml aliquot was removed from each culture. The cells were harvested by centrifugation (3 min at 13,000 × g) and washed in an equal volume of sterile 0.1% (wt/vol) buffered peptone water (Diño Laboratories, Becton Dickinson). After a second washing, the cell fractions of each strain were combined and resuspended to approximately 10^9 cells/ml in sterile 0.1% buffered peptone water, thereby producing six five-strain culture compositions, each of the susceptible and multiantimicrobial-resistant *Salmonella* strains, each grown in the presence or absence of the fermentable carbohydrate (i.e., glucose at 0.25 or 1.00%).

**Heat resistance.** Thermal inactivation experiments were performed in accordance with previously reported procedures for evaluation of the thermal resistance of *Salmonella* (4, 21), *L. monocytogenes* (11, 28), and *Aeromonas hydrophila* (37) strains in liquid whole eggs, egg fractions, and phosphate buffer. The capillary tubes (Kimax-51; 0.8 to 1.1 mm) were wound with paraffin and then washed with a long, thin, hypodermic needle with a deflected (septum) point (Popper & Sons, Inc., New Hyde Park, N.Y.). The capillary tubes were manually heat sealed with a propane torch, and care was taken to avoid heating the cell suspension. Immediately after sealing, capillary tubes were suspended in a thermosatically controlled circulat-

<table>
<thead>
<tr>
<th>TABLE 1. Serotypes and antimicrobial susceptibility patterns of susceptible and multiantimicrobial-resistant <em>Salmonella</em> strains used in this study</th>
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<tbody>
<tr>
<td><strong>Salmonella serotype</strong></td>
</tr>
<tr>
<td>Susceptible*</td>
</tr>
<tr>
<td>Saint-Paul</td>
</tr>
<tr>
<td>Anatum</td>
</tr>
<tr>
<td>Mbonda</td>
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<tr>
<td>Agona</td>
</tr>
<tr>
<td>Agona</td>
</tr>
<tr>
<td>Resistant†</td>
</tr>
<tr>
<td>Typhimurium DT104 (ATCC 700408)</td>
</tr>
<tr>
<td>Reading</td>
</tr>
<tr>
<td>Typhimurium DT104 (var. Copenhagen)</td>
</tr>
<tr>
<td>Typhimurium DT104 (var. Copenhagen)</td>
</tr>
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</table>

* With the exception of *S. enterica* serovar Typhimurium DT104 ATCC 700408, all of the *Salmonella* strains used in this study were derived from beef animal hide samples collected in four commercial packing plants.

† ACSSuT, ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline.
ing-water bath (Isotemp 2013S; Fisher Scientific, Pittsburgh, Pa.). At each temperature and glucose level, susceptible and multiantimicrobial-resistant cultures were simultaneously challenged. Capillary tubes were completely submerged in the water bath with a suspended, screen-covered test tube rack. For each cell suspension at each challenge temperature, duplicate capillary tubes were removed at equally spaced time intervals for each of the following challenge temperatures. (i) At 55°C, samples were removed at 0, 3, 6, 9, 12, 15, and 18 min. (ii) At 57°C, samples were removed at 0, 45, 90, 135, 180, 225, and 270 s. (iii) At 59°C, samples were removed at 0, 30, 60, 90, 120, 150, and 180 s. (iv) At 61°C, samples were removed at 0, 10, 20, 30, 40, and 50 s. After heating, capillary tubes were cooled in a beaker containing an ice-water mixture and then sanitized by immersion in sodium hypochlorite (500 ppm, pH 6.5). Residual sanitizer was removed by two sequential immersions in sterile, distilled water. Following rinsing, each capillary tube was aseptically transferred with sterile forceps into a test tube (16 by 125 mm) containing 9 ml of sterile 0.1% buffered peptone water. Capillary tubes were finely crushed with a sterile glass rod while contained within the test tubes (16 by 125 mm). A homogeneous sample was then removed, and serial dilutions were made with sterile 0.1% buffered peptone water. Viable-cell populations were enumerated by plating (0.1 ml) appropriate dilutions, in duplicate, on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) and 1.0% sodium pyruvate (TSAYE/H11001P; Difco Laboratories, Becton Dickinson). Plates were incubated at 28 ± 1°C for 72 h, and then colonies were manually counted and recorded as numbers of CFU per milliliter.

Data analysis. Thermal inactivation experiments were performed in triplicate, during each of which duplicate samples (capillary tubes) were removed and analyzed at each sampling time. Resulting Salmonella populations (numbers of CFU per milliliter) were transformed to log 10 numbers of CFU per milliliter for statistical analyses. The minimum detection limit for the plated sample volume (0.1 ml) was 3.3 log CFU/ml (1 CFU/ml); this was based on the maximum sensitivity, without further dilution, of the sample contained in the capillary tube (50 μl) after the tube was crushed. Samples without detectable colonies (<1) were recorded as containing 3.2 log CFU/ml (0.9 CFU/ml) so that statistical analysis could be performed. For each experimental replicate conducted at each challenge temperature and glucose level and for each Salmonella culture composite, survival curves were constructed by plotting the log of surviving counts versus the corresponding challenge times (Fig. 1). Following linear regression
analysis by the REG procedure of SAS (36), only survivor curves with more than five values in the straight portion and having a coefficient of determination ($r^2$) of greater than 0.90 were used (18, 19). The decimal reduction time ($D$ value) was calculated as the negative reciprocal of the slope of the linear regression line of the survivor curve ($D = -\text{slope}^{-1}$) (11, 21, 28, 37). Because of the number of variables involved in the study, it would be very difficult to determine and compare the heat resistances of individual strains. Therefore, it was decided to thermally challenge composites of strains and determine their $D$ values as a means of comparing the heat resistances of the various inocula prepared under different conditions.

Data ($D$ values) were evaluated by analysis of variance (AOV) at each challenge temperature (55, 57, 59, and 61°C) with the model $y = a + x_1 + x_2 + x_1x_2$; least-squares mean $D$ values were computed for fixed main effects of glucose level ($x_1$) and Salmonella culture composite ($x_2$) and for the glucose level-Salmonella culture composite ($x_1x_2$) interaction with the General Linear Models procedure of SAS (36).

Data also were evaluated to determine $D$ value differences between challenge temperatures. Preliminary AOV indicated no difference ($P = 0.7179$) between Salmonella composites when they were evaluated across all of the glucose levels and challenge temperatures used. Therefore, Salmonella composites were pooled and data were evaluated by AOV with the model $y = a + x_1 + x_2 + x_1x_2$; least-squares means were computed for $D$ values by glucose level ($x_1$), challenge temperature ($x_2$), and the glucose level-challenge temperature interaction ($x_1x_2$). Because of the interaction ($P < 0.05$), subclass least-squares means were reported and used for subsequent $Z_{50}$ value and $E_6$ value determinations. Interaction subclass least-squares means were separated with models including the following challenge temperature comparisons because of heterogeneity of variance: (i) 55 and 57°C; (ii) 57 and 59°C; and (iii) 59 and 61°C. When AOV detected effects ($P < 0.05$) within or between challenge temperatures, $D$ values were separated by the pairwise $t$ test of SAS (36).

$log_{10} D$ values were plotted versus the corresponding temperatures, and $Z_{50}$ values were calculated as the negative reciprocal of the slope of the linear regression line of the thermal death-time curve ($Z_{50} = -\text{slope}^{-1}$). $E_6$ values also were determined by constructing Arrhenius plots [log $k$ versus temperature in Kelvin $^\circ$], where the rate constant ($k$) = $2.303D/R$, from which the slopes of the lines were used to calculate $E_6$ as $E_6 = -\frac{2.303D}{R}(\text{slope})$, where $R$ is the gas constant (8.31 J K$^{-1}$ mol$^{-1}$) (11, 37).

RESULTS AND DISCUSSION

Salmonellae were grown in the presence or absence of a fermentable carbohydrate (i.e., glucose at 0.25 or 1.00%) in order to evaluate the heat tolerance of stationary-phase, susceptible and multiantimicrobial-resistant cultures with and without prior acid adaptation (5, 42). Distinct from the pH-independent, alternative sigma factor $\sigma^B$-dependent general stress response system induced by entry into stationary phase, a pH-dependent system known as the stationary-phase ATR is induced by low-pH exposure (12, 25). Wilde et al. (42) reported RpoS-independent induction of stationary-phase acid tolerance among S. enterica serovar Enteritidis PT4 isolates following overnight growth in tryptic soy broth, nutrient broth, and Tryptone soya broth, all containing 0.25% glucose.

In the present study, overnight growth (18 h; 37°C) of Salmonella strains in TSBYE (9 ml, pH 7.3) containing 0.00, 0.25 or 1.00% glucose resulted in ultimate pH values of approximately 8.1 to 8.3, 7.2 to 7.4, and 4.7 to 4.8, respectively, because of differences in fermentable carbohydrate availability (data not presented in tabular form). As indicated, individual strains were combined to produce six five-strain culture composites as a result of limitations associated primarily with the simultaneous removal of duplicate samples at each predetermined time interval. Further, previously collected data on the individual growth and inactivation kinetics of the Salmonella strains evaluated in this study suggested no differences (data not presented). However, it is worth noting that a strain-dependent difference in stress tolerance is a recognized phenomenon.

Following thermal stress, viable populations were recovered and enumerated on TSA YE+$P$, which has been reported to increase the recovery of low-pH-stressed cells by approximately 1,000-fold because of the protective action afforded against oxygen radicals (26, 30). Furthermore, plates were incubated at 28°C for 72 h prior to colony counting, as suboptimum temperatures have been reported to enhance repair of heat damage (18, 19, 22).

AOV for glucose level (0.00, 0.25, and 1.00%) and culture type (susceptible and multiantimicrobial resistant) main effects, at each of the four challenge temperatures (i.e., 55, 57, 59, and 61°C), indicated that thermal resistance ($D$ value) depended ($P < 0.05$) on previous growth in the presence or absence of glucose (acid adaptation) at 59°C and on the interaction ($P < 0.05$) between previous acid adaptation and culture composite at 61°C (Table 2). The $D_{61\text{C}}$ values of antimicrobial-susceptible Salmonella strains increased ($P < 0.05$) from 0.14 to 0.19 as the glucose level increased from 0.00 to 1.00%, respectively, while the multiantimicrobial-resistant Salmonella strain composite, the corresponding $D$ values were not different ($P > 0.05$), as growth in both TSBYE–G and TSBYE+G resulted in a decimal reduction time of 0.21 min. At 61°C, the thermal resistances of susceptible and multiantimicrobial-resistant composite cultures were not different ($P > 0.05$) when the bacteria were grown in the presence of 0.25% glucose. At 59°C, when averaged across culture composites, the decimal reduction times increased ($P < 0.05$) from 0.50 min for Salmonella strains grown in the absence of glucose to 0.58 min for Salmonella strains grown in 0.25% glucose; the $D_{60\text{C}}$ values also increased ($P < 0.05$) from 0.58 min following growth in 0.25% glucose to 0.66 min for Salmonella strains grown in the presence of 1.00% glucose, respectively (Table 3).

Data also were analyzed to determine $D$ value differences
TABLE 3. Decimal reduction times, $z_D$ values, and $E_a$ values for salmonellae grown to stationary phase with or without glucose

<table>
<thead>
<tr>
<th>Glucose</th>
<th>$D$ value (SD)$^a$ at challenge temp ($°C$) of:</th>
<th>$z_D$ value (°C)$^b$</th>
<th>$E_a$ value (kJ/mol)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>0.00</td>
<td>4.23' (0.77)</td>
<td>1.81' (0.67)</td>
<td>0.58'' (0.03)</td>
</tr>
<tr>
<td>0.25</td>
<td>4.38' (0.95)</td>
<td>1.47' (0.16)</td>
<td>0.58*** (0.06)</td>
</tr>
<tr>
<td>1.00</td>
<td>5.39' (0.55)</td>
<td>1.73' (0.51)</td>
<td>0.66*** (0.04)</td>
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</table>

$^a$ Decimal reduction times ($D$ values; min) were calculated from the survivor curves of triplicate trials. At 59°C, $D$ values in the same column with the same superscript letter ($a$, $b$, or $c$) are not different ($P > 0.05$). $D$ values in the same row with the same superscript letter ($w$, $x$, $y$, or $z$) are not different ($P > 0.05$).

$^b$ Salmonellae were grown overnight with (0.25 or 1.00%) or without (0.00%) glucose.

$^c$ $z_D$ values, or the temperature changes necessary to effect a 10-fold change in the $D$ value, were calculated from the decimal death time curves ($\log_{10} D$ values plotted versus temperature).

$^d$ Arrhenius plots were constructed, and $E_a$ values were calculated from the slopes by using the equation $E_a = -2.33(R(slope))$, where $R = 8.31$ J K$^{-1}$ mol$^{-1}$.

among challenge temperatures. After pooling of culture composite data because of a lack of difference ($P = 0.7179$) between susceptible and resistant Salmonella strains, both the glucose level and challenge temperature fixed effects and the glucose-level-challenge temperature interaction were significant ($P < 0.05$). Decimal reduction times decreased ($P < 0.05$) as the challenge temperature increased from 55 to 57°C, from 57 to 59°C, and from 59 to 61°C for Salmonella strains grown in 0.00, 0.25, or 1.00% glucose (Table 3). Collectively, $D$ values ranged from 4.23 to 5.39, 1.47 to 1.81, 0.50 to 0.66, and 0.16 to 0.20 min for Salmonella strains inactivated at 55, 57, 59, and 61°C, respectively (Table 3). For each of the glucose levels (0.00, 0.25, and 1.00%), the corresponding $z_D$ values, or the changes in temperature required to effect a 10-fold change in the decimal reduction time, were 1.49, 1.48, and 1.20°C, respectively, while the corresponding $E_a$ values were 494, 493, and 497 kJ/mol (Table 3).

Results of this study suggested a positive relationship between acid adaptation and the ability to resist thermal stress. Decimal reduction times associated with heating at 59 and 61°C increased ($P < 0.05$) following previous acid adaptation, and despite greater variability, a similar relationship was also suggested at 55°C ($P = 0.0511$; Table 2). It has been reported that, in addition to withstand low-pH exposure, acid-adapted S. enterica serovar Typhimurium exhibits cross-protection from heat, osmotic, and oxidative stresses (27, 42). Leyer and Johnson (27) reported that acid adaptation during logarithmic growth alters cellular resistance to a wide range of environmental stresses, including temperature and salt, by inducing the synthesis of specific outer membrane proteins, subsequently resulting in increased outer surface hydrophobicity in addition to intracellular proton homeostatic activities. They reported ≥10-fold differences in survivor counts between acid-adapted and nonadapted S. enterica serovar Typhimurium following prolonged exposure to 50.0, 55.0, and 57.5°C and enumeration on tryptose-phosphate agar containing at least 0.1% sodium pyruvate (27). Wilde et al. (42) evaluated heat and acid tolerance among S. enterica serovar Enteritidis PT4 strains possessing normal and mutant rpoS alleles following fermentative stationary-phase growth in complex media containing glucose. When blood agar was used to enumerate viable cells, induction of the RpoS-independent ATR resulted in significantly higher ($P < 0.001$) tolerance to heat among cells grown in tryptic soy broth or nutrient broth (no. 2) containing 0.25% glucose compared to cells grown in the absence of glucose (42). It is worth noting that, much as the physiological state of a bacterium has been shown to influence stress survival (25), so may nutrient availability. Cells grown under laboratory conditions in a medium containing an abundant supply of nutrients may not accurately simulate the physiology of cells found in the food environment (27). Lack of available nutrients has been found to induce heat shock protein production in S. enterica serovar Typhimurium and E. coli, resulting in starvation-induced thermal cross-protection (17, 27).

The relationship between acid adaptation and the ability to resist thermal stress may prove relevant to the production and manufacturing of certain foods. Commercially available brands of semidry and summer-type sausages are associated with pH values ranging from 4.5 to 5.2 and 4.5 to 4.7, respectively (16). In this study, overnight growth of Salmonella strains in TSBYE (pH 7.3) containing 1.00% glucose resulted in pH values of approximately 4.7 to 4.8. Microorganisms may also encounter sublethal, low-pH stress during nonfermented-food manufacturing (e.g., animal-to-muscle food conversion). The use of organic acids to decontaminate food surfaces has been extensively examined, and as a result of recognized antimicrobial efficacy, acid solution rinsing is used throughout the commercial beef industry during the slaughtering and dressing process (8). Coupled with this application is the opportunity for sublethal, transient low-pH exposure and subsequent acid tolerance development among associated bacterial populations (e.g., salmonellae). The occurrence of localized, sublethal pH microenvironments within a slaughtering facility is not only plausible but probable, as in-plant organic acid solution rinsing typically occurs following ambient temperature carcass washing (water), a subject of previous discussion (35). The effects of organic acids on the pH of microenvironments within commercial slaughtering facilities are not fully understood but provide a potential means by which acid tolerant bacteria may be developed and reintroduced to carcass surfaces. The ability of salmonellae to mount an ATR also may prove important during pathogenesis, as acid-adapted cells may survive subsequent acid stress exposure(s) associated with food ingestion (i.e., stomach, intestinal, and intracellular environments), especially among individuals with compromised gastrointestinal tracts, ultimately resulting in infection (15, 43).

Although obvious differences exist between laboratory and commercial industry settings, acid adaptation resulting from exposure(s) to sublethal low-pH stress may result in cross-protection against heat stress, subsequently increasing pathogen survival in fresh or fermented undercooked or underprocessed foods (27, 34). Results of this study comparing the...
thermal tolerances of susceptible and multiantimicrobial-resistant Salmonella strains challenged following preparation under acid tolerance-inducing and -noninducing conditions suggested no association between antibiotic susceptibility and the ability to survive or repair damage associated with heat stress. Additional work is required to further understand the relationships among antimicrobial susceptibility, stationary-phase ATR induction, and sensitivity to environmental stresses and how these relationships are intertwined with current food manufacturing and processing practices.

ACKNOWLEDGMENTS

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REFERENCES

37. Schuman, J. D., B. W. Sheldon, and P. M. Foegeding. 1997. Thermal resis-
42. Wilmes-Riemsberg, M. R., B. Bearson, J. W. Foster, and R. Curtiss III. 1996. Role of the acid tolerance response in virulence of Salmonella typhi-