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Thermal Inactivation *D*- and *z*-Values of Multidrug-Resistant and Non–Multidrug-Resistant *Salmonella* Serotypes and Survival in Ground Beef Exposed to Consumer-Style Cooking

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ABSTRACT

There has been speculation that multidrug-resistant (MDR) strains are generated by subtherapeutic antibiotic use in food animals and that such strains result in increased resistance to lethality by food processes such as heat and irradiation. The objective of this study was to evaluate the heat resistance of 20 strains, namely an MDR and a non–multidrug-resistant (NMDR) strain of each of 10 *Salmonella* serotypes isolated from cattle or cattle environments. MDR and NMDR *Salmonella* serotypes studied included Montevideo, Typhimurium, Anatum, Muenster, Newport, Mbandaka, Dublin, Reading, Agona, and Give. For phase I, stationary-phase cultures of the strains were aliquoted into sterile capillary tubes and immersed in a temperature-controlled water bath at 55, 60, 65, and 70°C for appropriate times. Survivor curves were plotted for each temperature, and a best-fit linear regression was derived for each temperature. *D*-values (decimal reduction times) and *z*-values (changes in temperature required to change the *D*-values) were calculated for each strain. Although there was no overall significant difference in the heat resistance of MDR and NMDR serotypes, NMDR serotypes generally appeared to have slightly higher heat resistance than NMDR serotypes, especially at 55 and 60°C. The highest relative heat resistance (highest *z*-values) was exhibited by *Salmonella* Anatum. Notably, the relative heat resistance of NMDR *Salmonella* Agona was similar to that of NMDR *Salmonella* Anatum and had the highest *D*-values at all four temperatures. For phase II, three serotypes (regardless of resistance profile) with the highest relative heat resistance and their drug-resistant counterparts were selected for thermal inactivation in ground beef patties cooked to endpoint temperatures. *Salmonella* Agona was able to survive in ground beef cooked to an internal temperature of 71°C. Results of these studies suggest drug resistance does not affect the heat resistance of *Salmonella* and that serotype or strain is an important consideration in risk assessment of the pathogen with regard to survival at cooking temperatures.

Increased emergence of antimicrobial-resistant pathogens, including *Salmonella,* has become a public health concern worldwide, especially considering the potential for increased rates in morbidity and mortality *(5).* The routine use of antimicrobials in domestic livestock for disease treatment and growth promotion could potentially lead to widespread dissemination of antimicrobial-resistant bacteria *(26)* and transfer to humans through the food chain *(2).* Ground meats are more likely to be internally contaminated with pathogenic bacteria, and indeed, antibiotic-resistant *Salmonella* has been isolated with increasing frequency from retail ground beef *(19, 25, 31, 33)* and implicated in outbreaks *(4, 15).*

Federal regulations have addressed *Salmonella* contamination of fully cooked meat products, calling for a 6.5-log reduction in beef *(27)*; however, the pathogen is still found in cooked meat *(28).* Furthermore, the U.S. Department of Agriculture Food Safety and Inspection Service *(27)* has recommended that ground beef be cooked to an internal temperature of 71.1°C because it is a hazard likely to occur, and salmonellae attached to meat surfaces are more resistant to heat than those unattached or dispersed in a food or liquid *(10).* Although regulators have defined critical limits for pathogen reduction in cooked beef, different strains of bacteria react differently to identical heat stress *(3, 17).* Various factors can influence the heat resistance of bacteria, including strain, metabolic phase, nutrient availability, growth conditions (temperature), intrinsic and extrinsic factors of the substrate, and previous exposure to the same or unrelated stresses.

The ability of bacteria to exhibit increased resistance when either the same or a seemingly unrelated stress is reapplied, cross-protection, has been studied extensively *(20, 21, 24, 25).* Exposure of bacteria to environmental pressures, such as the presence of antibiotics, resulted in their developing strategies to survive in the presence of the stress. Microorganisms respond to these stresses by inducing specific sets of proteins to protect against damage *(30).* Induction of stress proteins by exposure to nonlethal levels of a stress (i.e., the application of subtherapeutic levels of antibiotics) may confer protection to subsequent exposure to otherwise lethal levels of the same or unrelated stress *(6, 35).* There is sparse and conflicting evidence of the relationship between the antimicrobial resistance profile of *Salmonella* and heat resistance. Bacon et al. *(3)* found no relationship between antimicrobial resistance and heat resistance, while Walsh et al. *(32)* found that multidrug-resistant

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TABLE 1. *Multidrug-resistant (MDR) and non–multidrug-resistant (NMDR)* Salmonella *serotypes isolated from cattle and cattle environments*

Salmonella serotype	Serogroup	Resistance profile[a]	Source
Montevideo	C1	Susceptible (NMDR)	Feces
Montevideo	C1	ACSSuTAmc (MDR)	Environment
Typhimurium	B	Suscepticle (NMDR)	Feces
Typhimurium	B	ASSuTK (MDR)	Feces
Anatum	E1	Susceptible (NMDR)	Hide swab
Anatum	E1	ACSSuT (MDR)	Feces
Muenster	E1	Susceptible (NMDR)	Environment
Muenster	E1	ACSSuTSxtKG (MDR)	Intestine
Newport	C2	Susceptible (NMDR)	Environment
Newport	C2	ACSSuTAmc (MDR)	Hide swab
Mbandaka	C1	Susceptible (NMDR)	Feces
Mbandaka	C1	ASSuTK (MDR)	Feces
Dublin	D1	Susceptible (NMDR)	Necropsy
Dublin	D1	ACSSuTK (MDR)	Feces
Reading	B	Susceptible (NMDR)	Feces
Reading	B	ACSSuTAmc (MDR)	Environment
Agona	B	Susceptible (NMDR)	Slurry
Agona	B	SSuT (MDR)	Feces
Give	E1	Susceptible (NMDR)	Feces
Give	E1	ACSSuTAmc (MDR)	Environment

[a] A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfonamide; T, tetracycline; Amc, amoxicillin–clavulanic acid; K, kanamycin; Sxt, sulfamethoxazole-trimethoprim; G, gentamicin.

(MDR) *Salmonella* Typhimurium DT104 had higher heat resistance than susceptible *Salmonella* Typhimurium and *Salmonella* Enteritidis.

The purpose of this study was to (i) investigate the role of drug resistance in heat resistance of *Salmonella* serotypes isolated from cattle and cattle environments, (ii) compare the heat resistance of different *Salmonella* serotypes isolated from cattle and cattle environments, and (iii) determine the survival of *Salmonella* serotypes with different drug resistance profiles in ground beef exposed to typical consumer cooking treatments.

MATERIALS AND METHODS

***Salmonella* strains.** MDR and non–multidrug-resistant (NMDR) strains of 10 *Salmonella* serotypes were used in this study (Table 1). Strains representing the predominant serotypes isolated from the ground beef national baseline samples from 1998 to 2005 *(29)* were obtained from the Field Disease Investigation Unit, Department of Veterinary Clinical Sciences at Washington State University (Pullman). Single colonies were tested by the disc diffusion method for antibiotic resistance according to NCCLS (now Clinical and Laboratory Standards Institute) standards *(16).* The antibiotics used were as follows: ampicillin (10 μg), chloramphenicol (30 μg), streptomycin (30 μg), sulfonamide (300 μg), tetracycline (30 μg), ciproflaxin (5 μg), sulfamethoxazole-trimethoprim (25 μg), kanamycin (30 μg), gentamicin (10 μg), amoxicillin–clavulanic acid (30 μg), cefotaxime (30 μg), and nalidixic acid (30 μg).

Culture preparation. Each *Salmonella* strain was subcultured twice for 24 h at 35°C in 10 ml of tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.). Following overnight incubation, the cells were harvested by centrifugation (20 min at 1,840 × *g*) and washed in 10 ml of 0.1% buffered peptone water (BPW; Difco, Becton Dickinson). The cells were resuspended with 10 ml of sterile 0.1% BPW and kept at 25°C for immediate use.

Phase I: thermal-death-time of MDR and NMDR *Salmonella* serotypes in capillary tubes. Thermal inactivation was performed according to methods described in Bacon et al. *(3)* for evaluation of the thermal resistance of *Salmonella* with modification. The glass capillary tubes (200 μl; Drummond Scientific Co., Broomall, Pa.) used in this study were sealed on one end with an acetylene torch, placed in screw-cap test tubes (16 by 125 mm), and sterilized in a 180°C oven for 24 h. Each *Salmonella* culture was dispensed (100 μl) into capillary tubes with a 22-gauge, 7.6-cm hypodermic needle with a deflected (septum) point (Popper & Sons, Inc., New Hyde Park, N.Y.), giving a final concentration of approximately 10^9 CFU/ml. After filling, the open end of the capillary tubes was sealed as previously described, taking care to avoid inactivating the cell suspension (preliminary studies with inoculated, unheated controls were used to verify that the method did not result in bacterial inactivation following sealing). Immediately after sealing, the cells were subjected to heat treatment in a static water bath. Capillary tubes were completely submerged in the water bath with thin copper wire. The *Salmonella* strains were heat challenged at 55°C for 0, 360, 720, and 1,080 s; at 60°C for 0, 20, 40, and 60 s; at 65°C for 15, 30, and 45 s; and at 70°C for 2, 4, and 6 s (exposure times were selected on the basis of results of preliminary research). Ten replicates were conducted for each test case (temperature-time combination for each strain).

Immediately after each time point exposure, capillary tubes were placed in ice water for 10 min and then sanitized by immersion in a 10% sodium hypochlorite solution. Capillary tubes were rinsed twice by immersions in sterile distilled water. After rinsing, both ends of capillary tubes were briefly passed through a flame, taking care to avoid inactivating surviving populations. Both ends of the capillary tubes were clipped open with sterilized pliers. The cell suspension inside the capillary was aseptically transferred into a test tube containing 9 ml of sterile 0.1% BPW, and the cells remaining in the capillary tube were rinsed with approximately 0.9 ml of sterile 0.1% BPW with a 1-ml sterile syringe and needle (Becton Dickinson, Franklin Lakes, N.J.). Cell suspensions were homogenized by vortexing, and serial dilutions were made. Enumeration of survivors was performed by plating 1 ml of the appropriate dilutions on aerobic count plates (3M Petrifilm, 3M Microbiology Products, St. Paul, Minn.). Petrifilms were incubated at 35°C for 48 h, and colonies were manually counted and recorded.

Phase I: statistical analysis. For each strain, the survivor curves were developed at each temperature by least-squares regression by SAS software *(22).* The slopes for individual survivor curves at each temperature were determined, following which the *D*-values were calculated by taking the negative reciprocal of the survivor curve slope. The general form of the regression equation used for estimating the *D*-values is as follows:

$$\log(N/N_0) = \beta_0 + \beta_1 \times (\text{time})$$

where N_0 = initial number of organisms; N = number of survivors; β_0 = intercept; and β_1 = slope of the survivor curve or $-1/D$, where D is the decimal reduction time.

Mean log *D*-values versus temperatures were also plotted for

TABLE 2. *Heat resistance of multidrug-resistant (MDR) and non–multidrug-resistant (NMDR)* Salmonella *serotypes inoculated into sterile capillary tubes and submerged in preheated water baths set to 55, 60, 65, and 70°C*[a]

Resistance profile	*Salmonella* serotype	Thermal inactivation			
		55°C	60°C	65°C	70°C
MDR	Montevideo	156.9 (0.95)	13.6 (0.90)	5.0 (0.87)	1.2 (0.89)
	Typhimurium	136.1 (0.98)	15.7 (0.87)	3.8 (0.91)	1.8 (0.93)
	Anatum	181.2 (0.96)	23.2 (0.89)	4.2 (0.70)	2.6 (0.97)
	Muenster	194.6 (0.92)	9.4 (0.85)	6.0 (0.65)	0.7 (0.73)
	Newport	182.4 (0.87)	14.3 (0.91)	5.3 (0.81)	1.4 (0.94)
	Mbandaka	205.6 (0.90)	18.3 (0.92)	5.0 (0.92)	0.6 (0.92)
	Dublin	287.5 (0.96)	14.6 (0.79)	5.2 (0.92)	1.2 (0.78)
	Reading	223.3 (0.96)	18.6 (0.63)	5.1 (0.86)	0.9 (0.86)
	Agona	251.0 (0.83)	20.4 (0.69)	5.2 (0.90)	0.9 (0.83)
	Give	174.3 (0.99)	17.3 (0.94)	5.3 (0.91)	0.7 (0.73)
	Pooled *D*-value	215.7 A[b]	18.5 B	5.5 C	1.2 D
NMDR	Montevideo	228.4 (0.94)	26.3 (0.96)	5.1 (0.88)	1.2 (0.90)
	Typhimurium	204.2 (0.92)	13.0 (0.67)	6.1 (0.62)	1.1 (0.73)
	Anatum	222.6 (0.99)	19.3 (0.99)	5.5 (0.86)	1.9 (0.98)
	Muenster	215.7 (0.98)	23.5 (0.91)	5.7 (0.83)	0.7 (0.75)
	Newport	269.3 (0.99)	16.8 (0.91)	5.4 (0.90)	1.0 (0.80)
	Mbandaka	252.1 (0.92)	25.2 (0.88)	5.0 (0.92)	1.3 (0.86)
	Dublin	235.6 (0.96)	29.0 (0.89)	5.1 (0.77)	1.1 (0.95)
	Reading	238.9 (0.97)	16.2 (0.94)	5.1 (0.88)	1.3 (0.98)
	Agona	784.3 (0.91)	148.1 (0.82)	20.2 (0.75)	8.4 (0.94)
	Give	265.9 (0.99)	16.1 (0.89)	5.3 (0.80)	1.8 (0.72)
	Pooled *D*-value	472.3 A	32.3 B	6.9 C	1.9 D

[a] Values are *D*-values expressed in seconds (R^2).
[b] *D*-values in the same row with different letters are significantly different ($P < 0.05$).

each strain, and *z*-values were calculated as the negative inverse of the slope obtained in each plot. The relative heat resistance between MDR and NMDR strains was tested by the two-sample *t* test. The level of significance used to determine difference between treatments means was 5%.

To verify that *D*-values decrease significantly with increasing temperatures, the *D*-values for each strain were pooled at different temperatures for both MDR and NMDR groups. These data were analyzed by analysis of variance to determine if the mean *D*-values differed significantly across the chosen temperatures. When differences between means were found to be significant, the Duncan multiple range test was used to rank the order of the individual treatment (temperature) effects. The level of significance used to determine the difference between treatment means was 5%. The results were analyzed by SAS software *(22).*

The results of this study were evaluated, and three serotypes (regardless of drug-resistant profile) with the highest heat resistance and their drug-resistant counterparts were selected for inoculation in ground beef patties for phase II.

Phase II: survival of heat-resistant MDR and NMDR *Salmonella* in ground beef patties. Six strains, MDR and NMDR strains of *Salmonella* Typhimurium, *Salmonella* Anatum, and *Salmonella* Agona (Table 2), were selected for survival studies on the basis of the three highest *z*-values observed in phase I.

Preformed ground beef patties containing 25% fat with a mean weight of 68.5 g, a thickness of 1 cm, and a diameter of 9 cm were obtained from the Department of Animal Science at Colorado State University (Fort Collins). Patties were left uninoculated or inoculated (each with 250 μl) immediately prior to heat treatment with *Salmonella* with a sterile syringe and needle (Becton Dickinson) inserted into the geometric center, resulting in a final concentration of approximately 10^6 CFU/g. Inoculated patties were stored at 5 ± 2°C prior to heat treatment. The pH values of the ground beef patties were determined by a spear-type combination electrode (Accumet model AP50, Oakton-Fisher Scientific, Vernon Hill, Ill.).

George Foreman electric household grills (Next Grilleration GRP99, George Foreman Grilling Machine, Lake Forest, Ill.) with double-sided (clam-shell) griddles were used for heating the patties. A type K thermocouple (model USB TC-08, Pico Technology Limited, Cambridgeshire, UK) was inserted into the geometric center of the patties to monitor the internal temperature. By means of the thermocouple datalogger software, the temperature of patties was monitored and recorded every 3 s. Patties were removed from the grill when internal temperatures reached 48.9, 54.4, 60.0, 65.6, and 71.1°C *(18)* and immediately placed into separate sterile Whirl-Pak bags (Nasco, Modesto, Calif.) immersed in ice water. Control (inoculated, untreated) patties were analyzed to determine initial populations.

After cooling, an equal weight of sterile 0.1% BPW (Difco, Becton Dickinson) was aseptically added into the Whirl-Pak bags containing patties. Samples were homogenized for 60 s with a masticator (IUL Instruments, Barcelona, Spain). Serial dilutions were made in sterile 0.1% BPW, and 100 μl of the sample or diluent was spread plated onto tryptic soy agar (TSA; Difco, Becton Dickinson) and xylose-lysine-tergitol 4 (XLT4) agar (Hardy Diagnostics, Santa Maria, Calif.). Plates were incubated at 35°C for 24 to 48 h, and colonies were enumerated.

Phase II: statistical analysis. For each strain, the effect of temperature on pathogen survival was determined by analysis of variance with the General Linear Models procedure to compare differences between treatment (temperature) means. When the dif-

TABLE 3. *Relative heat resistance (z-values) of multidrug-resistant (MDR) and non–multidrug-resistant (NMDR)* Salmonella *serotypes inoculated into sterile capillary tubes and submerged in preheated water baths set to 55, 60, 65, and 70°C*

Resistance profile	*Salmonella* serotype	RMSE[a]	R^2	*z*-Value (increasing order)
MDR	Mbandaka	0.141	0.988	6.127
	Agona	0.159	0.984	6.340
	Reading	0.164	0.982	6.513
	Give	0.141	0.987	6.558
	Dublin	0.283	0.948	6.569
	Muenster	0.323	0.930	6.692
	Montevideo	0.167	0.980	6.832
	Newport	0.226	0.958	7.355
	Typhimurium	0.129	0.986	7.357
	Anatum	0.197	0.964	7.757
NMDR	Muenster	0.102	0.994	6.194
	Newport	0.221	0.969	6.371
	Dublin	0.085	0.995	6.445
	Mbandaka	0.148	0.985	6.585
	Montevideo	0.110	0.992	6.613
	Reading	0.239	0.958	6.894
	Typhimurium	0.258	0.950	6.993
	Give	0.289	0.936	7.160
	Anatum	0.157	0.979	7.386
	Agona	0.221	0.959	7.387

[a] RMSE, root mean square error.

ferences between treatment means were found to be significant, the Duncan multiple range test was used to rank the order of the individual treatment effects. The level of significance used to determine the difference between treatment means was 5%. The results were analyzed by SAS software *(22).*

RESULTS AND DISCUSSION

Serotypes used in this study were derived from cattle or cattle environments and chosen to represent the 10 predominant serotypes isolated from the ground beef national baseline samples from 1998 to 2005 *(29).* Strain-dependent differences in stress tolerance were tested by exposing 10 different *Salmonella* serotypes to thermal treatments in order to characterize the relative heat resistance of serotypes commonly isolated from ground beef.

Effect of drug resistance profile on *Salmonella* heat resistance. Thermal-death-time studies were conducted to determine the decimal reduction time (*D*-value) at specific temperatures and the temperature rise for a 1-log reduction of *D* (*z*-value) of the *Salmonella* serotypes. Analysis of variance indicated that *D*-values decreased significantly ($P < 0.05$) with increases in temperature, as would be expected (Table 2). Overall, heat resistance of MDR serotypes did not differ ($P \geq 0.05$) from that of NMDR serotypes with *D*-values of 215.7 to 472.3 at 55°C, 18.5 to 32.3 at 60°C, 5.5 to 6.9 at 65°C, and 1.2 to 1.9 at 70°C (Table 2). This was confirmed by comparing the relative heat resistances in terms of *z*-values with the values of 6.127 to 7.757 for MDR serotypes and 6.194 to 7.387 for NMDR serotypes (Table 3). Although there was no overall significant difference in the heat resistance of MDR and NMDR serotypes, NMDR serotypes generally appeared to have slightly higher heat resistance than NMDR serotypes, especially at 55 and 60°C (Tables 2 and 3). These results support previous findings *(3, 32)* that there is no apparent association between antibiotic resistance and heat resistance of *Salmonella* strains. It should be noted that the study conducted by Bacon et al. *(3)* did not consider antibiotic-resistant and -susceptible profiles of the same serotypes and, as such, evaluation of antibiotic resistance is somewhat inconclusive. Walsh et al. *(32)* compared the heat resistance of susceptible and antibiotic-resistant *Salmonella* strains generated in the laboratory. This is, to our knowledge, the first report to compare the heat resistance of susceptible and MDR counterparts of *Salmonella* serotypes isolated from the environment. This study suggests that the NMDR *Salmonella* strains evaluated have higher, albeit not statistically significant, heat resistance than their MDR counterparts. There is limited research evaluating the role of antibiotic resistance in cross-protection of bacteria to other stresses, such as heat, and no literature proposing a mechanism for such an occurrence. One possible hypothesis is that up-regulation of genes for maintaining antibiotic resistance also results in up-regulation of genes, including alternative sigma factor (σ^s) for general stress response, or heat shock proteins *(36).* Considering that results of this study suggest a slightly negative relationship between the antibiotic resistance and heat resistance of *Salmonella,* the explanation may be that the energy expended for regulation of housekeeping genes to maintain antibiotic resistance rendered the MDR strains more susceptible to the effects of heat than their NMDR counterparts. This is merely speculation, and further research is needed to understand the relationship between antibiotic resistance and heat resistance of bacteria as a whole.

Effect of serotype on *Salmonella* heat resistance. Individual *Salmonella* serotypes had different heat resistance, irrespective of antibiotic resistance profile (MDR or NMDR). This was expected, because strain-dependent differences are well-known phenomena; however, the purpose of this study was to assess the relative heat resistance of the serotypes included in this study, because they represent serotypes commonly isolated from ground beef *(29).* Overall, relative heat resistance of individual serotypes did not differ significantly in this study (Table 3); however, certain serotypes had substantially higher heat resistance than the rest. It should be noted that the differences or similarities in heat resistance of the serotypes may not necessarily indicate serotype differences but rather strain-specific differences. Overall, the highest relative heat resistance was demonstrated by *Salmonella* Anatum (highest *z*-values for both MDR and NMDR strains). Notably, the relative heat resistance of NMDR *Salmonella* Agona was similar to that of NMDR *Salmonella* Anatum (Table 3) and had the highest *D*-values at all four temperatures (55, 60, 65, and 70°C) (Table 2). This is an important finding, because it may signify the presence of a resistant subpopulation of cells *(13)* that is not necessarily genetically different from the major portion of the population but rather has manufactured more

TABLE 4. *Survival of total bacterial populations (tryptic soy agar) in ground beef patties inoculated with multidrug-resistant (MDR) and non–multidrug-resistant (NMDR)* Salmonella *serotypes and exposed to consumer cooking with a commercial grill*[a]

		Endpoint temp (°C):				
Salmonella serotype	Control[b]	48.9	54.4	60	65.6	71.1
NMDR Typhimurium	6.4 AZ[c] (0.3)	5.5 BZ (0.9)	5.3 BZ (0.6)	4.7 CY (0.4)	2.6 DZY (0.7)	0.1 EY (0.1)
MDR Typhimurium	6.4 AZ (0.3)	5.5 BZ (0.6)	5.2 BZ (0.7)	4.3 CYX (0.5)	2.5 DY (1.4)	0.3 EY (0.5)
NMDR Anatum	6.4 AZ (0.3)	5.4 BZ (0.2)	5.2 BZ (0.5)	4.0 CX (0.8)	2.7 DZY (1.9)	0.3 EY (0.5)
MDR Anatum	6.3 AZ (0.4)	5.8 ABZ (0.3)	5.4 BZ (0.5)	4.2 CYX (0.9)	1.4 DY (1.4)	0.1 EY (0.2)
NMDR Agona	6.4 AZ (0.4)	5.6 BZ (0.3)	5.5 BZ (0.4)	5.4 BZ (0.5)	4.0 CZ (1.4)	1.0 DZ (0.8)
MDR Agona	6.4 AZ (0.4)	5.7 BZ (0.3)	5.5 BZ (0.5)	5.4 BZ (0.3)	2.1 CY (1.6)	0 DY (0.0)

[a] Values are expressed in log CFU per gram (standard deviation).
[b] Inoculated, untreated control (patties kept at 5 ± 2°C prior to cooking).
[c] Means in the same row with different letters (A through E) are significantly different ($P < 0.05$). Means in the same column with different letters (X through Z) are significantly different ($P < 0.05$).

heat shock proteins *(34).* The relatively high heat resistance of these strains and their frequent isolation from ground beef are risk factors that highlight the importance of further characterizing their thermal inactivation in actual products.

Survival of *Salmonella* in ground beef patties. It is imperative to obtain quantitative information on the cooking lethality of different *Salmonella* serotypes in ground beef, as these organisms may be protected by meat surfaces *(9)* and natural microflora *(11).* For this reason, the three serotypes (regardless of resistance profile) with the highest relative heat resistance (Table 3), i.e., NMDR *Salmonella* Agona, MDR *Salmonella* Anatum, and MDR *Salmonella* Typhimurium, and their drug-resistant counterpart were selected for thermal inactivation in ground beef patties. In this study, *Salmonella* was inoculated (6.4 log CFU/g) in ground beef and cooked to endpoint temperatures, including 48.9, 54.4, 60, 65.6, and 71.1°C. In general, the recovery of inoculated *Salmonella* from heat-treated patties was up to 0.4 log CFU/g higher ($P < 0.05$) on TSA (Table 4) than on XLT4 (Table 5), which is to be expected, because XLT4 is a selective recovery medium and not conducive to the recovery of heat-injured cells. Because the natural bacterial populations on ground beef were relatively low (1.3 ± 0.2 log CFU/g), there was no detectable *Salmonella,* and the difference in recovery between the two media was not substantial, survival data are discussed in terms of TSA counts. Irrespective of media and serotype or strain, *Salmonella* was significantly ($P < 0.05$) reduced in ground beef patties as follows: inoculated untreated (control) < 48.9°C, 54.4°C < 60°C < 65.6°C < 71.1°C. At higher temperatures (65.6 and 71.1°C), the heat resistance of NMDR strains was higher ($P < 0.05$) than that of MDR. Furthermore, the injury (difference in survival on TSA compared with XLT4 agar) experienced by NMDR strains was less than that by MDR strains, further supporting the finding that NMDR strains have slightly higher heat resistance than MDR strains (results from phase I of this study). Reduction of *Salmonella* in ground beef patties cooked to an internal temperature of 71.1°C ranged from 5.4 (NMDR *Salmonella* Agona) to 6.1 to 6.4 log CFU/g (Table 4). NMDR *Salmonella* Agona had the highest ($P < 0.05$) survival (1.0 log CFU/g) in ground beef, even when cooked to an internal temperature of 71.1°C. This is critical, because it is accepted that cooking meat such as ground beef to an internal temperature of 71.1°C is sufficient to eliminate at least 6.5 log of *Salmonella,* and although this may be the case for the majority of serotypes and strains, including those used in this study, the existence of heat-resistant serotypes and strains should be considered. Although *Salmonella* Agona has not been implicated in outbreaks re-

TABLE 5. *Survival of multidrug-resistant (MDR) and non–multidrug-resistant (NMDR)* Salmonella *serotypes (XLT4 agar) inoculated into ground beef patties and exposed to consumer cooking with a commercial grill*[a]

		Endpoint temp (°C):				
Strain	Control[b]	48.9	54.4	60	65.6	71.1
NMDR Typhimurium	5.8 AZ[c] (0.4)	5.5 AZ (0.5)	5.2 AZY (0.7)	4.2 BZY (1.0)	1.9 CY (1.9)	0 DY (0.0)
MDR Typhimurium	5.9 AZ (0.4)	4.9 BY (0.6)	4.7 BY (0.5)	3.9 CYX (0.7)	2.2 DZY (1.9)	0 EY (0.0)
NMDR Anatum	5.8 AZ (0.7)	5.1 AZY (0.5)	5.0 AZY (0.5)	3.1 BX (1.3)	2.4 BZY (1.7)	0 CY (0.0)
MDR Anatum	5.6 AZ (0.6)	5.6 AZ (0.3)	5.0 AZY (0.5)	3.8 BYX (0.5)	1.5 CY (1.6)	0 DY (0.0)
NMDR Agona	5.7 AZ (0.5)	5.3 AZY (0.7)	5.4 AZ (0.7)	4.7 BZ (0.8)	3.6 CZ (0.6)	0.5 DZ (0.6)
MDR Agona	5.4 AZ (0.7)	5.4 AZ (0.4)	5.0 AZY (0.7)	4.9 AZ (0.4)	1.5 BY (1.5)	0 CY (0.0)

[a] Values are expressed in log CFU per gram (standard deviation).
[b] Inoculated, untreated control (patties kept at 5 ± 2°C prior to cooking).
[c] Means in the same row with different letters (A through E) are significantly different ($P < 0.05$). Means in the same column with different letters (X through Z) are significantly different ($P < 0.05$).

sulting from the consumption of ground beef, the serotype has frequently been isolated from ground beef and may be introduced into the food supply.

Adequate cooking of ground beef is generally effective at destroying vegetative pathogens such as *Salmonella*; however, numerous foodborne outbreaks implicating MDR *Salmonella* resulted from the consumption of contaminated hamburger meat that was possibly cooked inadequately *(8, 25).* Ground beef is thought to be heated as a result of steam-vapor flow rather than conduction of heat, and once a given temperature is reached, it tends to be maintained for several seconds and, thus providing a sufficiently high internal temperature (i.e., 71.1°C) is reached, will allow optimal kill without specifying holding times *(12).* There are several methods for cooking ground beef, and although the main objective is to achieve a specific internal temperature, there are factors that need to be considered, which may affect the cooking process, including the following: the type of product (higher fat content may increase thermal resistance of pathogens), the thickness (thicker products slow heat flow), the rate and type of heat, and the type of equipment *(1, 7, 14).* Failure to consider these factors when selecting a cooking regimen, combined with the occurrence of a heat-resistant serotype or strain such as the NMDR *Salmonella* Agona described in this study, may result in inadequate thermal inactivation. This could have severe ramifications, given that many consumers prefer ''juicy'' (undercooked) hamburgers, and according to the results of this study, NMDR *Salmonella* Agona was reduced by only ≤2.4 log CFU/g when undercooked (<71.1°C), thereby increasing the risk of foodborne illness from consumption of such product. Furthermore, incomplete inactivation of the pathogen may result in the selection of the more hardy *Salmonella* populations that display cross-protection and increased potential to survive the acidic conditions encountered in the human gastrointestinal tract *(23).* These data are important, because the frequent occurrence of more heat-resistant serotypes may pose a public health concern and should be considered for inclusion in risk assessments of this pathogen in ground beef.

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