International Journal of Biology Research
www.biologyjournal.in
ISSN: 2455-6548
Received: 15-11-2021, Accepted: 30-11-2021, Published 16-12-2021
Volume 6, Issue 4, 2021, Page No. 24-28

Thermal resistance of multidrug-resistant (MDR) and non-multidrug-resistant (NMDR) *salmonella* serovars isolated from chicken gizzards in Abidjan, Côte D'ivoire

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Abstract
To evaluate the possibility of a link between MultiDrug-Resistance (MDR) and thermal resistance of some serovars of *Salmonella*, the study was conducted on strains isolated from ready-to-eat (RTE) chicken gizzards in Abidjan, Côte d'Ivoire.
One MDR *Salmonella* strains (*S.* Hadar) and two NMDR *Salmonella* strains (S. Enteritidis and S. Typhimurium) isolated from chicken gizzards were tested for their thermal resistance. Bacterial suspension of 10^6Salmonella/mL in buffered peptone water (BPW) were heated at 50, 55 and 60°C in capillary tubes (6.5 mm diameter and 10 cm long) immersed in a thermostatically controlled circulating water bath. Decimal reduction time (D_{values}) were calculated from survival curves having r2 values of >0.90 as means of comparing thermal tolerance among variables. At 50°C, D_{values} were 2.68, 3.16 and 2.18 min respectively for S. Enteritidis, S. Hadar and S. Typhimurium. We obtained at 55°C, 2.54, 2.08 and 1.73 min and at 60°C, 1.89, 1.54 and 1.17 min respectively for S. Enteritidis, S. Hadar and S. Typhimurium.
S. Enteritidis was the most resistant serovar to the thermal treatment and S. Typhimurium, the most susceptible to the heat. S. Hadar the most MDR was less resistant to the heating; the MDR faculty was not correlated to the heat resistance.
Significance and Impact of the Study: results of this study suggest a probable relationship between Multidrug-Resistance and ability to resist to thermal stress. Decimal reduction times associated with heating at temperatures 50, 55 and 60°C decreased from MDR S. Hadar to NMDR strains (S. Enteritidis and S. Typhimurium). As thermal treatments are critical in controlling food-borne pathogens in many foods of animal origin, data presented in this study can raise antimicrobial susceptibility test on pathogens such as *Salmonella* when they reveal a thermal stress resistance.

Keywords: thermal resistance, enumeration; *salmonella*; multidrug-resistance, chicken, gizzards, Abidjan

Introduction
Salmonella is a leading cause of bacterial food-borne disease outbreaks in developed countries (Roberts, 1988; D'Aoust, 1994; White *et al.*, 1997; Bean *et al.*, 1997; Hedberg *et al.*, 1999; Poppe, 2000; Voetsch *et al.*, 2004; Clark, 2005; Flint *et al.*, 2005) [42, 14, 48, 6, 22, 40, 47, 11, 18]. In developing countries, *Salmonella* is of a public health concern (Anyanwu et Jukes, 1990; Wouaffo *et al.*, 1996; Dosso *et al.*, 1998; Nola *et al.*, 1998; Medeiros *et al.*, 2001; Fonkoua et Wouafo, 2002; Tibaijuka *et al.*, 2003; Seydi *et al.*, 2005) [2, 49, 15, 36, 32, 19, 46, 44]. Diarrhoea, the common enteric disease caused by *Salmonella* causes death of millions and millions of children each year in many countries (Archer et kvenberg, 1985; Roberts, 1988; Motarjemi *et al.*, 1993; Swartz, 2002; Voetsch *et al.*, 2004; Flint *et al.*, 2005) [3, 42, 34, 45, 47, 18]. In Africa especially, *Salmonella* is an important opportunistic cause of infection in patients with AIDS (Seydi *et al.*, 2005) [44]. Treatment of *Salmonella* infections, in both animals and humans has become more difficult with the emergence of multidrug-resistant (MDR) *Salmonella* strains. Foodborne infections and outbreaks with MDR *Salmonella* are also increasingly reported (Angulo, 1997; Casin *et al.*, 1999; Helm *et al.*, 1999; Lederberg, 2000; Rabatsky-Ehr *et al.*, 2004) [6, 10, 23, 31, 41]. Foods of animal origin, especially poultry and poultry products, are widely acknowledged to be the reservoir of *Salmonella* due to the ability of *Salmonella* to proliferate in the gastrointestinal tract of chickens (Keller *et al.*, 1995; Baeumler *et al.*, 2000; Poppe, 2000; Barrow *et al.*, 2004) [30, 40, 5]. In Côte d'Ivoire, chicken gizzards are very much valued by the population who consumes them cooked braised and sold in the streets or cooked in sauce. These products of very big consumption in Côte d'Ivoire are reported to carry pathogens such as *Salmonella* with multidrug-resistant strains (Ouattara, 2005; Bonny *et al.*, 2011; Karou *et al.*, 2013) [37, 7, 28]. It is known the heat resistance of pathogens can be affected by any intrinsic and environment parameters (Murphy *et al.*, 1999; Fernandez et Peck, 1999) [35, 17]. Thermal treatments are critical in controlling foodborne pathogens in many foods of animal origin. Multidrug-resistance is generated by subtherapeutic antibiotic use in food animals we wonder if thermal resistance could be linked to this parameter in pathogens such as *Salmonella*. The objective of this study was to evaluate the thermal resistance of a multidrug-resistant *S.* Hadar and Non-multidrug-resistant *Salmonella* (S. Enteritidis and S. Typhimurium) isolated from ready-to-eat (RTE) braised chicken gizzards.

Material and Methods
The strains of *Salmonella* used in this study were previously isolated from ready-to-eat (RTE) braised chicken gizzards. From 960 samples of TRE chicken gizzards collected from retail street vendors in Abidjan, 32 strains (3.33%) were isolated, serotyped according to the Kauffmann-White scheme (Kauffmann et White, 1934) [29] and their

susceptibility to 15 antimicrobials (Table 1) was determined according to the CLSI (2008) [12] method. Among the 32 strains, S. Hadar was found multidrug-resistant (MDR) while S. Enteritidis and S. Typhimurium were found non-multidrug-resistant (NMDR) (Karou *et al.*, 2013). [28]

Thermal inactivation experiments were conducted according to previously reported procedures for evaluation of the thermal resistance of *Salmonella* (Jung et Beuchat, 2000; Brackett *et al.*, 2001) [27, 8]. Tubes (6.5 mm diameter for 100 mm long) previously sterilized by dry heat by placement of 11 tubes wrapped in Kraft paper into a dry heat sterilizer. For each culture, 1.5 mL was dispensed into a tube; the tubes were manually sealed with the flame of the Bunsen burner taking care to avoid heating the cell suspension. Immediately after sealing, tubes were suspended in a thermostatically controlled circulating-water bath. At each tested temperature (50, 55 and 60°C), cultures of the MDR S. Hadar strain and the NMDR S. Enteritidis and S. Typhimurium were challenged. Tubes were completely submerged in the water bath. For each of the cell suspensions at each challenged temperature, duplicate tubes were removed at equally spaced time (3 min) intervals for each of the following challenged temperatures. After heating, tubes were cooled in an ice-water mixture bath for two minutes and then tubes were cut with a sterile glass saw and 1.0 mL from the tube containing cell suspension was aseptically transferred into a tube containing 9.0 mL of sterile buffered peptone water (BPW). Then serial decimal dilutions were made with 1.0 mL into 9.0 mL of BPW. Viable-cell populations were enumerated by plating 0.1 mL appropriate dilution in duplicate on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) and 1.0% sodium pyruvate (TSAYE + P, Difco laboratories, Becton Dickinson). Plates were incubated at 37°C for 24h and then colonies were manually counted and recorded as numbers of (colony forming unit (CFU) per milliliter (mL). Resulting *Salmonella* population (numbers of CFU per mL) were transformed to $\log_{10}$ numbers of CFU per mL for survival curves construction. For each experimental replicate conducted at each challenge temperature (50, 55 and 60°C), for MDR S. Hadar and the NMDR S. Enteritidis and S. Typhimurium, survival curves were constructed by plotting the log of surviving counts versus the corresponding challenge (heating) time. The decimal reduction time (D_{values}) was determined using GInaFiT software (Log - Linear + Shoulder + Tail) Geeraerd *et al.* (2000) [20] for each strain and as the negative reciprocal of the slope of the linear regression line of the survivor curve ($D= -slope^{-1}$) when values (Lag time, log10 (N0), D_{values}) of the survivor curves were modulated. For $Z_D$values, $\log_{10}D_{values}$ were plotted versus the corresponding temperature and then $Z_D$values were determined as the negative reciprocal of the linear regression line of the thermal death-time curve ($Z_D$values $= -slope^{-1}$).

Table 1: List of antimicrobials tested for the antibiotic susceptibility of the strains of *Salmonella*.

Classes of antibiotics	antimicrobials	Charge of the disk of antibiotic (µg)	Items of the antibiotic
β-Lactamines	Amoxicillin	(10µg)	AMX
	Amoxicillin+ Clavulanic acid	(10/20µg)	AMC
	Ticarcillin	(75µg)	TIC
Cephalosporines	Cefalotine (C1G)	(10µg)	CF
	Cefoxitine (C2G)	(10µg)	FOX
	Cefotaxime (C3G)	(10µg)	CTX
Phenicoles	Chloramphenicol	(10µg)	C
Quinolones	Nalidixicacid	(10µg)	NA
	Ciprofloxacin	(10µg)	CIP
Aminosides	Gentamicin	(10µg)	GM
	Streptomycin	(10µg)	STR
Cyclines	Tetracycline	(10µg)	TE
Sulphonamides	Sulfamide	(10µg)	SSS
	Trimethoprime	(10µg)	TMP
	Sulfamide + trimethoprime	(10/20µg)	SXT

Table 2: Characteristic parameters of surviving *Salmonella* at 50, 55 and 60°C heating

	Parameters		
Serovars of *Salmonella*	**$\log_{10}N_0$**	**Lag Time(min)**	**D**
Heating temperature (50°C)			
S. Hadar	5.971	2.744	2.905
S. Enteritidis	6.008	2.682	2.68
S. Typhimurium	6.023	2.813	2.183
Heating temperature (55°C)			
S. Hadar	6.001	2.623	2.538
S. Enteritidis	5.935	2.574	2.097
S. Typhimurium	6.005	1.200	1.736
Heating temperature (60°C)			
S. Hadar	6.030	2.338	1.893
S. Enteritidis	5.807	0.830	1.543
S. Typhimurium	5.908	0.799	1.170

Results

Survival curves constructed by plotting the log10 of surviving counts versus corresponding challenge (heating) times are presented in figures 1, 2 and 3. Based on the linear portion of these survivor curves, the (DRT) decimal reduction times (D-values) determined for each challenge

(heating) time are summarized in table 2. Plotting $\log_{10}$(D-values) from the survivor curves of the MDR S. Hadar and the NMDR S. Enteritidis and S. Typhimurium at the challenge temperatures (50, 55 and 60°C) curves are presented in figure 4; $Z_{Dvalues}$ determined as the negative reciprocal of the linear regression line of the thermal death-time curve from figure 4 are summarized in table 3.

Discussion

Excluding the initial microbial counts (from 6.03log10 (UFC/mL) to 5.807log10 (UFC/mL), these survivor curves showed a linear decline with shoulders and tails in log10 number of surviving bacteria as a function of heating time. Our observation is in accordance with publications that report that thermal destruction curves for salmonellae exhibit significant tailing due to the presence of two populations of cells, one more heat sensitive than the other (Humpheson *et al.*, 1998; Peleg et Cole, 1998) [24, 38]. The D_{50}, D_{55} and D_{60} of the MDR S. Hadar were 2.905, 2.53 and 1.893 min respectively with 5.376°C as the Z-value. For the NMDR S. Enteritidis D-values (50, 55 and 60°C) were2.68, 2.097 and 1.543 min with a Z-value of 4.166°C and 2.183, 1.736 and 1.170 min respectively for the NMDR S. Typhimurium with a Z-value of 3.690°C. We noticed decreasing D-values as the challenge temperature increased from 50 to 60°C for the MDR S. Hadar and the NMDR S. Enteritidis and S. Typhimurium; these data were in agreement with Gould (1989) [21] who reported that thermal inactivation was generally known to increase exponentially with an increase in the temperature. In a phosphate buffer, Breeuwer *et al.* (2003) [9] reported a Z-value of 4.30°C at D60°C for S. Enteritidis. In ground chicken and turkey, Juneja *et al.* (2001) [26] reported Z-values of 5.5 and 6.1°C respectively for *Salmonella* spp. The thermal inactivation experiments showed that the decimal reduction times (D-values) of the MDR S. Hadar were higher than those (D-values) of the NMDR strains (S. Enteritidis an S. Typhimurium) for the challenge temperature (50, 55 and 60°C). The MDR S. Hadar appeared to be more resistant to thermal stress than the NMDR S. Enteritidis and S. Typhimurium. Among the NMDR Salmonellae, S. Enteritidis was more thermal resistant than S. Typhimurium. It's reported (Doyle et Mazzotta, 2000; Pflug, 2003) [16, 39] that heat resistance of Salmonella is highly influenced by strain tested, the type of experiment method used, culture conditions prior to the experiment, heating menstruum and recovery conditions. But Juneja *et al.* (2001) [26] found 4.87 min and 1.30 min as D(55°C) and D(60°C) values respectively in chicken broth while Jin *et al.* (2008) [25] reported D(52°C) and D(54°C) values of 6.12 and 1.51min respectively for S. Enteritidis in liquid egg white and D(54°C) and D(58°C) values of 5.70 and 0.82 min respectively in liquid white egg. Is it a link between the MDR and the thermal resistance? Our data shows that the MDR S. Hadar appeared more resistant to the thermal stress than the two NMDR salmonellae tested. But the number of MDR strains was not enough to establish a formal link at this step. As for Russell (2003) [43], lethal effects on bacterial physiology and structure could be strain-depend and be influenced by several factors. For Moats *et al.* (1971) [33] variations in heat resistance appear to be physiological rather than genetic since subculture of heat-resistant cells were no more heat resistant than the parent culture.

Conclusion

Results of this study suggest a probable relationship between Multidrug-Resistance and ability to resist thermal stress. Decimal reduction times associated with heating at 50, 55 and 60°C decreased from MDR S. Hadar to NMDR strains (S. Enteritidis and S. Typhimurium). As thermal treatments are critical in controlling food-borne pathogens in many foods of animal origin, antimicrobial susceptibility should be tested on pathogens such as *Salmonella* when they reveal a thermal stress resistance.

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