RESEARCH REPORT
PORK SAFETY

Title: Impact of organic acids and Quaternary Ammonium Compounds on *Salmonella* serovars with SGI1- mediated multi-antibiotic resistance - **NPB # 07-200**

Investigator: Mark Harrison

Institution: University of Georgia (Athens)

Date Submitted: July 29, 2009

Industry Summary

Multidrug-resistant (MDR) *Salmonella* has been implicated in several infections and outbreaks worldwide; they have been linked to the consumption of contaminated products involving pork as well. Despite the interventions of HACCP in abattoirs, *Salmonella* is still isolated from carcasses, and retailers (NARMS, 2005). The objective of this study was to compare the relative efficacy of organic acids and sanitizers against multiple drug-resistant (MDR) serovars of *Salmonella* and non-resistant *Salmonella* in order to gain more information on the potential effectiveness of intervening chemical treatments in pork slaughter and processing. The study also determined if exposure of normal and MDR *Salmonella* to organic acids affects sensitivity to sanitizers. Observed changes in *Salmonella* isolates which either had or did not have the *qacE*Δ1 gene were compared.

This research was conducted in four steps. First, MDR-*Salmonella* were exposed to two different organic acids (lactic and acetic acid) with samples withdrawn at different times to determine survival due to acid exposure. Bacteria were first pre-adapted to acid conditions, since it has been found that acid adapted bacteria are more resistant to further treatments. The second stage of the project was performed by exposing the MDR-*Salmonella* to three different quaternary ammonium compounds (QACs), two pure compounds (benzalkonium chloride, cetylpyridinium chloride) and one commercial compound (S.S.4) to determine survival due to exposure to these sanitizers. Samples were taken at different exposure times and neutralized in DE neutralizing buffer before enumerating by plating. The third phase of the project involved the possibility of organic acids conferring cross-protection against further treatment with quaternary ammonium compounds. This objective was developed using the same strains and QACs from the previous objectives. Strains were pre-adjusted to acid conditions in the same way that it was done in the first objective of the project and subsequently treated with QACs. Samples were withdrawn after 10 minutes of treatment and neutralized in DE buffer and plated in order to determine the survival rates. The last part of the research involved the susceptibility of MDR and non-MDR *Salmonella* in biofilms to treatment with quaternary ammonium compounds. Briefly, planktonic and biofilm cells were prepared according to the procedure of Ren and Frank (1993) with slight modifications and subsequently treated with QACs. Survival rates were determined.

Acid adapted and non-adapted *Salmonella* were both sensitive to the organic acid treatments; however, the acid adapted *Salmonella* were more resistant than the non-adapted when challenged with lactic and acetic acid 2%. It is known, acid adaptation can induce cross-protection to subsequent treatments such as organic acids, heat, osmotic pressure and oxidizers (Davidson and Taylor, 2007; Greenacre et al., 2006; Foster 1991). However, acid adaptation did not appear to induce cross-protection to further quaternary ammonium compounds treatment.

Quaternary ammonium compounds were more effective after 600 sec at 200 ppm. There was no significant difference in response to QACs between MDR and non-MDR *Salmonella*. MDR and non-MDR *Salmonella* in biofilms were more resistant to QACs treatment than planktonic cells, but the response to QAC treatments did not vary for MDR or non-MDR cells when in biofilms.

According to our findings we concluded that treatments that are available in food processing plants are equally effective against MDR and non-MDR *Salmonella*.

Contact information: Dr. Mark Harrison, phone number: 706-542-1088, e-mail address: mahfst@uga.edu. Department of Food Science and Technology, University of Georgia, Athens, USA 30605.

Scientific Abstract

Multidrug-resistant (MDR) *Salmonella* emerged early in the 1980s in the United Kingdom, and since then several infections and outbreaks have been reported worldwide.
In this study, six MDR *Salmonella* and two non-MDR *Salmonella* were evaluated against organic acids, quaternary ammonium compounds, possible organic acid cross-protection to subsequent treatment with quaternary ammonium compounds, and survival of biofilm cells after quaternary ammonium compounds treatments.

According to the findings of this study, acid adapted bacteria survived better than non-adapted bacteria when challenged with 2% of either acid at pH 3.5. Lactic acid was more effective than acetic acid after 4 h of exposure. Pre-adjustment with organic acids did not confer cross-protection against further treatments with quaternary ammonium compounds. There was no significant difference ($p > 0.05$) in response between MDR and non MDR-*Salmonella* that were planktonic or in biofilms quaternary ammonium treatments.

Key words: Multidrug resistant *Salmonella*, quaternary ammonium compounds, organic acids, cross-protection

Introduction

It has been estimated that 1.4 million cases of salmonellosis, leading to 16,000 hospitalizations and about 600 deaths occur each year in the United States (Zhao et al., 2003). In 2008, according to the Center for Disease Control and Prevention (CDC) *Salmonella* species accounted for 7,444 foodborne infections from a total of 18,499 cases per 100,000 people in the U. S. *Salmonella* serotypes Enteritidis, Typhimurium, Newport, Javiana and Saint Paul were the most frequently isolated during that year. *Salmonella* infections with nontyphoid serotypes usually appear 8 to 72 h after contact with the pathogen. Generally the illness is self-limiting and the use of antibiotics is not necessary; however, invasive cases such as meningitis and septicemia might require antimicrobial therapy (D'Aoust and Maurer, 2007; Willford et al., 2007).

Over the last two decades, there has been an increase in the emergence and prevalence of antimicrobial resistant bacteria; among them *Salmonella* (Miko et al., 2005; Gupta et al., 2003). Infections with multidrug resistant bacteria represent a public health concern, since failure to treatment increases morbidity and mortality in humans (Ladely et al., 2007; WHO, 2002). The intensive use of antimicrobials in different fields, such as human, veterinary medicine, and as food animal growth promoting agents has been associated with the

emergence of antimicrobial resistant microorganisms (Karatzas et al., 2008; Miko et al., 2005; Guerra et al., 2000).

Antibiotic resistance in *Salmonella* is attributed to mobile genetic elements such as plasmids, transposons and integrons. There are at least eight classes of integrons, with class 1 integrons the most frequently found in human and animal pathogens. Class 1 integrons also have a truncated quaternary ammonium resistance gene, qacΔE, and the sul1 gene which confers resistance to sulfonamide. Genes associated with class 1 integrons are connected with resistance to aminoglycosides, β-lactams, phenicols, macrolides, quaternary ammonium and trimethoprim (Gaze et al., 2005; Cloeckaert et al., 2006; D'Aoust and Maurer, 2007).

Salmonella is an ubiquitous microorganism and infections have been related to the consumption of contaminated products, such as poultry, beef, pork, eggs, milk, seafood, fresh produce and direct contact with animals (Miko et al., 2005; Zhao et al., 2007; WHO, 2008a). Several interventions, such as chemical dehairing, washing and sanitizing animal carcasses have been developed in order to minimize microbial contamination from food animals coming from abattoirs. Hot water, chlorine, short-chain organic acids and multiple hurdles are frequently used as part of the treatments. (Dickson and Anderson, 1992; Koohmaraie et al., 2005).

Sanitizers, such as quaternary ammonium compounds are usually used to prevent and eliminate contamination in food processing plants. However, there are some conditions such as slow growth rate, nutrient depletion, and surface attachment (biofilms) that modify the cell envelope of microorganisms and can modify resistance to antimicrobial agents (Frank and Koffi, 1990).

This research was intended to compare the effectiveness of organic acids against multidrug resistant (MDR) and non-MDR *Salmonella* when they have been adapted to acid conditions, to compare the effectiveness of quaternary ammonium treatment against MDR and non-MDR *Salmonella,* to determine whether organic acid treatments confer cross-protection against subsequent quaternary ammonium treatment on MDR and non-MDR *Salmonella,* and finally to determine survival of MDR and non-MDR *Salmonella* in biofilms after quaternary ammonium compound treatment.

Objectives

1. To compare the effectiveness of organic acids against multidrug resistant (MDR) and non-MDR *Salmonella* when they have been adapted to acid conditions.

2. To compare the effectiveness of quaternary ammonium treatment against MDR and non-MDR *Salmonella.*

3. To determine whether organic acid treatments confer cross-protection against subsequent quaternary ammonium treatment on MDR and non-MDR *Salmonella.*

4. To determine survival of MDR and non-MDR *Salmonella* in biofilms after quaternary ammonium compounds treatment.

Materials and Methods

Bacterial strains

MDR resistant *Salmonella* used included: *S.* Typhimurium DT104S, *S.* Typhimurium DT104565, *S.* Typhimurium DT104S/960081, *S.* Newport 01-2174, *S.* Agona 1146SA97, and *S.* Newport (Athens). Non-MDR resistant *Salmonella* used included: *S.* Typhimurium DT104S/921495 and *S.* Agona 0059SA98. *Pseudomonas aeruginosa* ATCC 15442 was used as quality control for the quaternary ammonium studies. The first seven *Salmonella* strains were donated by Dr. Axel Cloeckaert (French National Center for *Salmonella*) and the last one by Dr. John Maurer (UGA). The French strains were originally isolated from stools of patients

with gastroenteritis in Europe.
The Athens strain was isolated from cattle. Stock strains were maintained in brain heart infusion broth (BHI, pH 7.3; Becton Dickinson, Sparks, MD) containing 15% glycerol and were frozen at -80°C until they were used. Prior to each experiment, loop inocula were transferred from the frozen stocks to tryptic soy agar (TSA, pH 7.2; Becton Dickinson) plates and incubated at 37°C for 24 h.

Sanitizers and neutralizing solution
Food grade lactic (85%) and acetic acids (99.5%) were purchased from SAFC (St. Louis, MO). Cetylpyridinium chloride (100%) was purchased from Sigma-Aldrich (St. Louis, MO), benzalkonium chloride (50%) was purchased from Spectrum Laboratories (Gardena, CA), and S.S.4 (10%; Saratoga Food Safety; Northlake, IL) commercial quaternary ammonium was donated by Dr. Peter Taormina. Quaternary ammonium compounds (QACs) were neutralized with D/E neutralizing broth, containing lecithin, polysorbate 80, sodium bisulfite, sodium thiosulfate, sodium thioglycollate, dextrose, yeast extract, and pancreatic digest of casein (pH 7.4; Becton Dickinson).

Adaptation of *Salmonella* to acid and determination of survival
For acid adaptation *Salmonella* strains were grown statically in tryptic soy broth (pH 7.2) without glucose at 37°C for 24 h and sequentially transferred in tryptic soy broth (TSB) without glucose whose pH had been adjusted with concentrated lactic acid to pH 6.5 and 6.0. Acid adapted *Salmonella* were kept refrigerated (4°C) on TSA slants which had been adjusted to pH 6.0.

A loopful of acid adapted and nonadapted *Salmonella* were grown into 9 ml of TSB adjusted to pH 6.0 and 7.2, respectively, incubated at 37°C for 24 h, and transferred to a sterile beaker. The pH of each strain was measured (Accumet Basic, AB15 Fisher Scientific) and then decreased to 3.5 with either 2% lactic or acetic acid. Beakers were continuously stirred on a magnetic stirrer, and samples were removed at different times from 0 to 4 h. Cellular viability was determined by spreading onto TSA (pH 7.2) with 0.1% sodium pyruvate (Leyer and Johnson., 1997) to restore injured cells and incubated at 37°C for 48 h. Each pH (6.0 and 7.2) was tested with both acids. This experiment was replicated three times.

Susceptibility to Quaternary Ammonium Compounds (QACs)
All strains were grown in TSB (pH 7.2), incubated at 37°C for 24 h, centrifuged (Beckman coulter Allegra X-22R) twice at 3,500 rpm for 5 min and suspended in 5 ml of saline solution 0.85%. One ml of each bacterial suspension was mixed with 9 ml of QAC solution prepared to give a final concentration of 0 (no QAC), 100 and 200 ppm. Solutions were continuously shaken on an orbital shaker (VWR Scientific Product) and samples were removed at 15, 120, 300 and 600 s of exposure. Once removed, the samples were immediately mixed with 9 ml of D/E neutralizing broth. Surviving cells were calculated by enumerating surviving cells on TSA plates containing 0.1% sodium pyruvate which were incubated at 37°C for 48 h. This experiment was replicated three times.

Organic acid cross protection to QACs treatments
The procedure to acid adapt was followed as above. Acid adapted and nonadapted bacteria were grown into 9 ml of TSB without glucose pH 6.0 (acid adapted) and pH 7.2 (nonadapted), incubated at 37°C for 24 h, centrifuged (Beckman Coulter Allegra X-22R) twice at 3500 rpm for 5 min, and suspended in 3 ml of saline solution 0.85%. Portions (0.1 ml) of the suspensions were dispensed on two different sterile stainless steel coupons (type 304, No. 4 finish, 2 x 5 cm) inside a sterile Petri dish. One served as the control and the other treatment. The suspensions were mixed with 200 µl of the QAC to obtain a final concentration of 33 ppm with an exposure of 10 min. After the exposure time, the volume of sample was collected with a pipette and transferred to a tube containing 9 ml of D/E neutralizing broth, serial dilutions were prepared, and surviving cells were enumerated for the control (no sanitizer) and treatment coupons by using spread plates of TSA with 0.1% sodium pyruvate and incubated at 37°C for 48 h. This experiment was replicated three times.

Biofilms
The procedure of Ren and Frank (1993) was followed for the planktonic and biofilms part with slight modifications.

Preparation of biofilms - Stainless steel coupons (type 304, No. 4 finish, 2 x 5 cm) were used as the support for the biofilms. New stainless steel coupons were degreased with acetone, washed with alkaline jet-clean detergent (Fisher-Scientific) and rinsed thoroughly with distilled water.

The coupons were placed into two (one control and one treatment) 25 x 180 mm culture tubes with 25 ml of TSB and autoclaved. Each tube was inoculated with 20 µl of an overnight TSB culture, and incubated at 37^{o}C for 24 h. The coupons were removed from the cultures tubes with sterile forceps, rinsed with sterile PBS, and placed into other tubes containing fresh sterile TSB, and incubated for another 24 h. After 48 h incubation, the coupons were rinsed with sterile PBS and ready for the inactivation experiments. The remaining suspension in the tube was used for the planktonic studies.

Inactivation of planktonic cells - One milliliter of the planktonic cells was added to 9 ml of the correspondent QAC that was prepared to obtain a final concentration of 200 ppm. The solution was continuously shaken on an orbital shaker (VWR Scientific Product) for 10 min. After the 10 min, exposure time, one ml of the solution was removed and added to 9 ml of D/E neutralizing broth. Control samples were mixed directly with D/E neutralizing broth without exposure to QAC. Surviving cells were calculated by spreading onto TSA plates containing 0.1% sodium pyruvate and incubated at 37^{o}C for 48 h. This experiment was replicated three times.

Inactivation of biofilm cells - Stainless steel coupons were removed from the culture tubes with sterile forceps, rinsed with sterile PBS to remove unattached cells, dipped into 45 ml of 200 ppm QAC solution, and continuously shaken on an orbital shaker for 10 min. At the end of the exposure time coupons were placed into sterile D/E neutralizing broth for 1 min. Control coupons (no QAC) were rinsed with sterile PBS and placed immediately into sterile D/E neutralizing broth for the same time. Biofilm cells were removed from the neutralized coupons by swabbing both sides of the coupon 10 times with a sterile calcium alginate swab. The swab was transferred to a 9 ml sterile D/E neutralizing broth tube, serial dilutions made, viable cells enumerated by spreading onto TSA plates containing 0.1% sodium pyruvate, and incubated at 37^{o}C for 48 h. This experiment was replicated three times.

Statistical Analysis
The $\log_{10}$ reduction of bacterial populations for the acid studies were calculated as $\log_{10}$ (cfu count after acid treatment/initial cfu count at time -1) where t = 0, 1, 2, 3 and 4. For the quaternary ammonium portion, bacterial populations were calculated as $\log_{10}$ (initial at time - 1/ cfu count after QAC treatment) where t = 20, 120, 300 and 600 sec and for the cross-protection and biofilms part it was calculated as $\log_{10}$ (control cfu count/ treatment cfu count). All the experiments were analyzed using analysis of variance (ANOVA) with Tukey's multiple comparison method. The level of significance throughout the project was $p < 0.05$.

Results

1. **Effectiveness of organic acids against multidrug resistant (MDR) and non-MDR *Salmonella* when adapted to acid conditions.**

Acid adapted and nonadapted *Salmonella* were treated with lactic acid and acetic acid. Overall, the acid adapted bacteria survived better ($p< 0.05$) than nonadapted bacteria when challenged with 2% of both acids at pH 3.5. There was no statistical difference between the effect of the different acids, so the means are combined (Table 1).

There was no statistical difference ($p > 0.05$) between the effectiveness of the organic acid at the 2 challenge pHs, so the means of the population decrease for the particular acid are combined for the challenge pH levels (Table 2). Overall, Lactic acid was more effective than acetic acid after 4 h of exposure (Table 2; Fig. 1).

There was no significant difference ($p > 0.05$) in response to acid treatment (lactic or acetic) between *Salmonella* MDR (R) and non-MDR (S). However, *S.* Typhimurium DT104565, *S.* Typhimurium DT104S/921495, and *S.* Typhimurium DT104s were more susceptible to the treatments than the others (Fig. 2).

Table 1. Survival of acid adapted and nonadapted *Salmonella* after subsequent challenge with either lactic or acetic acids.

pH	Mean population decrease
6.0	-2.59[a]
7.2	-2.76[b]

Means in the same column with different superscripts were statistically different at significance level 0.05.

Table 2. Survival of acid adapted and nonadapted *Salmonella* after subsequent challenge at either pH 6.0 or 7.2.

Acid	Mean population decrease
Acetic	-2.58
Lactic	-2.75

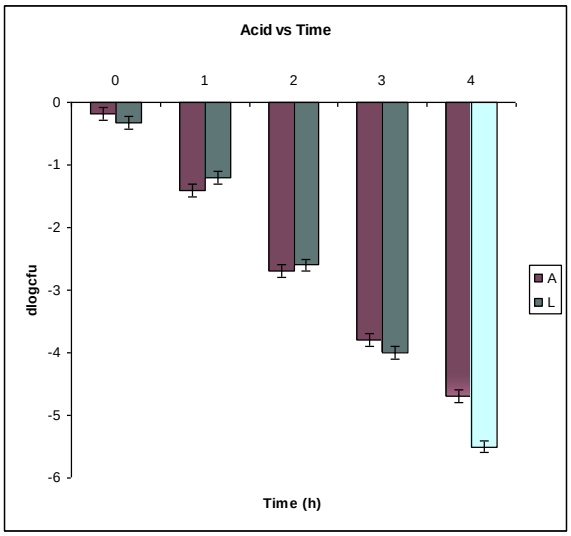

Fig. 1. Survival of acid adapted and nonadapted *Salmonella* after 4 h exposure to lactic or acetic acid. There was no significant difference ($p > 0.05$) in the effect of the acid, so the means of the population decrease for the particular acid are combined for the challenge pH levels

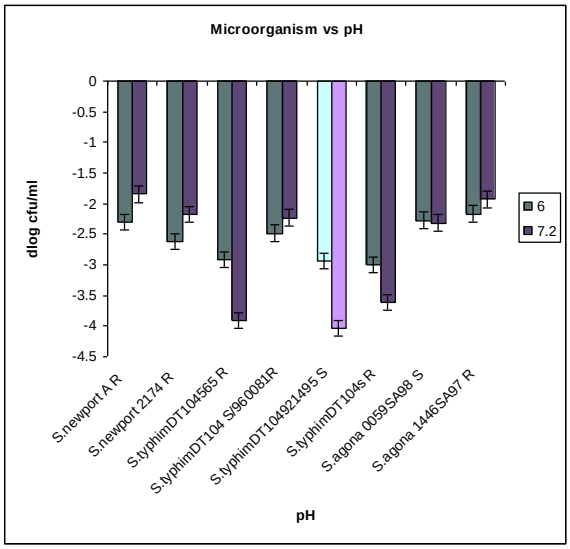

Fig. 2. Mean population decreases for individual MDR and non-MDR *Salmonella* isolates that were acid adapted and nonadapted in response to acid (lactic or acetic) challenge at pH 6.0 or 7.2.

2. Effectiveness of quaternary ammonium compounds against MDR and non-MDR *Salmonella.*

Overall there is no significant difference ($p > 0.05$) in response to quaternary ammonium treatment between MDR and non-MDR. Since there was no difference in the population reductions for the different QACs at different concentrations and exposure times the data was combined (Fig. 3). The inactivation of the *Salmonella* strains was rapid with at least a 4.8 log decrease within the first 20 sec (Table 3). Greater inactivation was achieved with additional time. *Pseudomonas aeruginosa*, which is more resistant to QAC exposure than *Salmonella*, is commonly used as a quality control organism for the QAC studies and was included for comparative purposes.

Between the *Salmonella* strains, there was no difference in the inhibitory response when exposed to the different QACs. However, there was a significant ($p<0.05$) difference in inactivation between the QACs overall. Benzalkonium chloride and SS4 were more effective than cetylpyridinium chloride ($p< 0.05$) (Fig. 5). There appears to be slight differences in the inactivation of the strains over time, but the *Salmonella* strains responded in similar fashions (Fig. 6). Again *P. aeruginosa* was included for comparative purposes.

Non-MDR *Salmonella* were more resistant ($p < 0.05$) to quaternary ammonium compounds than MDR *Salmonella* (Table 4). It should be noted that while there was a statistical difference, the QACs were able to reduce the populations of both groups by at least 5.9 logs from the populations at time 0.

Table 3. Response of MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds over time.

Time (sec)	Mean difference from time 0 population (log cfu/ml)	Std Dev
20	4.8	1.20
120	5.8	1.20
300	6.3	0.96
600	6.5	0.84

Table 4. Response of MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds.

Group	Mean difference from time 0 population (log cfu/ml)	Std Dev
MDR	6.2[a]	0.93
Non-MDR	5.9[b]	1.11

Means in the same column with different superscripts were statistically different at significance level 0.05.

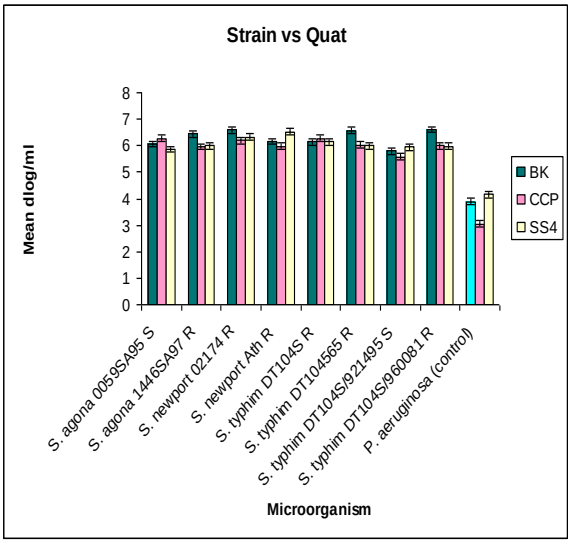

Fig. 3. MDR and non-MDR *Salmonella* response to quaternary ammonium compound treatments. BK: benzalkonium chloride, CCP: cetylpyridinium chloride, SS4: commercial QAC

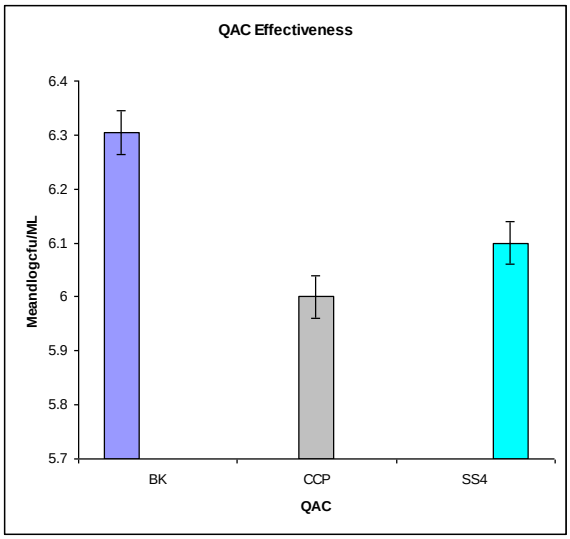

Fig. 4. Comparison of population reductions (mean dlog cfu/ml) of MDR and non-MRD *Salmonella* when exposed to benzalkonium chloride (BK), a commercial quaternary ammonium compound (SS4), and cetylpyridinium chloride (CCP).

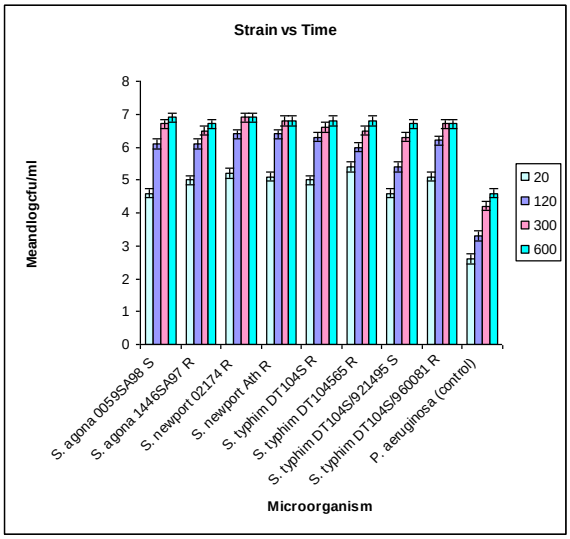

Fig. 5. Comparison of population reductions (mean dlog cfu/ml) of MDR and non-MRD *Salmonella* when exposed to benzalkonium chloride (BK), a commercial quaternary ammonium compound (SS4), and cetylpyridinium chloride (CCP) over time (sec).

3. Organic acids treatment conferring cross-protection against subsequent quaternary ammonium treatment on MDR and non-MDR *Salmonella.*

In order to determine a possible difference in the survivor rate in this portion of the project, lower than recommended levels (33 ppm rather than 200 ppm) of the QACs were used. Overall, there was no significant difference ($p > 0.05$) between the two pH levels (Table 5), meaning that pre-adjustment with organic acids (lactic or acetic) did not confer cross-protection against further treatment with quaternary ammonium compounds. In addition, there was no significant difference ($p > 0.05$) between MDR and non-MDR *Salmonella* in response to QAC treatment after pre-adjustment to acid conditions (Table 6). Overall, cetylpyridinium chloride (CPC) was the less effective ($p < 0.05$) of the three QACs used in this part of the study (Table 7). Each *Salmonella* strain behaved differently with each quaternary ammonium compound, but overall, cetylpyridinium chloride was the less effective ($p < 0.05$) (Fig. 6).

Table 5. Comparison of the response of pre-acid adapted MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds (33 ppm) challenged with lactic or acetic acids at pH 6.0 or 7.2.

pH	Mean difference from time 0 population (log cfu/ml)	Std Dev
6.0	2.79	1.12
7.2	2.98	1.21

Table 6. Comparison of the response of pre-acid adapted MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds (33 ppm) challenged with lactic or acetic acids.

Group	Mean difference from time 0 population (log cfu/ml)	Std Dev
MDR	2.85	1.16
Non-MDR	2.99	1.21

Table 7. Comparison of the response of pre-acid adapted MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds (33 ppm) challenged with lactic or acetic acids.

QAC	Mean difference from time 0 population (log cfu/ml)	Std Dev
BK	3.57[a]	1.00
SS4	3.15[a]	1.00
CPC	1.93[b]	0.75

Means in the same column with different superscripts were statistically different at significance level 0.05.

BK: benzalkonium chloride, CCP: cetylpyridinium chloride, SS4: commercial QAC

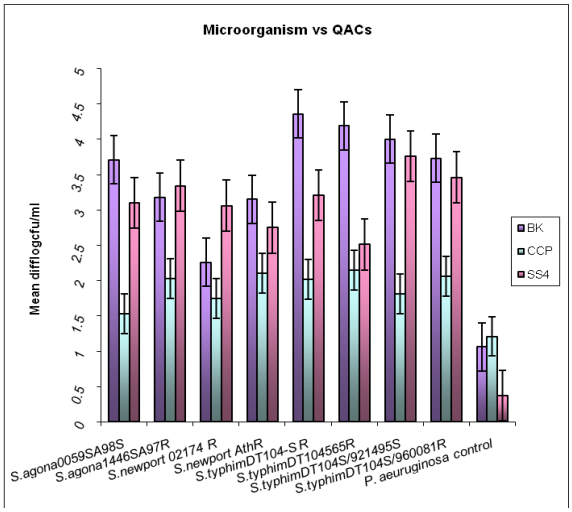

Fig. 6. Comparison of the response of individual strains of pre-acid adapted MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds (33 ppm) challenged with lactic or acetic acids. BK: benzalkonium chloride, CCP: cetylpyridinium chloride, SS4: commercial QAC

4. Survival of MDR and non-MDR *Salmonella* in biofilms after quaternary ammonium compounds treatment.

4.1. Planktonic

Overall there was no significant difference ($p > 0.05$) between MDR and non-MDR *Salmonella* planktonic cells after treatment with 200 ppm QACs (Table 8). There was no individual significant difference ($p > 0.05$) among *Salmonella* strains. *P. aeruginosa* was included for comparative purposes (Fig. 7). Planktonic cells were killed more effectively ($p < 0.05$) with benzalkonium chloride and cetylpyridinium chloride than when SS 4 was used (Table 9).

Table 8. Comparison of the response of planktonic MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds (200 ppm).

Group	Mean difference from time 0 population (log cfu/ml)	Std Dev
MDR	6.00	0.52
Non-MDR	6.01	0.57

Table 9. Comparison of the response of planktonic MDR and non-MDR *Salmonella* populations to different quaternary ammonium compounds (200 ppm).

QAC	Mean difference from time 0 population (log cfu/ml)	Std Dev
BK	6.24[a]	0.75
SS4	5.20[b]	0.85
CPC	6.11[a]	0.65

Means in the same column with different superscripts were statistically different at significance level 0.05.

BK: benzalkonium chloride, CCP: cetylpyridinium chloride, SS4: commercial QAC

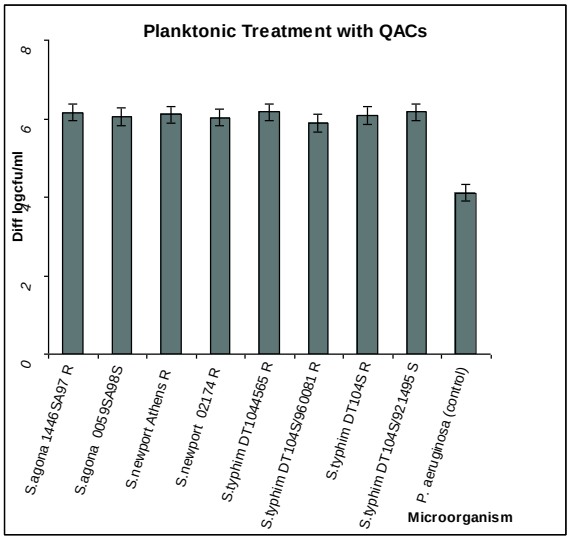

Fig. 7. Comparison of the response of individual strains of planktonic MDR and non-MDR *Salmonella* populations to quaternary ammonium compounds (200 ppm) challenged with lactic or acetic acids.

4.2. Biofilms

There was no significant difference ($p > 0.05$) between MDR and non-MDR *Salmonella* after treatment with QACs (Table 10). There was no individual significant difference ($p > 0.05$) among *Salmonella* strains; however, there was difference ($p < 0.05$) between *Salmonella* strains and the control strain, *P. aeruginosa* (Fig. 8). Biofilm cells were equally resistant to the benzalkonium chloride, cetylpyridinium chloride, and SS 4 (Table 11).

Table 10. Comparison of the response of MDR and non-MDR *Salmonella* populations in an established biofilm to quaternary ammonium compounds (200 ppm).

Group	Mean difference from time 0 population (log cfu/cm^2)	Std Dev
MDR	2.73	0.75
Non-MDR	2.75	0.80

Table 11. Comparison of the response of MDR and non-MDR *Salmonella* populations in an established biofilm to quaternary ammonium compounds (200 ppm).

QAC	Mean difference from time 0 population (log cfu/cm^2)	Std Dev
BK	2.61	0.89
SS4	2.64	0.78
CPC	2.45	0.99

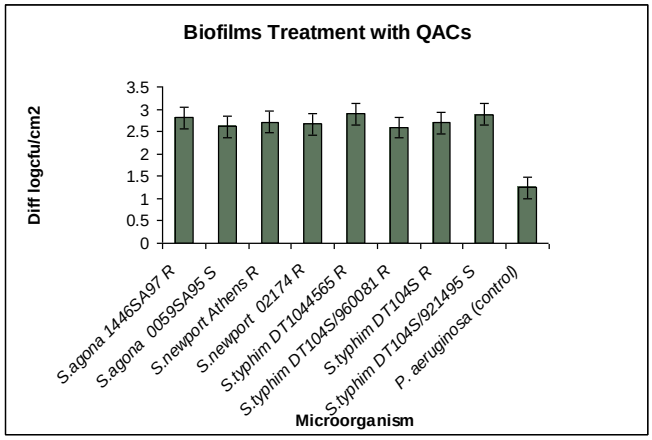

Fig. 8. Comparison of the response of individual strains of MDR and non-MDR *Salmonella* populations in an established biofilm to quaternary ammonium compounds (200 ppm).

Discussion

In this study, acid adapted and nonadapted *Salmonella* were both sensitive to the organic acid treatments. However, the acid adapted *Salmonella* were more resistant than the nonadapted populations when challenged with lactic and acetic acid 2%. These results appear to disagree with a previous research (Dickson and Kunduru, 1995) where acid adapted *S.* Typhimurium was more sensitive to lactic acid (1.5 and 3.0%) than the nonadapted cultures. In the present study, the microbial populations decreased gradually with time being through 4 hours of exposure. Lactic acid was slightly more effective than acetic acid. This might be explained by the lower pH and pKa of the lactic acid (Davison and Taylor, 2007).

Organic acids can induce cross-protection to subsequent treatments (Davidson and Taylor, 2007; Greenacre et al., 2006; Foster 1991). In contrast, according to our findings, acid adaptation did not appear to induce cross-protection to exposure to subsequent quaternary ammonium compounds treatments. In general, there was no significant difference ($p > 0.05$) in response to acid treatment between *Salmonella* MDR and non-MDR. However, *S.* Typhimurium DT104565, *S.* Typhimurium DT104S/921495, and *S.* Typhimurium DT104S were more susceptible to the treatments than the other *Salmonella* strains. Further research needs to be done to explain the susceptibility of these strains.

Results of this study agree with previous reports of bacterial capability to attach to surfaces such as stainless steel and their ability to be more resistant to sanitizers than planktonic bacteria (Ronner and Wong, 1993; Ren and Frank, 1993; Frank and Koffi, 1990). In our findings, planktonic cells were more effectively reduced than the biofilm cells, with benzalkonium chloride and SS4, the commercial QAC, being more effective against planktonic cells. In contrast, there was no significant difference ($p > 0.05$) among the effectiveness of quaternary ammonium compounds when they were used on biofilm cells. In this study, we used stainless steel as the attachment surface since this material is widely used in food processing plants and microorganisms attach easily to it (Frank, 2001).

In general, under most of the conditions tested there was no difference between the MDR and non-MDR *Salmonella* populations survival rates. Benzalkonium chloride was the most effective QAC followed in most cases by SS4, the commercial QAC, and then cetylpyridinium chloride. *Pseudomonas aeruginosa* was included in the QACs experiments as a quality control strain due to its intrinsic resistance to QACs (Tabata et al., 2003). In all cases, it was significantly more resistant to QACs than the *Salmonella* strains.

It has been speculated that some microorganisms, including *Salmonella*, might acquire adaptive resistance to quaternary ammonium compounds (Chaplin, 1959; Nishikawa et al., 1979; Maxcy et al., 1971; Mangalappalli-Illathu and Korber, 2006). Several genes have been implicated in conferring resistant to cationic biocides such as quaternary ammonium compounds. Some of them are qacA, qacB, qacg, qacH, smr, qacE, and qacΔE (Russell, 2003). The qacΔE gene is present in integrons class 1 that make part of MDR *Salmonella*. Our findings show that this particular gene was not involved in conferring resistant to QACs since there was little, if any, difference in susceptibility to the QAC treatments between the MDR and non-MDR *Salmonella* that were tested.

Reference List

1. Center for Disease Control and Prevention. 2009. Preliminary Foodnet data on the incidence of infection with pathogens transmitted commonly trough food - 2008. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5813a2.htm.
2. Chaplin, E.C. 1951. Bacterial resistance to quaternary ammonium compounds. J. Bacteriol. 63:453:458.
3. Cloeckaert, A., Praud, K., Doblet, B., Demartin, M., and Weill, F. 2006. Variant *Salmonella* genomic island 1-L antibiotic resistance gene cluster in *Salmonella enterica* serovar Newport. Antimicrob. Agents Chemother. 50:3944-3946.
4. D'Aoust, J-Y., Maurer, J., and Bailey J. 2007. *Salmonella* species. In:M.P. Doyle and L.R. Beuchat. Food Microbiology - Fundamentals and Frontiers. 3rd ed. ASM Press. Washington, D.C. pg. 187-236.
5. Davidson, M. and Taylor, M. 2007. Chemical preservatives and natural antimicrobial compounds. In: M.P. Doyle and L.R. Beuchat. Food Microbiology - Fundamentals and Frontiers. 3rd ed. ASM Press. Washington, D.C. pg. 713-745.
6. Dickson, J. and Anderson, E. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Prot. 55:133-140.
7. Dickson, J. and Kunduru, M. 1995. Resistance of acid-adapted *Salmonella* to organic acid rinses on beef. J. Food Prot. 58:973-976.
8. Foster, W. 1991. *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. J. Bacteriol. 173:6896-6902.
9. Frank, J. 2001. Microbial attachment to food and food contact surfaces. In: S.L. Taylor. Advances in Food and Nutrition Research. vol 43. Academic Press. San Diego, CA. pg. 320-357.
10. Frank, J. and Koffi, A. 1990. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. J. Food Prot. 53:550-554.
11. Gaze, H., Abdouslam, N., Hawkey, M., and Wellington, M. 2005. Incidence of class 1 integrons in a quaternary ammonium compound-polluted environment. Antimicrob. Agents Chemother. 49:1802-1807.
12. Greenacre, J., Lucchini, S., Hinton, C., and Brocklehurst. 2006. The lactic acid induced acid tolerance response in *Salmonella enterica* serovar Typhimurium induces sensitivity to hydrogen peroxide. Appl. Environ. Microbiol. 72:5623-5625.
13. Guerra, B., Junker, E., Schroeter, A., Helmuth, R., Guth, E., and Beutin, L. 2006. Phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* O111 isolates. J. Antimicrob. Chemother. 57:1210-1214.
14. Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S., Hoekstra, P., Whichard, M., Barrett, J., and Angulo J. 2003. Emergence of multidrug resistant *Salmonella enterica* serovar Newport infections resistant to expanded-spectrum cephalosporins in the United States. J. Infec. Dis. 188:1707-1716.
15. Hickey, W. and Hirshfield, N. 1990. Low pH induced effects on patterns of protein synthesis and on internal pH in *Escherichia coli* and *Salmonella* Typhimurium. Appl. Environ. Microbiol. 56:1038-1045.
16. Jung, S. and Beuchat, L. 2000. Sensitivity of multidrug resistant *Salmonella* Typhimurium DT104 to organic acids and thermal inactivation in liquid eggs. Food Microbiol. 17: 63-71.
17. Karatzas, A., Randall, P., Webber, M., Piddock, J., Humphrey, J., Woodward, J., and Goldhman, G. 2008. Phenotypic and proteomic characterization of multiply antibiotic resistant variants of *Salmonella enterica* serovar Typhimurium selected following exposure to disinfectants. Appl. Environ. Microbiol. 74:1508-1516.
18. Koohmaraie, M., Arthur, T., Bosilevac, J., Guerini, M., Shackelford, S., and Wheeler, T. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. Meat Science. 71:79-91.
19. Mangalappalli-Illathu, K. and Korber, R. 2006. Adaptive resistance and differential protein expression of *Salmonella enterica* serovar Enteritidis biofilms exposed to benzalkonium chloride. Antimicrob. Agents Chemother. 50:3588-3696.
20. Maxcy, R., Tiwari, N., and Soprey, P. 1971. Changes in *Escherichia coli* associated with acquired tolerance for quaternary ammonium compounds. Appl. Microbiol. 22:229-232.

21. Miko, A., Pries, K., Schoeter, A, and Helmuth, R. 2005. Molecular mechanisms of resistance in multidrug resistant serovars of *Salmonella enterica* isolated from foods in Germany. J. Antimicrobial. Chemoth. 56:1025-1033.
22. Nishikawa, K., Oi, S., and Yamamoto, T. 1979. A bacterium resistant to benzalkonium chloride. Agric. Biol. Chem. 43:2473-2478.
23. Ren, T. and Frank, J. 1993. Susceptibility of starved planktonic and biofilm *Listeria monocytogenes* to quaternary ammonium sanitizer as determined by direct viable and agar plate counts. J. Food Prot. 56:573-576.
24. Ronner, B. and Wong, C. 1993. Biofilm development and sanitizer inactivation of *Listeria monocytogenes* and *Salmonella* Typhimurium on stainless steel and buna-n rubber. J. Food. Prot. 56:750-758.
25. Russell, A. 2003. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect. Dis. 3:794-803.
26. Willford, J., Manley, W., Rebelein, T., and Goodridge, L. 2007. Pulsed field, ribotype and integron analysis of multidrug resistant isolates of *Salmonella enterica* serovar Newport. Food Res. Internat. 40:975-981.
27. Zhao, S., Qaiyumi, S., Friedman, R., Foley, S., White, D., McDermott, P., Donkar, T., Bolin, C., Munro, S., Baron, R., and Walker, R. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J. Clin. Microbiol. 41:5366-5371.