The Journal of Microbiology, April 2009, p. 142-146 Vol. 47, No. 2
DOI 10.1007/s12275-008-0237-5

Adaptation and Cross-Adaptation of *Listeria monocytogenes* and *Salmonella enterica* to Poultry Decontaminants

Alicia Alonso-Hernando, Rosa Capita*, Miguel Prieto, and Carlos Alonso-Calleja

Área de Nutrición y Bromatología, Escuela Superior y Técnica de Ingeniería Agraria. Avenida de Astorga, s/n. 24400-Ponferrada, Spain

(Received September 24, 2008 / Accepted December 19, 2008)

Information on the potential for acquired reduced susceptibility of bacteria to poultry decontaminants occurring is lacking. Minimal Inhibitory Concentrations (MICs) were established for assessing the initial susceptibility and the adaptative and cross-adaptative responses of four bacterial strains (*Listeria monocytogenes* serovar 1/2a, *L. monocytogenes* serovar 4b, *Salmonella enterica* serotype Typhimurium, and *S. enterica* serotype Enteritidis) to four poultry decontaminants (trisodium phosphate, acidified sodium chlorite -ASC-, citric acid, and peroxyacetic acid). The initial susceptibility was observed to differ among species (all decontaminants) and between *Salmonella* strains (ASC). These inter- and intra-specific variations highlight (1) the need for strict monitoring of decontaminant concentrations to inactivate all target pathogens of concern, and (2) the importance of selecting adequate test strains in decontamination studies. MICs of ASC (0.17±0.02 to 0.21±0.02 mg/ml) were higher than the U.S. authorized concentration when applied as a pre-chiller or chiller solution (0.05 to 0.15 mg/ml). Progressively increasing decontaminant concentrations resulted in reduced susceptibility of strains. The highest increase in MIC was 1.88 to 2.71-fold (ASC). All decontaminants were shown to cause cross-adaptation of strains between both related and unrelated compounds, the highest increase in MIC being 1.82-fold (ASC). Our results suggest that the in-use concentrations of ASC could, in certain conditions, be ineffective against *Listeria* and *Salmonella* strains. The adaptative and cross-adaptative responses of strains tested to poultry decontaminants are of minor concern. However, the observations being presented here are based on *in vitro* studies, and further research into practical applications are needed in order to confirm these findings.

***Keywords*:** poultry decontaminants, adaptation, cross-adaptation, *Listeria monocytogenes*, *Salmonella enterica*

Poultry consumption has increased considerably over the last few years. At present, approximately 30% of the world's total meat consumption is poultry, only exceeded by pork (FAOSTAT, 2006). This high consumption of poultry adds to concerns for marketing a product safe for consumers. However, poultry is often a vector for pathogenic microorganisms, and is frequently implicated in foodborne diseases (Sofos, 2008).

In the European Union, the report of trends and sources of zoonoses, zoonotic agents, antimicrobial resistance, and food-borne outbreaks shows that in 2006 salmonellosis was the second most common zoonosis, after campylobacteriosis, accounting for 160,649 confirmed human cases, with poultry being among the main foodstuffs where *Salmonella* was detected. Listeriosis is also an important food-borne zoonosis, due to the severity of the disease and the high mortality rate related to it. The number of listeriosis cases has increased significantly in the EU in recent years, and in 2006 a total of 1,583 human cases were reported. Ready-to-eat products of meat origin (including broiler meat) are among the main sources of human listeriosis (EFSA, 2007).

Strict and continuous adherence to good hygiene practices (GHP) throughout the food chain, in association with the application of Hazard Analysis and Critical Control Point (HACCP) principles, is the basis for controlling microbial contamination in meat and poultry. Moreover, the microbiological status of carcasses can be improved substantially by the application of carcass decontamination technologies (Del Río *et al.*, 2007a, 2007b). For many years, European legislation (Directives 71/118/EEC, 92/116/EEC, and 97/79/EEC) limited the use of substances, other than clean water, for the removal of microbial surface contamination of products of animal origin. Currently, article 3 (2) of the Regulation (EC) N° 853/2004 of the European Parliament and of the Council, laying down specific hygiene rules for food of animal origin (OJEC, 2004) provides a legal basis for the use of chemical substances for decontamination purposes, provided they are approved in accordance with the procedure referred to in the said Regulation. The European Food Safety Authority is responsible for scientific evaluation of the safety and efficacy of substances used for the removal of microbial surface contamination of foods of animal origin, and a guidance document for the submission of data has been developed (EFSA, 2006). According to this document, available scientific information on the potential for acquired reduced susceptibility of bacteria to the substances occurring is one of the criteria given in the guidance document on the submission of data for the evaluation of decontami-

* To whom correspondence should be addressed.
(Tel) 34-987-44-2000; (Fax) 34-987-44-2070
(E-mail) rosa.capita@unileon.es

nant safety and efficacy. Four chemicals (trisodium phosphate, acidified sodium chlorite, chlorine dioxide and peroxyacids) have been currently revised for final approval by the EU authorities as poultry decontaminants. In a recent report, the EFSA concluded that there are currently no published data which would enable an assessment of whether the application of the four abovementioned antimicrobials could lead to the occurrence of acquired reduced susceptibility to these substances, and encouraged further research on the likelihood of the emergence of bacteria adaptation to poultry decontaminants (EFSA, 2008).

The main objective of this study was to investigate the incidence and magnitude of adaptative and cross-adaptative responses of *L. monocytogenes* and *S. enterica* isolates to four poultry decontaminants. A further aim was to explore the inter- and intra-specific differences in susceptibility of strains to chemicals.

Materials and Methods

Bacterial isolates

Four strains were used in this study: *Listeria monocytogenes* serovar 1/2a (L1), *Salmonella enterica* serotype Typhimurium (S1), *Salmonella enterica* serotype Enteritidis (S2) (poultry isolates), and *L. monocytogenes* serovar 4a (L2) (obtained from a sheep's spinal cord). All strains were previously isolated in our laboratory. Stock cultures were maintained through monthly transfers onto tryptic soy agar (TSA, Oxoid) plates and stored at 3±1°C.

Decontaminants

Four chemical decontaminants were included: trisodium phosphate (TSP, Merck, Germany), sodium chlorite (Fluka, Spain) acidified to pH 2.7 by adding citric acid (Panreac, Spain) (acidified sodium chlorite; ASC), citric acid (CA), and peroxyacetic acid (PA, Inspexx 100, Ecolab, USA). All solutions were aseptically prepared in sterile distilled water. Further dilutions were freshly prepared before each experiment.

Minimum Inhibitory Concentrations (MICs)

The MIC values were established using a microdilution broth method following the NCCLS Standard (Anonymous, 1999). Five colonies of each organism were taken from TSA plates, inoculated into 10 ml of Mueller-Hinton (MH) broth (Oxoid) and incubated at 37°C for 24 h. These bacterial cultures contained approximately 10^8 CFU/ml. The decontaminants' dilution range was prepared using sterilised distilled water and added to microwell plates to a final volume of 20 µl, and each dilution was inoculated with 180 µl of appropriate dilutions (in MH broth) of inocula in order to give a final concentration in the well of 5×10^5 CFU/ml. The inoculum concentration was confirmed by plating. The microwell plates were incubated at 37°C, and the MIC was established as the lowest decontaminant concentration necessary to prevent growth after 24 h of incubation. The absence of growth was determined by visual inspection and, if necessary, confirmed by plating. Both positive (200 µl of inoculum 5×10^5 CFU/ml) and negative (180 µl of MH broth + 20 µl of chemical compound) controls were included in each experiment.

Adaptation and cross-adaptation to poultry decontaminants

Isolates were tested for their ability to adapt to chemical decontaminants by exposing the strain to increasing concentrations of a compound. The test was performed in the same manner as described for determining MIC. The decontaminant starting concentration was MIC/2. When growth was observed, 20 µl of the suspension were aseptically transferred to the next well, which contained 160 µl of MH broth and 20 µl of decontaminant solution. After the transfer, each well contained a decontaminant concentration 1.5 times stronger than the previous well. This procedure was continued until no growth was observed after 3 days of incubation at 37°C. The suspension in the last well with recorded growth was centrifuged (8,600 rpm, 2 min), and the pellet was washed with phosphate-buffered saline to remove the compound. The pellet was re-suspended in 10 ml of MH broth and incubated at 37°C for 24 h. Cultures were streaked on TSA plates and stored (3±1°C) after incubation at 37°C for 48 h. MICs of all the four decontaminants were measured for all the 16 adapted strains (four strains adapted to four chemical compounds). The stability of adaptation to decontaminants was determined by measuring MICs of decontaminants for adapted strains after storage for three months on TSA plates (3±1°C) with monthly transfers.

Statistical analysis

MIC values were evaluated using analysis of variance techniques. Mean separations were obtained using Duncan's multiple range test. The level of significance was taken as $P=0.05$. For statistical calculations, the Statistica® 6.0 (Statsoft Ltd., USA) software package was used.

Results

Decontaminant MIC values for all non-adapted (initial) and adapted (after progressively increasing decontaminant concentrations at 37°C) strains are shown in Tables 1~4. The initial MICs (mg/ml) varied from 14.00±0.10 to 15.06 ±0.25 (TSP), from 0.17±0.02 to 0.21±0.02 (ASC), from 0.95±0.01 to 3.62±0.02 (CA), and from 0.07±0.03 to 0.11 ±0.01 (PA). MICs differed between species, with higher values observed in *Listeria* than in *Salmonella* for TSP and PA. By contrast, MICs of CA were significantly higher for *Salmonella* strains than for *Listeria* strains. MICs of ASC were higher for *Salmonella* Typhimurium than for all other strains tested.

Progressively increasing decontaminant concentrations resulted in an increase in MICs of TSP and ASC in all strains (Tables 1 and 2). Only some strains showed adaptation to CA (strain L1) and PA (L2 and S1). The magnitude of the adaptative response was greatest with ASC, where the increase in MICs was observed to be from 1.88-fold (L2) to 2.71-fold (L1). Only marginal increases in the MICs (lower than 1.72-fold) of TSP, CA, and PA agents were observed.

All chemicals were observed to cause cross-adaptation in both *L. monocytogenes* and *S. enterica* strains to various decontaminants. Cross-adaptative responses were greatest

Table 1. Minimum inhibitory concentrations (MICs) of trisodium phosphate (mg/ml) before and after exposing four bacterial strains to increasing concentrations of poultry decontaminants

Strain	L1	L2	S1	S2
Non-adapted	$15.06\pm0.25^{a}{}_{a}$	$14.90\pm0.28^{a}{}_{a}$	$14.10\pm0.00^{b}{}_{a}$	$14.33\pm0.05^{c}{}_{a}$
Adapted to TSP	$16.80\pm0.09^{a}{}_{b}$	$17.07\pm0.05^{b}{}_{b}$	$16.63\pm0.10^{c}{}_{b}$	$16.97\pm0.05^{d}{}_{b}$
Adapted to ASC	$17.00\pm0.09^{a}{}_{b}$	$16.20\pm0.54^{b}{}_{c}$	$15.53\pm0.36^{c}{}_{c}$	$\underline{15.47\pm0.27}^{c}{}_{c}$
Adapted to CA	$17.10\pm0.57^{a}{}_{b}$	$16.93\pm0.43^{a}{}_{b}$	$16.27\pm0.14^{b}{}_{b}$	$\underline{16.70\pm0.42}^{ab}{}_{b}$
Adapted to PA	$15.90\pm0.54^{ab}{}_{c}$	$16.27\pm0.81^{a}{}_{c}$	$15.40\pm0.18^{bc}{}_{c}$	$\underline{15.10\pm0.26}^{c}{}_{d}$

L1, *Listeria monocytogenes* serovar 1/2a (poultry isolate); L2, *L. monocytogenes* serovar 4b (sheep's spinal cord isolate); S1, *S. enterica* serotype Typhimurium PT 193 (poultry isolate); S2, *S. enterica* serotype Enteritidis PT 1 (poultry isolate); TSP, trisodium phosphate; ASC, acidified sodium chlorite; CA, citric acid; PA, peroxyacetic acid. Mean values (n=6) in the same row with no letters in common (superscript) are significantly different ($P<0.05$). Mean values (n=6) in the same column with no letters in common (subscript) are significantly different ($P<0.05$). Strains with reduced susceptibility maintained after 90 days with periodic transfers are underlined.

with ASC. Thus, up to a 1.82-fold increase in ASC MICs was observed in strains adapted to different decontaminants compared with initial strains. For most compounds and strains, the MICs of decontaminants due to cross-adaptative responses were similar to or smaller than the MICs of decontaminants resulting from adaptative responses.

The reduced susceptibility to ASC and CA obtained with progressively increasing decontaminant concentrations was shown to persist over three months for most strains. However, the MIC values for TSP and PA in adapted strains returned to those of non-adapted strains in most cases. The stability of the reduced susceptibility to decontaminants as a result of cross-adaptation varied among strains and chemical compounds. In 36 out of 64 tested cases – 16 adapted strains tested against four decontaminants – (56%) the reduced susceptibility, acquired as a consequence of adaptation or cross-adaptation response, remained stable throughout the study period.

Discussion

As in the research being presented here, variations in the susceptibility of different *Listeria* and *Salmonella* strains to chemical biocides have been reported by other authors (Aase *et al.*, 2000; Charnock, 2003; Heir *et al*., 2004; Gradel *et al*., 2005; Aarnisalo *et al*., 2007; Capita, 2007; EFSA, 2008). The substantial differences in susceptibility to decontaminants between bacterial species observed for CA, with *Salmonella* having a 3-fold higher MICs than *Listeria*, support previous observations, where CA showed higher activity against Gram-positive than Gram-negative bacteria (Del Río *et al.*, 2007a, 2007b). The variation in the structure and chemical composition of the different bacterial strains could explain the different responses to biocides. Intrinsic tolerance of bacteria to biocides are related to penetration barriers (membranes, cell wall peptidoglucan, number, and size of porins) and cell capacity to reduce biocide accumulation (degradation/modification, presence of efflux systems) (Russell, 2001; Charnock, 2003).

The inter- and intra-specific differences in bacteria susceptibility to poultry decontaminants could influence the survival of strains on decontaminated carcasses, and highlight the importance of using sufficiently highly concentrated compounds in order to inactivate all target pathogens of concern. The US authorized in-use concentrations (mg/ml) of poultry decontaminants are: 80∼120 (TSP), 15∼25 (CA), and up to 0.22 (PA) (Del Río *et al*., 2007b; EFSA, 2008). ASC is typically used in poultry processing waters, applied as a spray or dip solution at concentrations of between 0.5 and 1.2 mg/ml and combined with a GRAS acid that achieves a pH of between 2.3 and 2.9 in the solution. According to US regulations, ASC may also be applied as a pre-chiller or chiller solution at levels that result in sodium chlorite concentrations of between 0.05 and 0.15 mg/ml, in combination with any acid considered GRAS at levels sufficient to achieve a pH of 2.8 to 3.2 (EFSA, 2008). Most of the MIC values were lower than those authorized. However, results in Table 2 show that MICs of ASC, for both adapted and non-adapted strains, are higher than the lowest authorized concentrations, and data for adapted strains (0.32±.02 to 0.46±0.01 mg/ml) are at the minimum interval limit for authorized concentrations in poultry processing waters. In these cases, bacterial strains probably encounter suboptimal concentrations of decontaminants that could provide selective pressure for the increase and dissemination of naturally tolerant strains. In such conditions it is possible that the organism's tolerance to the agent increases through adaptative responses, which may also be followed by cross-adaptation to other decontaminants. It is, however, important to bear in mind that the MICs tests were per-

Table 2. Minimum inhibitory concentrations (MICs) of acidified sodium chlorite (mg/ml) before and after exposing four bacterial strains to increasing concentrations of poultry decontaminants

Strain	L1	L2	S1	S2
Non-adapted	$0.17\pm0.01^{a}{}_{a}$	$0.17\pm0.02^{a}{}_{a}$	$0.21\pm0.02^{b}{}_{a}$	$0.17\pm0.01^{a}{}_{a}$
Adapted to TSP	$\underline{0.24\pm0.01}^{a}{}_{b}$	$\underline{0.22\pm0.01}^{a}{}_{b}$	$\underline{0.36\pm0.03}^{b}{}_{b}$	$\underline{0.22\pm0.01}^{a}{}_{b}$
Adapted to ASC	$\underline{0.46\pm0.01}^{a}{}_{c}$	$\underline{0.32\pm0.02}^{b}{}_{c}$	$\underline{0.45\pm0.02}^{a}{}_{c}$	$0.44\pm0.02^{a}{}_{c}$
Adapted to CA	$\underline{0.26\pm0.01}^{a}{}_{d}$	$\underline{0.25\pm0.01}^{a}{}_{d}$	$\underline{0.25\pm0.02}^{a}{}_{d}$	$0.31\pm0.01^{b}{}_{d}$
Adapted to PA	$\underline{0.28\pm0.01}^{a}{}_{e}$	$\underline{0.25\pm0.02}^{b}{}_{d}$	$0.31\pm0.01^{c}{}_{e}$	$\underline{0.31\pm0.01}^{c}{}_{d}$

For interpretation see Table 1

Table 3. Minimum inhibitory concentrations (MICs) of citric acid (mg/ml) before and after exposing

Strain	L1	L2	S1	S2
Non-adapted	$0.95\pm0.01^{a}{}_{a}$	$1.25\pm0.02^{b}{}_{a}$	$3.24\pm0.09^{c}{}_{a}$	$3.62\pm0.02^{d}{}_{a}$
Adapted to TSP	$\underline{1.13\pm0.02}^{a}{}_{b}$	$\underline{1.31\pm0.04}^{b}{}_{b}$	$\underline{3.27\pm0.07}^{c}{}_{a}$	$3.59\pm0.05^{d}{}_{b}$
Adapted to ASC	$\underline{1.09\pm0.01}^{a}{}_{b}$	$\underline{1.18\pm0.02}^{b}{}_{c}$	$\underline{3.21\pm0.00}^{c}{}_{a}$	$\underline{3.57\pm0.01}^{d}{}_{bc}$
Adapted to CA	$\underline{1.24\pm0.09}^{a}{}_{c}$	$\underline{1.25\pm0.09}^{a}{}_{a}$	$\underline{3.25\pm0.02}^{b}{}_{a}$	$\underline{3.27\pm0.02}^{b}{}_{d}$
Adapted to PA	$1.14\pm0.04^{a}{}_{b}$	$1.26\pm0.01^{b}{}_{a}$	$\underline{3.22\pm0.02}^{c}{}_{a}$	$3.55\pm0.02^{d}{}_{c}$

For interpretation see Table 1

formed in MH broth, which contains organic matter and causes inactivation of the agents (Aarnisalo *et al.*, 2007). The MICs observed for ASC could be also influenced by compound evaporation.

Sub-inhibitory concentrations of acid or alkaline compounds have previously been shown to lead to adaptative responses in various foodborne microorganisms (Lunden *et al.*, 2003; EFSA, 2008). Adaptation to oxidative stress and to chlorous compounds has been also demonstrated (Farr and Kogoma, 1991; Braoudaki and Hilton, 2004; Lear *et al.*, 2006). However, no specific studies of poultry decontaminants have been conducted so far. The final MICs of TSP, CA, and PA for adapted bacteria are much lower than the concentrations used in working solutions in poultry slaughterhouses plants. Therefore, according to our results, reduced susceptibility to these compounds due to adaptation does not appear to be of major importance in the survival of *Listeria* and *Salmonella* cells when the decontaminant treatment is performed adequately. Reduced susceptibility could, however, be of concern when bacteria encounter suboptimal concentrations of decontaminants. These insufficient concentrations could occur because of inadequate distribution or dosage of disinfectants, or excessive amounts of organic matter, known to inactivate disinfectants, in the dipping tank.

The main *in vitro* TSP action mechanism is related to the solution's high alkalinity, which causes the cells to leak intracellular fluid through the disruption of fatty molecules in the cell membrane. Moreover, ionic strength is high, which can cause bacterial cell autolysis. ASC (its activity is mainly derived from chlorous acid) and PA are oxiding biocides which kill microorganisms through direct action on the cellular membrane and disruption of fundamental cellular processes. A secondary mechanism of these compounds is the acidification of both the cell's environment and the cell's cytoplasm. The bactericidal effect of CA is largely due to the ability of this compound's undissociated form to diffuse through the cell membrane into the cytoplasm, where some of the acid dissociates, tending to cause proton and anion accumulation in cell cytoplasm (Capita *et al*., 2002; SCVPH, 2003; EFSA, 2008).

Strains were observed to undergo cross-adaptation to decontaminants with similar action mechanisms (e.g. oxiding compounds). However, decontaminants with different action mechanisms were also observed to cause cross-adaptation (e.g. TSP and ASC), suggesting a non-specific resistance mechanism such as increased impermeability due to outer membrane adaptation. Similar findings have been reported by other authors studying different chemical biocides (Braoudaki and Hilton, 2004; Lear *et al*., 2006). These results imply that rotation or combination of decontaminant agents with different action mechanisms in slaughterhouses may not have the desired effect.

As in the research being presented here, the maintenance of reduced susceptibility to biocides after several transfers in compound-free broth has been observed by other authors (Lundén *et al*., 2003). These observations suggest that cellular changes due to adaptation continue to have an effect on tolerance to decontaminants some time after exposure. According to Russell (2001), acquired reduced susceptibility to biocides may occur when bacteria are "trained" to grow in gradually increasing concentrations of a biocide, although this reduced susceptibility is not always stable. Temporary reduced susceptibility as a result of phenotypic adaptation has been known, but in general it is thought that a non-genetic adaptative type of resistance is unlikely to play an important role in determining the long-term survival of bacteria to biocides. Changes in membrane proteins and the presence of an active efflux system could be involved in the development of stable phenotypic tolerance to decontaminants.

To sum up, results in the present study show that the susceptibility to decontaminants of different *Listeria* and *Salmonella* strains differ, which may influence survival of the strains. These findings highlight the importance of (1) strictly monitoring the compound concentrations in order to ensure an anti-microbial effect on all pathogens of concern; (2) selecting adequate test strains when testing such compounds on *Listeria* and *Salmonella*, and (3) identifying the

Table 4. Minimum inhibitory concentrations (MICs) of peroxyacetic acid (mg/ml) before and after exposing four bacterial strains to increasing concentrations of poultry decontaminants

Strain	L1	L2	S1	S2
Non-adapted	$0.11\pm0.01^{a}{}_{a}$	$0.10\pm0.02^{a}{}_{a}$	$0.07\pm0.03^{b}{}_{ab}$	$0.08\pm0.01^{c}{}_{a}$
Adapted to TSP	$\underline{0.10\pm0.02}^{a}{}_{b}$	$\underline{0.10\pm0.00}^{a}{}_{a}$	$\underline{0.07\pm0.02}b^{b}{}_{a}$	$0.08\pm0.01^{c}{}_{a}$
Adapted to ASC	$\underline{0.11\pm0.08}^{a}{}_{a}$	$\underline{0.09\pm0.02}^{b}{}_{b}$	$0.08\pm0.05^{c}{}_{bc}$	$0.10\pm0.02^{d}{}_{b}$
Adapted to CA	$\underline{0.11\pm0.03}^{a}{}_{a}$	$0.11\pm0.01^{a}{}_{c}$	$\underline{0.06\pm0.02}^{b}{}_{d}$	$0.08\pm0.01^{c}{}_{a}$
Adapted to PA	$\underline{0.11\pm0.01}^{a}{}_{a}$	$0.11\pm0.02^{a}{}_{c}$	$0.08\pm0.01^{b}{}_{c}$	$0.08\pm0.02^{b}{}_{a}$

For interpretation see Table 1

principal pathogenic bacteria of concern in a poultry plant before choosing a decontamination procedure.

It appears that the adaptative responses are not of concern when TSP, CA or PA are used at an authorized level, which is higher than MICs. Moreover, the low magnitude of adaptative responses found in these agents suggests that there is no cause for concern when strains encounter suboptimal concentrations of decontaminant. However, an adaptative response towards ASC could have an influence on the survival of *Listeria* and *Salmonella* strains when the compound is applied as a pre-chiller or chiller solution (authorized at lower concentrations than MICs obtained in this research), or when ASC is inadequately used (at suboptimal concentrations) in poultry processing waters. Cross-adaptative responses of strains were observed for all decontaminants tested, suggesting that tolerance to a decontaminant promotes the emergence of tolerance to other compounds. It should be pointed out, however, that the magnitude of adaptative and cross-adaptative responses was low (with maximum increase in MIC being 2.71-fold) suggesting that these responses are of minor concern.

This study provides a starting point for research into bacterial adaptation and cross-adaptation responses to poultry decontaminants. However, the findings presented here are based on limited laboratory studies, and additional research into practical applications would be needed to substantiate these findings.

Acknowledgements

The authors wish to thank the Spanish *Ministerio de Sanidad y Consumo* (*Instituto de Salud Carlos III*, Project FIS PI 040722) and the *Junta de Castilla y León* (*Consejería de Sanidad*, Project SAN673/LE05/08) for their financial support.

References

Aarnisalo, K., J. Lundén, H. Korkeala, and G. Wirtanen. 2007. Susceptibility of *Listeria monocytogenes* strains to disinfectants and chlorinated alkaline cleaners at cold temperatures. *LWT-Food Sci. Technol*. 40, 1041-1048.

Aase, B., G. Sundheim, S. Langsrud, and L. Rørvik. 2000. Occurrence of and possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. *Int. J. Food Microbiol*. 62, 57-63.

Anonymous. 1999. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated in animals; approved standard. NCCLS document M31-A, National Committee on Clinical Laboratory Standards, Wayne, PE, USA.

Braoudaki, M. and A.C. Hilton. 2004. Adaptative resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *J. Clin. Microbiol.* 42, 73-78.

Capita, R. 2007. Variation in *Salmonella* resistance to poultry chemical decontaminants, based on serotype, phage type, and antibiotic resistance patterns. *J. Food Prot.* 70, 1835-1843.

Capita, R., C. Alonso-Calleja, M.C. García-Fernández, and B. Moreno. 2002. Review: Trisodium phosphate treatment for decontamination of poultry. *Food Sci. Technol. Int*. 8, 11-24.

Charnock, C. 2003. The antibacterial efficacy of Norwegian hand dishwashing detergents. *Food Prot. Trends* 23, 790-796.

Del Río, E., R. Muriente, M. Prieto, C. Alonso-Calleja, and R. Capita. 2007a. Effectiveness of trisodium phosphate, acidified sodium chlorite, citric acid, and peroxyacids against pathogenic bacteria on poultry during refrigerated storage. *J. Food Prot*. 70, 2063-2071.

Del Río, E., M. Panizo-Morán, M. Prieto, C. Alonso-Calleja, and R. Capita. 2007b. Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry. *Int. J. Food Microbiol.* 115, 268-280.

EFSA (European Food Safety Authority). 2006. Guidance document on the safety and the efficacy of substances for the removal of microbial surface contamination of foods of animal origin. *The EFSA J*. 388, 1-9.

EFSA (European Food Safety Authority). 2007. The Community Summary Report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistante and foodborne outbreaks in the European Union in 2006. *The EFSA J*. 130, 2-352.

EFSA (European Food Safety Authority). 2008. Assessment of the possible effect of the four antimicrobial treatment substances on the emergence of antimicrobial resistance. *The EFSA J.* 659, 2-26.

FAOSTAT. 2006. Internet site at: http://faostat.fao.org/DesktopDefault.aspx?PageID=410&lang=en.

Farr, S.B. and T. Kogoma. 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella* Typhimurium. *Microbiol. Rev.* 55, 561-585.

Gradel, K.O., L. Randall, A.R. Sayers, and R.H. Davies. 2005. Possible associations between *Salmonella* persistence in poultry houses and resistance to commonly used disinfectants and a putative role of *mar*. *Vet. Microbiol.* 107, 127-138.

Heir, E., B.A. Lindstedt, O.J. Røtterud, T. Vardund, G. Kapperud, and T. Nesbakken. 2004. Molecular epidemiology and disinfectant susceptibility of *Listeria monocytogenes* from meat processing plants and human infections. *Int. J. Food Microbiol*. 96, 85-96.

Lear, J.C., J.Y. Maillard, P.W. Dettmar, P.A. Goddard, and A.D. Russell. 2006. Chloroxylenol and triclosan-tolerant bacteria from industrial sources-susceptibility to antibiotics and other biocides. *Int. Biodet. Biodegr.* 57, 51-56.

Lundén, J., T. Autio, A. Markkula, S. Hellström, and H. Korkeala. 2003. Adaptative and cross-adaptative responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. *Int. J. Food Microbiol.* 82, 265-272.

OJEC. 2004. Regulation (EC) N° 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for the hygiene of foodstuffs. *Official Journal of the European Communities* 30.4.2004 L139/55. Available at: http://europa.eu.int/eur-lex/en/archive/2004/l_1392 0040430en.html.

Russell, A.D. 2001. Mechanisms of bacterial insusceptibility to biocides. *Amer. J. Infect. Control* 29, 259-261.

SCVPH (Scientific Committee on Veterinary measures relating to Public Health). 2003. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on the evaluation of antimicrobial treatments for poultry carcasses. April 2003.

Sofos, J.N. 2008. Challenges to meat safety in the 21st century. *Meat Sci.* 78, 3-13.