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Reduced susceptibilities to biocides and resistance to antibiotics in food-associated bacteria following exposure to quaternary ammonium compounds

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Abbreviated running headline : Adaptive cross-resistance in response to QAC

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Aims: Our aim was to assess the effects of step-wise exposure to didecyl dimethyl ammonium chloride (DDAC) on the antimicrobial (antibiotics and biocides) susceptibilities of food-associated bacterial strains.

Methods and Results: Adaptive responses of bacterial strains were investigated by exposing the strains daily to increasing sub-inhibitory concentrations of DDAC for 7 days. Following adaptation to DDAC, a 3-fold increase in the MIC values for this biocide was observed in 48 % of the *Escherichia coli* and *Listeria monocytogenes* strains, and 3% of the *Salmonella* strains. Reduced susceptibility to other biocides was found with the most important increase in MIC for benzalkonium chloride (BC) and a commercial biocide formulation (Galox Horizon) containing DDAC and glutaraldehyde, for all species except *Salmonella*. Increase in antibiotic MIC values was more pronounced in *Escherichia coli* in terms of antibiotic numbers and of magnitude (from 4- to 32-fold increase) and, to a lesser extent, in *Salmonella* strains. Most of these strains had acquired resistance to ampicillin, cefotaxime, ceftazidime, chloramphenicol and ciprofloxacin.

Conclusions: The effects of exposure to DDAC on biocides and antibiotics susceptibilities depend upon the bacteria species.

Significance and Impact of the Study: Extensive use of DDAC at sub-inhibitory concentrations may lead to the development of antibiotic-resistant bacteria and may represent a public health issue.

Keywords Adaptation, Disinfectants, Antibiotics, Decreased susceptibility, Resistance, QAC

Introduction

Biocides are chemical agents playing a crucial role in limiting the spread of infections and diseases. The quaternary ammonium compounds, such as benzalkonium chloride (BC) and didecyl dimethyl ammonium chloride (DDAC), are among the most widely used biocides in cleaning and disinfection procedures. There are growing concerns about the risk of selection of resistant bacteria to antibiotics with regard to the increased use of biocides. The main reasons for the increasing prevalence of antibiotic resistances are the widespread usage of antibiotics in human and veterinary therapy and the inclusion of antibiotics in animal feedstuffs (Ortega Morente *et al.*, 2013). The contribution of other factors such as disinfectant usage is nevertheless difficult to evaluate.

Disinfectants as biocides are generally effective to inhibit or kill bacteria when they are applied at the concentrations recommended by the manufacturer. They can be found at lower concentrations in contact with bacteria because of insufficient cleaning or rinsing before disinfection, under-dosing of applied disinfectant, and biofilm formation. Under such conditions, bacteria are exposed to sub-lethal concentrations of disinfectants, and this can lead to adaptation of initially susceptible bacteria (Davidson and Harrison, 2002). Repeated exposure to

sub-inhibitory concentrations of biocide may promote a decreased susceptibility or resistance to other antimicrobial agents. Bacteria may indeed elicit common cellular responses to counteract the effects of biocides and antibiotic : reduced permeability or uptake, enhanced efflux, enzymatic inactivation (Buffet-Bataillon *et al.*, 2011). This phenomenon has been known as cross-susceptibility or cross-resistance between biocides and antibiotics, but the mechanisms are still poorly understood.

The adaptation to sub-inhibitory concentrations of biocide has been documented for some bacteria (Lunden *et al.*, 2003, Condell *et al.*, 2012, Mavri and Mozina, 2013). DDAC is widely used but there were a few studies about its effect on different food-associated bacteria on the contrary to BC. These data will be useful in the framework of European discussion about the importance of cross-resistance between biocides and antibiotics as already reported by SCENIHR Committee (Scenihr, 2010).

The aim was to assess the effects of step-wise exposure to DDAC on the antimicrobial susceptibilities of food-associated bacterial strains. The analysis included the susceptibility to the biocide itself, the susceptibility to 4 other disinfectants and the cross-resistance to clinically important antimicrobials. Four species were chosen in this study (*Campylobacter coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*) as they belong to the nine biological hazards transmitted to human through the consumption of pork products (Efsa and Cdc, 2016).

Material and methods

Bacterial strains

A total of 136 strains were tested (Table 1). The species considered were *Escherichia coli* (n=54 strains), *Campylobacter coli* (n=16 strains), *Salmonella enterica* (n=35 strains) and *Listeria monocytogenes* (n=31 strains). They were isolated from pig faeces at slaughterhouse during the annual French antibiotic resistance monitoring program or from pork meat at retail during European surveys carried out between 2008 and 2012. All the strains were kept at -80°C in a nutritive solution containing 20% (v/v) glycerol.

Biocide susceptibility testing

We used disinfectants commonly found in biocide formulations in the French pork industry. They included didecyl dimethyl ammonium chloride (DDAC), benzalkonium chloride (BC), sodium hypochlorite (NaOCl) and hydrogen

peroxide (H_2O_2). A commercially available food-grade biocide formulation (Galox Horizon) was also used, containing DDAC and glutaraldehyde. Susceptibility tests against the 4 active substances and the commercial biocide formulation were performed using a standard microdilution method.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed using a standard microdilution method with the Sensititre® system on customized microtiter plates (Trek Diagnostic Systems, England). Antibiotics used for these tests are listed in Table S1. The strains were interpreted as susceptible or resistant to antibiotics according to the epidemiological resistance cut-off determined from EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CA-SFM (Antibiogram Committee of the French Society for Microbiology,) websites (http://mic.eucast.org last accessed 12/02/2016). The number of tested antibiotics differed depending on the species: 20 antibiotics for *Listeria monocytogenes*, 14 for *Salmonella enterica*, 7 for *Campylobacter coli* and 13 for *Escherichia coli*.

Adaptation experiments to DDAC

Adaptive responses of bacterial strains were investigated by exposing the strains daily to increasing sub-inhibitory concentrations of DDAC for 7 days (Soumet *et al.*, 2012). Briefly, 100 µl of a calibrated bacterial suspension (10^8 Colony-Forming Unit (CFU) per ml) was initially exposed to a starting concentration of disinfectant below the Minimum Inhibitory Concentration (MIC) for 24 h at 37 °C in a total volume of 10 ml of Mueller-Hinton (MH) broth. When growth was observed, a 10-fold diluted culture was transferred to fresh MH broth supplemented with a higher concentration of disinfectant. If no growth was observed, the previous concentration was used. As control, a bacterial suspension (100 µl) and MH broth (10 ml) without disinfectant were tested using the same protocol. After 7 days, bacteria were spread with a loop (10 µl) on MH agar and incubated for 24 h at 37 °C. They were then collected with 2.5 ml of storage nutritive solution and kept in cryotubes at −80 °C. MIC increase factor for each biocide and each antibiotic was determined for each strain as a ratio between MIC after adaptation and MIC before adaptation.

Statistical analysis

The distributions of susceptible and resistant strains before and after adaptation were compared by Chi^2 test using Excel program for Windows. Results were considered significant when $p < 0.05$.

Results

Reduced susceptibility to biocides

Before the adaptation experiment, DDAC MIC values varied from 0.5 to 1.5 µg ml^{-1} among *Listeria* strains, from 4 to 8 µg ml^{-1} among *Salmonella* and from 1.5 to 4 µg ml^{-1} among *Escherichia coli* strains, and from 0.37 to 0.75 µg ml^{-1} among *Campylobacter* strains (Figure S1). Following adaptation to DDAC, a minimum 3-fold increase in the MIC values for this biocide was observed for 3% of the *Salmonella* strains, for 48% of the *Listeria monocytogenes* strains, and also 50% of the *E coli* strains (Table 2, Figure S1). The increase was lower (2-fold) for *Campylobacter coli*.

Reduced susceptibility to other biocides was found with the most important increase in MIC (3-fold) for BC and Galox Horizon, for all species except *Salmonella*. No significant increase (less than 2-fold) was observed in the susceptibility to hydrogen peroxide and sodium hypochlorite for all strains tested.

Reduced susceptibility and resistance to antibiotics

Following adaptation to DDAC, increase in antibiotic MIC values was more pronounced in *Escherichia coli* in terms of antibiotic numbers (up to 8 antibiotics) and of magnitude (from 4- to 32-fold increase) and, to a lesser extent, in *Salmonella* strains. The evolution of antibiotic susceptibility was low for *Listeria monocytogenes* and *Campylobacter coli;* MICs increased from 4- to 8-fold for only 2 and 1 antibiotics, respectively (data not shown). Before adaptation, 39 of the 54 strains of *Escherichia coli* (72.2%) were resistant to at least one antibiotic (tetracycline and/or streptomycin and/or sulfomethosaxole). After step-wise exposure to DDAC, the MIC values exceeded the antibiotic resistance epidemiological cut-off for 50 of the 54 strains (92%). Most of them acquired resistance to one or two new antibiotics (Fig.1). Moreover, thirty two strains became multiresistant, defined as resistant to at least 3 antibiotics of different antimicrobial categories (Magiorakos *et al.*, 2012). Most of these strains had acquired resistance to chloramphenicol, ampicillin, cefotaxime, ceftazidime, and ciprofloxacin (Fig. 2).

Seven of the 35 *Salmonella* strains (20%) acquired resistance to various antibiotics (Table 3). They were mainly resistant to chloramphenicol (3 strains) and 5 out of 7 belonging to the serotype Derby.

Only one strain of *Listeria monocytogenes* and two strains of *Campylobacter coli* became resistant to tetracycline and streptomycin, respectively (data not shown).

Discussion

The step-wise exposure to didecyl dimethyl ammonium chloride (DDAC) modifies the susceptibilities to the biocide, to other ones and to antibiotics of food-associated bacteria strains belonging to *Escherichia coli*, *Salmonella* Typhimurium and Derby, *Campylobacter coli* and *Listeria monocytogenes* species. Adaptation to DDAC differed depending on the species studied.

The ability of food-associated bacteria to survive in increasing concentrations of quaternary ammonium compounds has been investigated, but mainly by using benzalkonium chloride (BC) (Lunden *et al.*, 2003, Braoudaki and Hilton, 2005). In this work, the effect of a repeated exposure to didecyl dimethyl ammonium chloride (DDAC) was studied because this biocide compound is frequently used in food industry. A 3-fold increase in the DDAC MIC values was observed in about half of the *Escherichia coli* and *Listeria monocytogenes* strains tested. Using the same adaptation experiment, Soumet *et al.* (2012) previously found similar levels of reduction in susceptibility to different QACs in *Escherichia coli* (with a mean 3-fold increase). Previous studies showed that the reduced susceptibility to BC varied from 10% to 46% in *Listeria monocytogenes* isolated from foods and food processing environments (Aase *et al.*, 2000, Soumet *et al.*, 2005, Xu *et al.*, 2014) and that adaptation to various disinfectants was common in this species (Lunden *et al.*, 2003). The high adaptation ability observed here suggests that the occurrence of decreased susceptibility to DDAC in *Listeria* is relatively high in food industry. Following adaptation, a low increase in the DDAC MIC values was observed in *Campylobacter coli* and *Salmonella* strains. No significant difference in the susceptibility to other biocides was found in *Salmonella* strains. These results are consistent with a previous study, where no stable decrease in the biocide susceptibility was detected in *Salmonella* after several rounds of *in vitro* selection by increasing concentrations of active biocides (Condell *et al.*, 2012). Concerning *Campylobacter coli*, Mavri and Mozina (2013) showed that a statistically significant difference was observed between one strain exposed to BC and the control strain after 15 days of exposure. The period of exposure in the current study may not be sufficient to observe significant biocide susceptibility changes in *Campylobacter coli.* The impact of DDAC exposure on reduced susceptibility to other biocides depended on the biocide type tested. Reduced susceptibilities were more pronounced for QAC disinfectants (BC and the commercial biocide formulation containing DDAC). Interestingly, adaptation to DDAC did not induce reduced susceptibilities to peroxydes (H_2O_2) and chlorine releasing agents (NaOCl) among the bacterial species tested. This phenomenon is explained by the different action mechanisms of DDAC and oxidizing agents. It was shown that peroxides like H_2O_2 generate hydroxyl radicals which may interact with metabolic pathways in microbial cells, probably leading to a lack of development of bacteria resistance or decreasing susceptibility (Ikai *et al.*, 2013)

A growing concern is the possibility that mechanisms providing reduced susceptibility to biocides may also provide cross-protection to clinically important antibiotics (Russell, 2002). Indeed, strains can move from the category of "sensitive" to that of "resistant" according to the CLSI (Clinical Laboratory Standard Institute) guidelines on antimicrobial susceptibility testing. In this study, MICs increased by 4-fold for erythromycin and ciprofloxacin in two *Campylobacter coli* strains following adaptation to DDAC. A previous work found that the resistance of ciprofloxacin increased only by 2-fold after 5 passages in presence of BC for one *Campylobacter coli* strain (Mavri and Mozina, 2013). Furthermore, the authors showed that in 29% of cases, after step-wise exposure to biocides, the *Campylobacter* strains were indeed more susceptible to the tested antimicrobials than the parent and the control non-exposed strains.

The impact of DDAC exposure on reduced susceptibility was stronger in *Escherichia coli* than in the other species, in terms of antibiotics numbers and of magnitude. The highest MIC increase factor was of 32-fold for chloramphenicol, and 85% of the tested strains having acquired the resistance to this antibiotic. Similar results were found in a previous study where 90% of *Escherichia coli* strains became resistant to phenicol compounds following adaptation to different QACs (Soumet *et al.*, 2012). Other classes of antibiotic showing a marked increase in resistance were penicillins (ampicillin), cephalosporins (cefotaxime and ceftazidime) and fluoroquinolones (ciprofloxacin). The three last classes were also identified by Capita *et al.* (2014), after exposure of *Escherichia coli* ATCC 12806 to sublethal concentrations of food-grade biocides. DDAC is able to induce resistance mechanisms that confer cross-resistance to different antibiotics categories due to common resistance mechanisms. These mechanisms may include increased efflux by AcrAB-TolC, AcrEF-TolC and YhiUV-TolC pumps of *Escherichia coli* present on chromosome or on mobile genetic elements resulting in expulsion of antibiotics and biocides out of the cell (Ortega Morente *et al.*, 2013). They also may be associated with cell permeability changes due to modifications of outer membrane fatty acid profiles or reduction of entry channels like porins. Our study revealed an increase in resistance to fluoroquinolones after adaptation to DDAC which are critically important drugs for treating serious *Escherichia coli* infections in humans. Furthermore, the movement of strains from the category of "sensitive" to that of "resistant" observed for cephalosporins is also a matter of concern, as these compounds are the front-line antimicrobials for treating serious bacterial human infections. The evolution of antibiotic susceptibility was low in *Listeria monocytogenes* strains in the present work (with only one strain on 31 becoming resistant to tetracycline). That is consistent with a previous study where no resistance to antibiotics was found in this species (Soumet *et al.*, 2005). Compared to *Listeria monocytogenes*, increases in

antibiotic MIC values were more pronounced in *Salmonella* strains (with the highest increase of 32-fold for nalidixic acid in one strain). Resistances to antibiotics were nevertheless acquired for only seven out of 35 *Salmonella* strains. Braoudaki and Hilton (2004) also found a low degree of resistance to a range of antibiotics in *Salmonella* serovar Typhimurium following adaptation to BC. Reduced susceptibility to several antibiotics was also observed after exposure of *Salmonella* Typhimurium strains to a biocidal formulation containing a QAC (Karatzas *et al.*, 2007). Some mutants of this study showed elevated *acrB* expression consistent with the Multi-Antibiotic Resistance (MAR) phenotype. In another study, the derepression of the AcrEF multidrug efflux pump genes generated MAR phenotype for strains of *Salmonella* Typhimurium exposed to four different biocides (Whitehead *et al.*, 2011).

This work has provided new data on susceptibilities to several biocides and antibiotics for a large number of strains isolated from pork production. However, more strains per bacterial species should be analysed as it was reported that reduced susceptibility to antimicrobials seemed to differ depending on the serotype of *Listeria monocytogenes* and *Salmonella enterica* (Aase *et al.*, 2000, Braoudaki and Hilton, 2004, Condell *et al.*, 2012, Xu *et al.*, 2014).

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Conflict of Interest

No conflict of interest declared.

References

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

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

Braoudaki, M. and Hilton, A. C. (2005) Mechanisms of resistance in *Salmonella enterica* adapted to erythromycin, benzalkonium chloride and triclosan. *Int J Antimicrob Agents,* **25,** 31-7.

Buffet-Bataillon, S., Branger, B., Cormier, M., Bonnaure-Mallet, M. and Jolivet-Gougeon, A. (2011) Effect of higher minimum inhibitory concentrations of quaternary ammonium compounds in clinical *E. coli* isolates on antibiotic susceptibilities and clinical outcomes. *J Hosp Infect,* **79,** 141-146.

Capita, R., Riesco-Peláez, F., Alonso-Hernando, A. and Alonso-Calleja, C. (2014) Exposure o*f Escherichia coli* ATCC 12806 to Sublethal Concentrations of Food-Grade Biocides Influences Its Ability To Form Biofilm, Resistance to Antimicrobials, and Ultrastructure. *Appl Environ Microbiol,* **80,** 1268-1280.

Condell, O., Iversen, C., Cooney, S., Power, K. A., Walsh, C., Burgess, C. and Fanning, S. (2012) Efficacy of biocides used in the modern food industry to control *Salmonella enterica*, and links between biocide tolerance and resistance to clinically relevant antimicrobial compounds. *Appl Environ Microbiol,* **78,** 3087-3097.

Davidson, P. M. and Harrison, M. A. (2002) Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. *Food Technol Chicago,* **56,** 69-78.

Efsa and Cdc (2016) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. . *EFSA Journal* **14,** 4380-4587.

Ikai, H., Odashima, Y., Kanno, T., Nakamura, K., Shirato, M., Sasaki, K. and Niwano, Y. (2013) In vitro evaluation of the risk of inducing bacterial resistance to disinfection treatment with photolysis of hydrogen peroxide. *PLoS ONE,* **8**.

Karatzas, K. A., Webber, M. A., Jorgensen, F., Woodward, M. J., Piddock, L. J. and Humphrey, T. J. (2007) Prolonged treatment of *Salmonella enterica* serovar Typhimurium with commercial disinfectants selects for multiple antibiotic resistance, increased efflux and reduced invasiveness. *Journal of Antimicrobrial Chemotherapy,* **60,** 947-55.

Lunden, J., Autio, T., Markkula, A., Hellstrom, S. and Korkeala, H. (2003) Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. *Int J Food Microbiol,* **82,** 265-272.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. and Monnet, D. L. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection,* **18,** 268-281.

Mavri, A. and Mozina, S. S. (2013) Development of antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* adapted to biocides *Int J Food Microbiol,* **160,** 304-312

Ortega Morente, E., Fernández-Fuentes, M. A., Grande Burgos, M. J., Abriouel, H., Pérez Pulido, R. and Gálvez, A. (2013) Biocide tolerance in bacteria. *International Journal of Food Microbiology,* **162,** 13-25.

Russell, A. D. (2002) Mechanisms of antimicrobial action of antiseptics and disinfectants : an increasingly important area of investigation *J Antimicrob Chem,* **49,** 597-599.

Scenihr (2010) Scientific Committee on Emerging and Newly Identified Health Risks - Research strategies to address the knowldge gaps on the antimicrobial resistance effects of biocides - http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_028.pdf accessed 23/11/2015.

Soumet, C., Fourreau, E., Legrandois, P. and Maris, P. (2012) Resistance to phenicol compounds following adaptation to quaternary ammonium compounds in *Escherichia coli*. *Vet Microbiol,* **158,** 147-152.

Soumet, C., Ragimbeau, C. and Maris, P. (2005) Screening of benzalkonium chloride resistance in *Listeria monocytogenes* strains isolated during cold smoked fish production. *Lett Appl Microbiol,* **41,** 291-6.

Whitehead, R. N., Overton, T. W., Kemp, C. L. and Webber, M. A. (2011) Exposure of *Salmonella enterica* serovar Typhimurium to high level biocide challenge can select multidrug resistant mutants in a single step. *PLoS ONE,* **6,** e22833.

Xu, D., Li, Y., Shamim Hasan Zahid, M., Yamasaki, S., Shi, L., Li, J.-R. and Yan, H. (2014) Benzalkonium chloride and heavy-metal tolerance in *Listeria monocytogenes* from retail foods. *Int J Food Microbiol,* **190,** 24-30.

Table 1: Bacterial species and origin of the strains tested

Bacterial species	Serotypes	N° of isolates from pig feces (slaughterhouse)	N° of isolates from pork meat (retail)	Total
Escherichia coli	-	54	0	54
Campylobacter coli *	-	16	0	16
Salmonella enterica	Derby†	7	8	15
	Typhimurium	2	18	20
Listeria monocytogenes	1/2 a	5	8	13
	1/2 b	3	5	8
	1/2 c	0	1	1
	4b	3	6	9
Total		90	46	136

*: All *Campylobacter* in the French pig production are *Campylobacter coli*.

†: The serotypes Derby and Typhimurium are two of the most prevalent serotypes of *Salmonella* in the French pig production

Table 2 Biocide MIC increase factor for different bacteria species after exposure to increasing concentrations of DDAC.

Species (N° of strains)	DDAC (%)	BC (%)	Galox Horizon (%)	NaOCl (%)	H_2O_2 (%)
Escherichia coli (54)*	≥ 3 (50) †	≥ 3 (22)	≥ 3 (65)	< 2 (100)	< 2 (100)
Listeria monocytogenes (31)	≥ 3 (48)	≥ 3 (45)	≥ 3 (35)	< 2 (100)	< 2 (100)
Salmonella enterica (35)	≥ 3 (3)	≥ 1.5 (37)	≥ 3 (6)	< 1.5 (100)	< 1.5 (100)
Campylobacter coli (16)	2 (31)	3 (31)	3 (31)	< 2 (100)	< 2 (100)

DDAC: didecyl dimethyl ammonium chloride; BC: benzalkonium chloride; NaOCl: sodium hypochlorite; H_2O_2: hydrogen peroxide; Galox Horizon: biocide formulation containing DDAC and glutaraldehyde

*: number of strains tested for a given species; †: percentage of strains with the biocide MIC increase factor indicated

Table 3: Antibiotic resistant patterns for strains of *Salmonella enterica* Derby and Typhimurium before and after exposure to DDAC

Serotypes of *Salmonella enterica*	Antibiotic resistance patterns		Number of acquired resistance
	Before adaptation	After adaptation	
Derby	STR-TET	STR-TET-**CHL**	1
	AMP-CHL-STR-TET-TMP	AMP-CHL-STR-TET-TMP-**FFN**	1
	No resistance	**STR**	1
	STR-TET	STR-TET-**CHL-FFN**	2
	No resistance	**CHL-NAL-STR**	3
Typhimurium	STR-TET-TMP	STR-TET-TMP-**CIP**	1
	TET-TMP	TET-TMP-**CIP**	1

AMP: ampicillin; CHL: chloramphenicol; FFN: florfenicol; STR: streptomycin; TET: tetracycline; TMP: trimethoprim; NAL: nalidixic acid; CIP: ciprofloxacin. Antibiotics in bold represent the acquired resistances after exposure to DDAC

Figure 1 Evolution of antibiotic resistance patterns of *Escherichia coli* strains after step-wise exposure to DDAC. (A) Distribution of the strains before (white bars) and after (grey bars) adaptation to DDAC depending on their number of antibiotic resistances. (B) Distribution of the strains depending on their number of antibiotic acquired resistances following adaptation to DDAC.

Figure 2 Number of *Escherichia coli* strains resistant to antibiotics before (white bars) and after (grey bars) adaptation to DDAC.
*:significant differences ($p<0.05$) - TET: tetracycline; STR: streptomycin; SMX: sulfamethoxazole; TMP: trimethoprim; AMP: ampicillin; CHL: chloramphenicol; NAL: nalidixic acid; CIP: ciprofloxacin; FOT: cefotaxime; GEN: gentamicin; TAZ: ceftazidime.

Table 1: Bacterial species and origin of the strains tested

Table 2 Biocide MIC increase factor for different bacteria species after exposure to increasing concentrations of DDAC.

Table 3: Antibiotic resistant patterns for strains of *Salmonella enterica* Derby and Typhimurium before and after exposure to DDAC

Table S1: Antibiotic susceptibility testing conditions for different bacterial species

Figure S1: Evolution of biocide MIC before and after adaptation to DDAC in different bacterial species

Table S1: Antibiotic susceptibility testing conditions for different bacterial species

Escherichia coli

Antibiotic	Concentration range (µg/ml)
Nalidixic acid (NAL)	2 - 128
Ampicillin (AMP)	1 - 128
Cefotaxime (FOT)	0.03 - 4
Ceftazidime (TAZ)	0.06 - 8
Chloramphenicol (CHL)	4 - 256
Ciprofloxacin (CIP)	0.008 – 8
Colistin (COL)	0.5 – 16
Gentamicin (GEN)	0.05 – 32
Meropenem (MER)	0.12 - 0.25
Streptomycin (STR)	4 – 256
Sulfamethoxazole (SMX)	8 – 1024
Tetracycline (TET)	1 – 128
Trimethoprim (TMP)	0.25 - 32

Salmonella enterica

Antibiotic	Concentration range(µg/ml)
Sulfamethoxazole (SMX)	8 -1024
Gentamicin (GEN)	0.25 – 32
Ciprofloxacin (CIP)	0.008 – 8
Ampicillin (AMP)	0.5 – 32
Cefotaxime (FOT)	0.06 – 4
Ceftazidime (TAZ)	0.25 – 16
Tetracycline (TET)	1 – 64
Streptomycin (STR)	2 – 128
Trimethoprim (TMP)	0.5 – 32
Chloramphenicol (CHL)	2 - 64
Colistin (COL)	2 – 4
Florfenicol (FFN)	2 – 64
Kanamycin (KAN)	4 – 128
Nalidixic acid (NAL)	4 - 64

Listeria monocytogenes

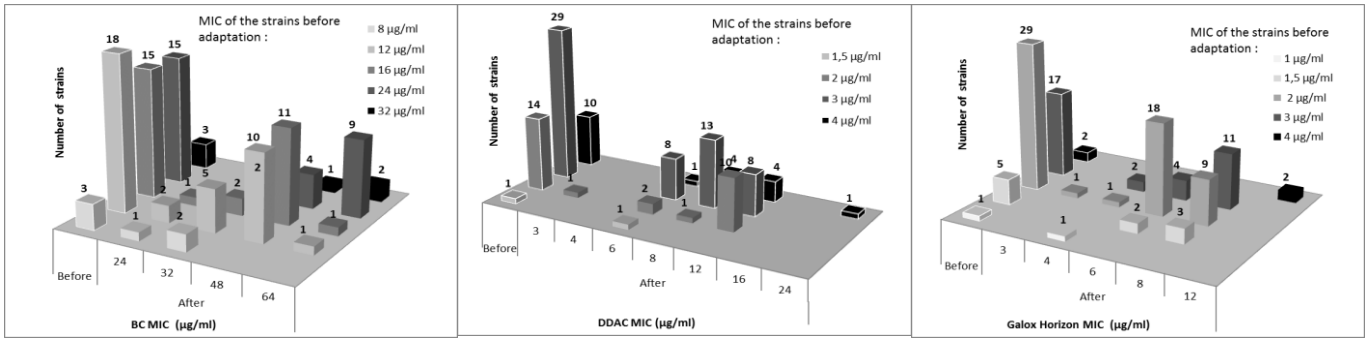
Antibiotic	Concentration range (µg/ml)
Moxifloxacin (MXF)	1 – 8
Levofloxacin (LEVO)	0.5 – 4
Tetracycline (TET)	1 – 8
Cefuroxime (FUR)	0.5 – 4
Ceftriaxone (AXO)	0.12 – 2
Cefotaxime (FOT)	0.12 - 4
Daptomycin (DAP)	0.06 – 2
Chloramphenicol (CHL)	1 – 32
Penicillin (PEN)	0.03 – 4
Meropenem (MER)	0.0.25 – 2
Ertapenem (ETP)	0.5 – 4
Amoxicillin/clavulanic acid 2/1 (AUG2)	2/1 – 16/8
Linezolid (LZD)	0.25 – 4
Clindamycin (CLI)	0.12 – 1
Cefepime (FEP)	0.5 – 8
Tigecycline (TGC)	0.015 – 0.12
Azithromycin (AZI)	0.25 – 2
Erythromycin (ERY)	0.25 – 2
Trimethoprim/sulfamethoxazole (SXT)	0.5/9.5 – 4/76
Vancomycin (VAN)	0.5 – 4

Campylobacter coli

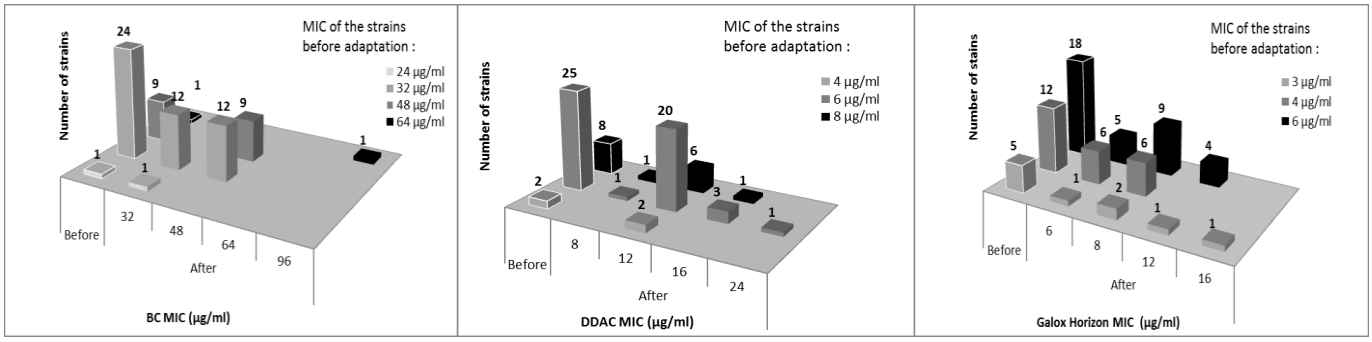
Antibiotic	Concentration range (µg/ml)
Gentamicin (GEN)	0.12 – 16
Streptomycin (STR)	1 – 16
Ciprofloxacin (CIP)	0.06 – 4
Tetracycline (TET)	0.25 – 16
Erythromycin (ERY)	0.5 – 32
Nalidixic acid (NAL)	2 – 64
Chloramphenicol (CHL)	2 – 64

Figure S1: Evolution of biocide MIC before and after adaptation to DDAC in different bacterial species

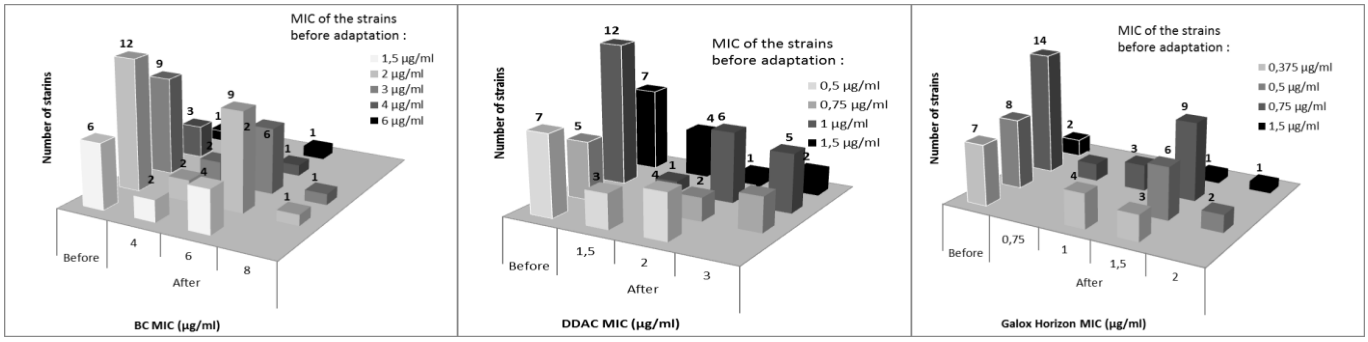
A- Escherichia coli



B- Salmonella enterica



C- Listeria monocytogenes



D- Campylobacter coli

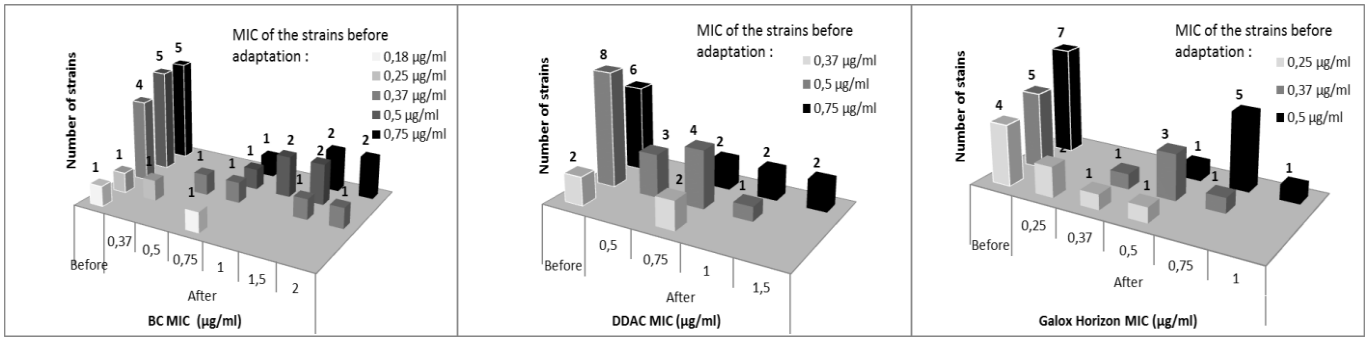


Figure 1

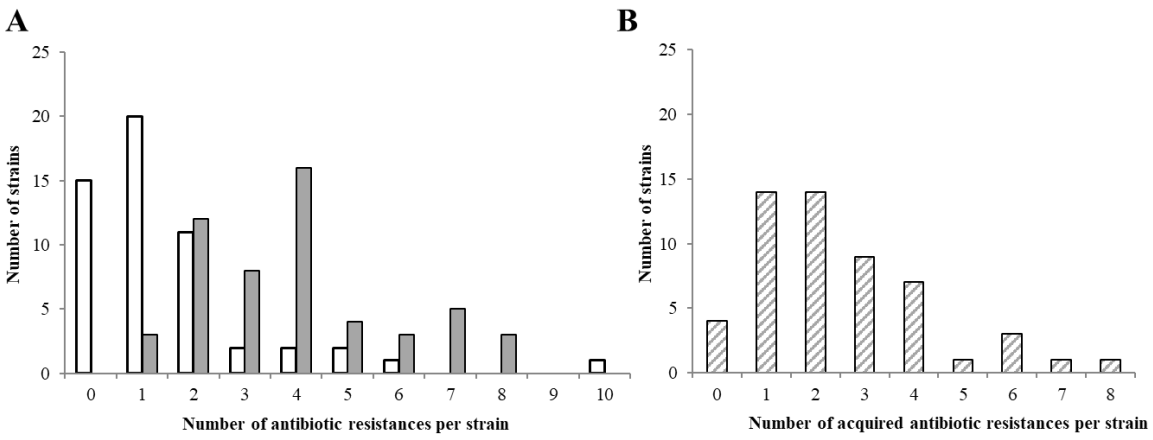
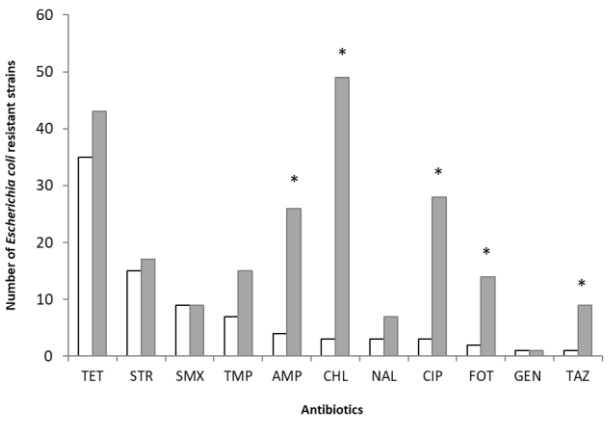



Figure 2