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

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

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

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

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

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

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

40 **Introduction**

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

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

53 sub-inhibitory concentrations of biocide may promote a decreased susceptibility or resistance to other
54 antimicrobial agents. Bacteria may indeed elicit common cellular responses to counteract the effects of biocides
55 and antibiotic : reduced permeability or uptake, enhanced efflux, enzymatic inactivation (Buffet-Bataillon *et al.*,
56 2011). This phenomenon has been known as cross-susceptibility or cross-resistance between biocides and
57 antibiotics, but the mechanisms are still poorly understood.

58

59 The adaptation to sub-inhibitory concentrations of biocide has been documented for some bacteria (Lunden *et al.*,
60 2003, Condell *et al.*, 2012, Mavri and Mozina, 2013). DDAC is widely used but there were a few studies about its
61 effect on different food-associated bacteria on the contrary to BC. These data will be useful in the framework of
62 European discussion about the importance of cross-resistance between biocides and antibiotics as already reported
63 by SCENIHR Committee (Scenih, 2010).

64

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

70 **Material and methods**

71 **Bacterial strains**

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

77 **Biocide susceptibility testing**

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

80 peroxide (H₂O₂). A commercially available food-grade biocide formulation (Galox Horizon) was also used,
81 containing DDAC and glutaraldehyde. Susceptibility tests against the 4 active substances and the commercial
82 biocide formulation were performed using a standard microdilution method.

83 **Antibiotic susceptibility testing**

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

91 **Adaptation experiments to DDAC**

92 Adaptive responses of bacterial strains were investigated by exposing the strains daily to increasing sub-inhibitory
93 concentrations of DDAC for 7 days (Soumet *et al.*, 2012). Briefly, 100 µl of a calibrated bacterial suspension (10⁸
94 Colony-Forming Unit (CFU) per ml) was initially exposed to a starting concentration of disinfectant below the
95 Minimum Inhibitory Concentration (MIC) for 24 h at 37 °C in a total volume of 10 ml of Mueller-Hinton (MH)
96 broth. When growth was observed, a 10-fold diluted culture was transferred to fresh MH broth supplemented with
97 a higher concentration of disinfectant. If no growth was observed, the previous concentration was used. As control,
98 a bacterial suspension (100 µl) and MH broth (10 ml) without disinfectant were tested using the same protocol.
99 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
100 collected with 2.5 ml of storage nutritive solution and kept in cryotubes at –80 °C. MIC increase factor for each
101 biocide and each antibiotic was determined for each strain as a ratio between MIC after adaptation and MIC before
102 adaptation.

103

104 **Statistical analysis**

105 The distributions of susceptible and resistant strains before and after adaptation were compared by Chi² test
106 using Excel program for Windows. Results were considered significant when $p < 0.05$.

107 **Results**

108 **Reduced susceptibility to biocides**

109 Before the adaptation experiment, DDAC MIC values varied from 0.5 to 1.5 $\mu\text{g ml}^{-1}$ among *Listeria* strains, from
110 4 to 8 $\mu\text{g ml}^{-1}$ among *Salmonella* and from 1.5 to 4 $\mu\text{g ml}^{-1}$ among *Escherichia coli* strains, and from 0.37 to 0.75
111 $\mu\text{g ml}^{-1}$ among *Campylobacter* strains (Figure S1). Following adaptation to DDAC, a minimum 3-fold increase in
112 the MIC values for this biocide was observed for 3% of the *Salmonella* strains, for 48% of the *Listeria*
113 *monocytogenes* strains, and also 50% of the *E coli* strains (Table 2, Figure S1). The increase was lower (2-fold)
114 for *Campylobacter coli*.

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

118 **Reduced susceptibility and resistance to antibiotics**

119 Following adaptation to DDAC, increase in antibiotic MIC values was more pronounced in *Escherichia coli* in
120 terms of antibiotic numbers (up to 8 antibiotics) and of magnitude (from 4- to 32-fold increase) and, to a lesser
121 extent, in *Salmonella* strains. The evolution of antibiotic susceptibility was low for *Listeria monocytogenes* and
122 *Campylobacter coli*; MICs increased from 4- to 8-fold for only 2 and 1 antibiotics, respectively (data not shown).
123 Before adaptation, 39 of the 54 strains of *Escherichia coli* (72.2%) were resistant to at least one antibiotic
124 (tetracycline and/or streptomycin and/or sulfomethosaxole). After step-wise exposure to DDAC, the MIC values
125 exceeded the antibiotic resistance epidemiological cut-off for 50 of the 54 strains (92%). Most of them acquired
126 resistance to one or two new antibiotics (Fig.1). Moreover, thirty two strains became multiresistant, defined as
127 resistant to at least 3 antibiotics of different antimicrobial categories (Magiorakos *et al.*, 2012). Most of these
128 strains had acquired resistance to chloramphenicol, ampicillin, cefotaxime, ceftazidime, and ciprofloxacin (Fig.
129 2).

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

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

134

135 **Discussion**

136

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

141 The ability of food-associated bacteria to survive in increasing concentrations of quaternary ammonium
142 compounds has been investigated, but mainly by using benzalkonium chloride (BC) (Lunden *et al.*, 2003,
143 Braoudaki and Hilton, 2005). In this work, the effect of a repeated exposure to didecyl dimethyl ammonium
144 chloride (DDAC) was studied because this biocide compound is frequently used in food industry. A 3-fold increase
145 in the DDAC MIC values was observed in about half of the *Escherichia coli* and *Listeria monocytogenes* strains
146 tested. Using the same adaptation experiment, Soumet *et al.* (2012) previously found similar levels of reduction in
147 susceptibility to different QACs in *Escherichia coli* (with a mean 3-fold increase). Previous studies showed that
148 the reduced susceptibility to BC varied from 10% to 46% in *Listeria monocytogenes* isolated from foods and food
149 processing environments (Aase *et al.*, 2000, Soumet *et al.*, 2005, Xu *et al.*, 2014) and that adaptation to various
150 disinfectants was common in this species (Lunden *et al.*, 2003). The high adaptation ability observed here suggests
151 that the occurrence of decreased susceptibility to DDAC in *Listeria* is relatively high in food industry. Following
152 adaptation, a low increase in the DDAC MIC values was observed in *Campylobacter coli* and *Salmonella* strains.
153 No significant difference in the susceptibility to other biocides was found in *Salmonella* strains. These results are
154 consistent with a previous study, where no stable decrease in the biocide susceptibility was detected in *Salmonella*
155 after several rounds of *in vitro* selection by increasing concentrations of active biocides (Condell *et al.*, 2012).
156 Concerning *Campylobacter coli*, Mavri and Mozina (2013) showed that a statistically significant difference was
157 observed between one strain exposed to BC and the control strain after 15 days of exposure. The period of exposure
158 in the current study may not be sufficient to observe significant biocide susceptibility changes in *Campylobacter*
159 *coli*. The impact of DDAC exposure on reduced susceptibility to other biocides depended on the biocide type
160 tested. Reduced susceptibilities were more pronounced for QAC disinfectants (BC and the commercial biocide
161 formulation containing DDAC). Interestingly, adaptation to DDAC did not induce reduced susceptibilities to
162 peroxydes (H₂O₂) and chlorine releasing agents (NaOCl) among the bacterial species tested. This phenomenon is
163 explained by the different action mechanisms of DDAC and oxidizing agents. It was shown that peroxides like
164 H₂O₂ generate hydroxyl radicals which may interact with metabolic pathways in microbial cells, probably leading
165 to a lack of development of bacteria resistance or decreasing susceptibility (Ikai *et al.*, 2013)

166

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

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

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

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

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 210 framework of the contract «Plan Etat – Région Bretagne».

211

212 **Conflict of Interest**

213 No conflict of interest declared.

214

215 **References**

216

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- 273

275

276 **Table 1:** Bacterial species and origin of the strains tested

277

Bacterial species	Serotypes	N° of isolates		Total
		from pig feces (slaughterhouse)	from pork meat (retail)	
<i>Escherichia coli</i>	-	54	0	54
<i>Campylobacter coli</i> *	-	16	0	16
<i>Salmonella enterica</i>	Derby†	7	8	15
	Typhimurium	2	18	20
<i>Listeria monocytogenes</i>	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

278

279 *: All *Campylobacter* in the French pig production are *Campylobacter coli*.280 †: The serotypes Derby and Typhimurium are two of the most prevalent serotypes of *Salmonella* in the French

281 pig production

282

283

284

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

287

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

288

289 DDAC: didecyl dimethyl ammonium chloride; BC: benzalkonium chloride; NaOCl: sodium hypochlorite;

290 H₂O₂: hydrogen peroxide; Galox Horizon: biocide formulation containing DDAC and glutaraldehyde

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

293

294

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

297

298

Serotypes of <i>Salmonella</i> <i>enterica</i>	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

299

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

303

304

305

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

310

311

312

313 **Figure 2** Number of *Escherichia coli* strains resistant to antibiotics before (white bars) and after (grey bars)
 314 adaptation to DDAC.

315 *:significant differences ($p < 0.05$) - TET: tetracycline; STR: streptomycin; SMX: sulfamethoxazole; TMP:
 316 trimethoprim; AMP: ampicillin; CHL: chloramphenicol; NAL: nalidixic acid; CIP: ciprofloxacin; FOT:
 317 cefotaxime; GEN: gentamicin; TAZ: ceftazidime.

318

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331 **Table S1:** Antibiotic susceptibility testing conditions for different bacterial species

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

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336 Table S1: Antibiotic susceptibility testing conditions for different bacterial species

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338 *Escherichia coli*

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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

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343 *Salmonella enterica*

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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

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364 *Listeria monocytogenes*

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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

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369 *Campylobacter coli*

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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

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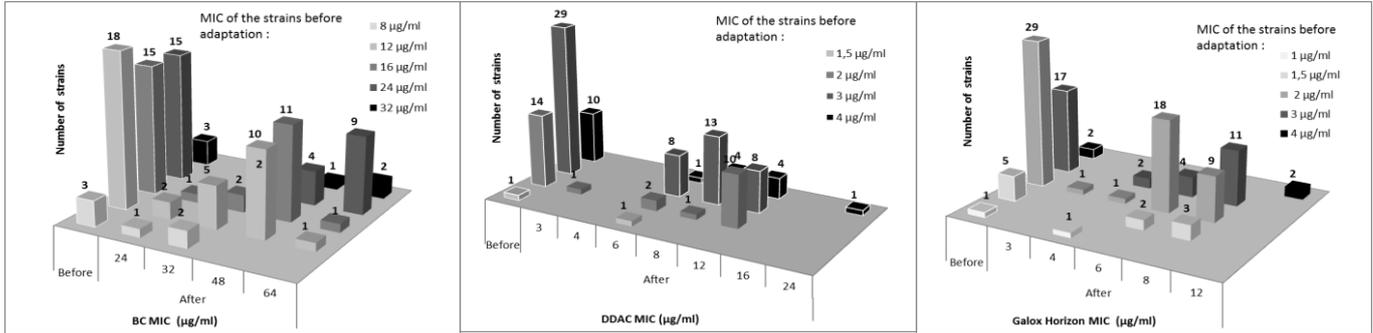
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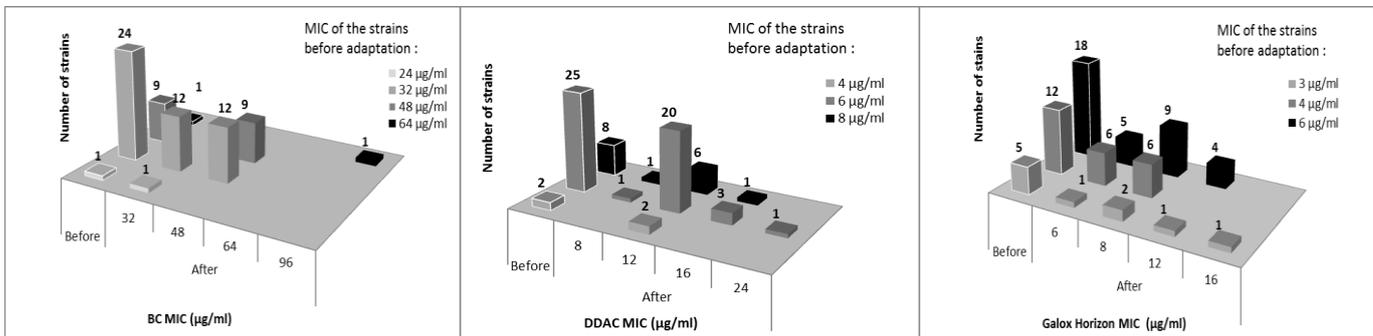
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Figure S1: Evolution of biocide MIC before and after adaptation to DDAC in different bacterial species

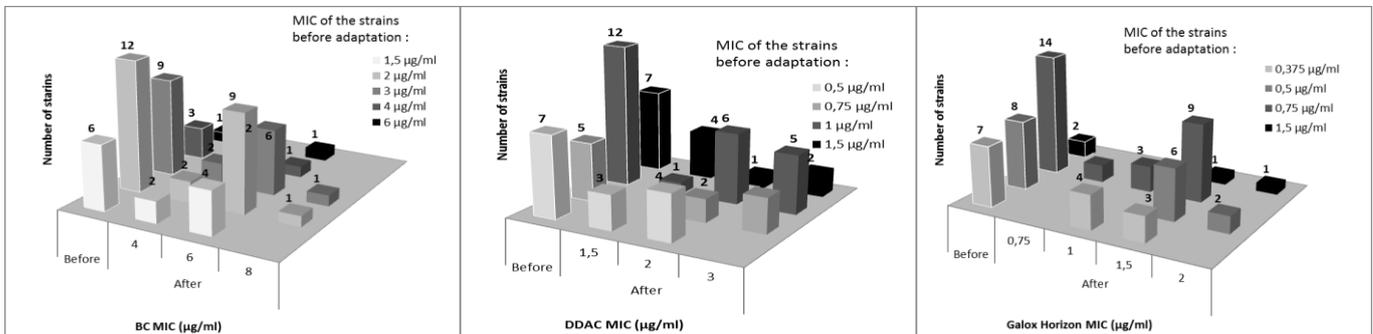
A- *Escherichia coli*



B- *Salmonella enterica*



C- *Listeria monocytogenes*



D- *Campylobacter coli*

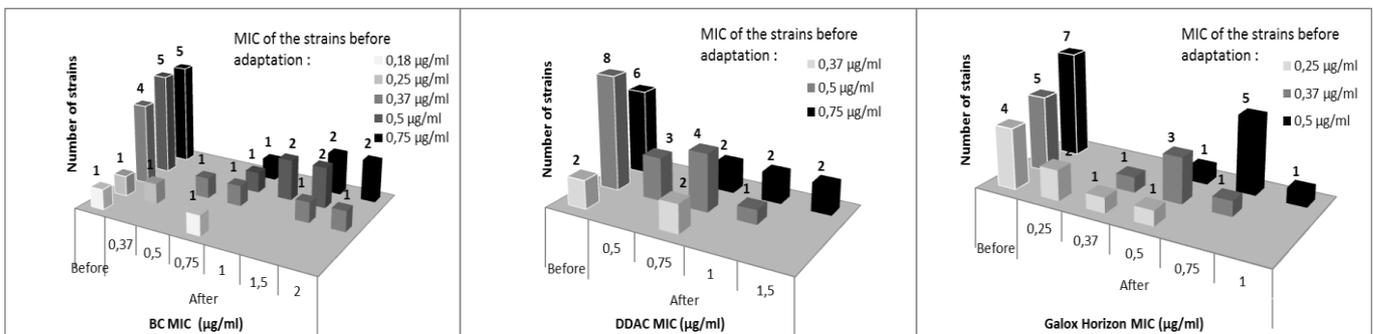


Figure 1

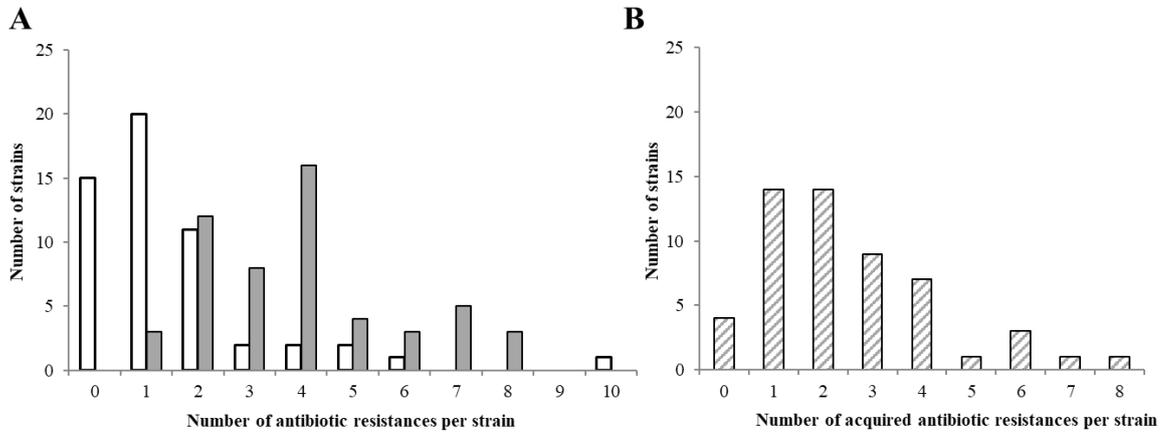


Figure 2

