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Resistance to phenicol compounds following adaptation to quaternary ammonium compounds in *Escherichia coli*

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ABSTRACT

Bacterial adaptation to quaternary ammonium compounds (QACs) is mainly documented for benzalkonium chloride (BC) and few data are available for other QACs. The aim of this study was to assess the effects of repeated exposure to different quaternary ammonium compounds (QACs) on the susceptibility and/or resistance of bacteria to other QACs and antibiotics. *Escherichia coli* strains ($n = 10$) were adapted by daily exposure to increasingly sub-inhibitory concentrations of a QAC for 7 days. Three QACs were studied. Following adaptation, we found similar levels of reduction in susceptibility to QACs with a mean 3-fold increase in the minimum inhibitory concentration (MIC) compared to initial MIC values, whatever the QAC used during adaptation. No significant differences in antibiotic susceptibility were observed between the tested QACs. Antibiotic susceptibility was reduced from 3.5- to 7.5-fold for phenicol compounds, β lactams, and quinolones. Increased MIC was associated with a shift in phenotype from susceptible to resistant for phenicol compounds (florfenicol and chloramphenicol) in 90% of *E. coli* strains. Regardless of the QAC used for adaptation, exposure to gradually increasing concentrations of this type of disinfectant results in reduced susceptibility to QACs and antibiotics as well as cross-resistance to phenicol compounds in *E. coli* strains. Extensive use of QACs at sub-inhibitory concentrations may lead to the emergence of antibiotic-resistant bacteria and may represent a public health risk.

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1. Introduction

Disinfectants are major compounds used in the food production chain during the steps of cleaning and disinfection to ensure microbiologically safe food products. Classified as biocides, they are commercialized as active substances alone or in combination. In the process of reviewing (Biocide Directive 98/8/EC), they must be evaluated for their potential unacceptable effects on target organisms, such as unacceptable resistance and cross-resistance. There are currently few data on resistance and cross-resistance following the use of biocides. Through standard *in vitro* bactericidal suspension tests, disinfectants have generally been demonstrated to be efficient in eliminating bacteria in food (Van de Weyer et al., 1993). However, in field conditions, bacteria are regularly exposed to sub-lethal concentrations of disinfectants and this may constitute a selective pressure driving the acquisition of resistant genes or adaptation of initially susceptible bacteria (Hegstad et al., 2010). This situation may be responsible for decreased susceptibility or resistance to not only commonly used disinfectants, but also to other biocides or even antibiotics used in human therapy. Resistance to biocides has only been documented for a limited number of biocides, such as triclosan and benzalkonium chloride and, in most cases, has been studied on few bacterial strains, making it impossible to

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conclude whether biocide resistance is strain-dependent or not (Langsrud et al., 2004). Results of studies on the potential link between the use of biocides and decreased susceptibility or resistance to antibiotics are controversial. Scientific evidence is therefore insufficient for correctly assessing the risks of biocide and antibiotic resistance as reported by the Scientific Committee for Emergent and Newly Identified Health Risks (SCENIHR, 2009). Regarding QACs, studies in over the past several years have been mainly done on adaptation to benzalkonium chloride (BC) in different bacteria species (Aase et al., 2000; Braoudaki and Hilton, 2004). Hence, to compensate for the lack of data on decreased susceptibility and potential resistance to antibiotics following bacterial adaptation to biocides, our aims were to assess the impact of two QACs (didecyl dimethyl ammonium chloride (DDAC) and dioctyl dimethyl ammonium chloride (OCDAC)) commonly used in the food industry and compare them to BC in 10 *Escherichia coli* strains.

2. Materials and methods

2.1. Strains

Nine different previously characterized avian and porcine *E. coli* strains and a reference strain *E. coli* ATCC®25922 were used in this study. They were selected from strains collected as part of the annual antibiotic resistance monitoring programs organized by the French Ministry of Agriculture. Table 1 lists the origin of these strains and their susceptibility to antimicrobial compounds commonly used in human and veterinary medicine and the minimum inhibitory concentration (MIC) value for BC. *E. coli* ATCC®25922 (AM10) was also used as quality-control strain for each antibiotic-susceptibility test (CLSI, Clinical and Laboratory Standards Institute 2008, M31-A3). The strains were kept at −80 °C in a storage nutritive solution (0.5% tryptone, 0.3% bovine extracts and 15% glycerol). Strains were spread with a loop on Mueller-Hinton (MH) agar (Becton Dickinson, Le-Pont-de-Claix, France) and incubated 24 h at 37 °C.

2.2. Disinfectants and antibiotics

The disinfectants used in this study are quaternary ammonium compounds (QACs) and are the most commonly used biocide formulations in the French agri-food industry. They included benzalkonium chloride (BC, BTC50, Stepan Europe, Voreppe, France), didecyl dimethyl ammonium chloride (DDAC, Bardac22, Lonza Bâle, Switzerland), dioctyl dimethyl ammonium chloride (OCDAC, Bardac LF, Lonza Bâle, Switzerland). Disinfectant stock solutions contained 50% of the tested QAC as the biocidal active substance. Customized microtiter plates containing dilution ranges of dehydrated antibiotics were purchased from Trek Diagnostic Systems (East Grinstead, England). The following antibiotics were included: ampicillin (AMP), ceftazidime (TAZ), cefotaxime (FOT), chloramphenicol (CHL), ciprofloxacin (CIP), florfenicol (FFN), gentamicin (GEN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SMX), tetracycline (TET) and trimethoprim (TMP).

2.3. Antibiotic and disinfectant susceptibility tests

Antibiotic susceptibility tests were performed using the microdilution method with the Sensititre® system on the customized microtiter plate. Two or three *E. coli* colonies from MH agar were suspended in sterile demineralized water and this suspension was adjusted to a 0.5 McFarland standard (bioMérieux, Marcy-l'Etoile, France). This bacterial suspension (10 μl) was diluted in 10 ml of MH broth and 50 μl were automatically inoculated by the Sensititre® in each well of the microtiter plates. *E. coli* ATCC®25922 (AM10) was used as a quality-control strain for each antibiotic-susceptibility test. After incubation for 24 h at 37 °C, bacteria growth was assessed by observing turbidity in the medium. The strains were interpreted as susceptible or resistant to antibiotics according to the epidemiological resistance cut-off determined from EUCAST and CA-SFM guidelines for *Enterobacteriaceae*. These cut-offs (breakpoint concentrations in μg/ml) are >2 (FOT); >8 (TAZ); >16 (NAL); >8 (TET); >16 (CHL); >4 (GEN); >16 (STR); >8 (TMP); >256 (SMX); >8 (AMP); >16 (FFN); >1 (CIP). Culture purity was checked by streaking 1 μl of suspension used for plate inoculation on MH agar supplemented with 5% (v/v) defibrinated sheep blood (AES Laboratoires, Combourg, France). Viable bacteria were enumerated from 100 μl of inoculation suspension diluted to 1/200 on MH agar and the value had to be in the range from 25 to 80 CFU/ml to validate the test.

Table 1
Antibiotics and BC susceptibilities for the studied *E. coli* strains.

Strain	Origin	Year sampled	MIC BC (μg/ml)	Antibiotic susceptibility profile
AM01	Pork	2005	32	Susceptible
AM02	Pork	2004	32	TET
AM03	Pork	2005	32	TET
AM04	Pork	2000	16	TET-STR-SMX
AM05	Poultry	2002	32	Susceptible
AM06	Poultry	2002	32	Susceptible
AM07	Poultry	2002	16	TET-STR-SMX
AM08	Poultry	2001	16	NAL
AM09	Poultry	2004	16	AMP-SMX
AM10	Control strain		32	Susceptible

TET: resistant to tetracycline; STR: resistant to streptomycin; SMX: resistant to sulfamethoxazole; NAL: resistant to nalidixic acid; AMP: resistant to ampicillin.