RESEARCH ARTICLE

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

Increasing antibiotic resistance in preservative-tolerant bacterial strains isolated from cosmetic products

Pilar Orús,[1] Laura Gomez-Perez,[2,3] Sonia Leranoz,[4] Mercedes Berlanga[2]*

[1]Beautyge-Revlon S.L., Barcelona, Spain. [2]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain. [3]School of Biological Sciences, University of East Anglia, Norwich, UK. [4]Supply Quality-Reckitt Benkiser Granollers S.L., Spain

Summary. To ensure the microbiological quality, consumer safety and organoleptic properties of cosmetic products, manufacturers need to comply with defined standards using several preservatives and disinfectants. A drawback regarding the use of these preservatives is the possibility of generating cross-insusceptibility to other disinfectants or preservatives, as well as cross resistance to antibiotics. Therefore, the objective of this study was to understand the adaptive mechanisms of *Enterobacter gergoviae*, *Pseudomonas putida* and *Burkholderia cepacia* that are involved in recurrent contamination in cosmetic products containing preservatives. Diminished susceptibility to formaldehyde-donors was detected in isolates but not to other preservatives commonly used in the cosmetics industry, although increasing resistance to different antibiotics (β-lactams, quinolones, rifampicin, and tetracycline) was demonstrated in these strains when compared with the wild-type strain. The outer membrane protein modifications and efflux mechanism activities responsible for the resistance trait were evaluated. The development of antibiotic-resistant microorganisms due to the selective pressure from preservatives included in cosmetic products could be a risk for the emergence and spread of bacterial resistance in the environment. Nevertheless, the large contribution of disinfection and preservation cannot be denied in cosmetic products. [**Int Microbiol** 2015; 18(1):51-59]

Keywords: *Enterobacter* · *Pseudomonas* · *Burkholderia* · cosmetic preservatives · antibiotics · cross-resistance

Introduction

Microbial contamination of cosmetic products is a matter of great importance to the industry and it is potentially a major cause of both product and economic losses. Water and nutri-

*Corresponding author: M. Berlanga
Department of Microbiology and Parasitology
Faculty of Pharmacy, University of Barcelona
Av. Joan XXIII, s/n
08028 Barcelona, Spain
Tel. +34-934024497. Fax +34-934024498
E-mail: mberlanga@ub.edu

ents present in cosmetics make them susceptible to microbial growth. Most often, microorganisms are the cause of organoleptic alterations, such as offensive odors, and changes in viscosity and color. Moreover, in a few cases, contaminating microorganisms or their activity may cause human health problems, such as skin irritation, allergic contact dermatitis and infection, especially in the eyes, mouth or wounds [10,25,33,35]. To ensure microbiological quality, consumer safety and the organoleptic properties of cosmetic products, manufacturers need to use disinfectants and preservatives. Therefore, cosmetics need preservatives that are able to reduce the microbial load to acceptable levels during the period

of validity [17]. Regulations in the EU and in other countries permit specified preservatives, define their maximum concentrations, and provide other controls specifically related to the type of cosmetic product. Preservatives are added to cosmetics in order to inhibit the proliferation of spoiling microorganisms. Microbial quality assurance in cosmetics aims to prevent the transmission of diseases by using these products properly, and ensures their stability and effectiveness [28].

Antibiotics are generally considered to be selectively toxic agents suitable for administration to patients, whereas biocides have been traditionally regarded as antiseptics, disinfectants or preservatives. Additionally, antibiotics are considered to have a specific target site within a bacterial cell, whereas biocides have multiple target sites. Unlike antibiotics, which are selectively toxic, most biocides do not act against a defined target cell [22,23,32,36]. For example, triclosan inhibits the synthesis of agents that bind to enoyl-acyl carrier protein reductase, causing inhibition of fatty acid biosynthesis and disruption of the membrane, thus precipitating the cytoplasmic compounds [13]. Formaldehyde-donors/releasers act against bacterial cells by releasing formaldehyde into the medium, and their biocidal effect is due to the cross-linking of proteins in the cell envelope and elsewhere in the cell, as well as cross-linking of RNA and DNA [34].

Exposure of bacteria to biocides can select for mutants with decreased biocide susceptibility, and these mutants often display a decreased susceptibility to various antibiotics, indicating that biocides can act as drivers of antibiotic resistance under laboratory conditions [7,37]. It has been suggested that cross-resistance to antimicrobial compounds, following exposure and adaptation to a biocide, could occur in a limited number of situations, such as when a biocide and antibiotic compound use the same entry mechanisms, have the same cellular target, can develop the same resistance mechanism and, finally, when biocide tolerance and antibiotic resistance are potentially carried by the same mobile genetic element [7]. Resistance to antimicrobial compounds can emerge following one or more target gene mutations, but it is difficult for bacteria to become resistant to the recommended concentrations used for many biocides, since mutations within a single gene will not usually confer resistance. In contrast, when tolerance to biocides arises, it is mediated by mechanisms that are less well characterized. Some of the modifications that can occur in a bacterial cell include upregulation of the efflux pump activity or structural alterations in the cell wall, which impact permeability. Efflux systems can export both antibiotics and many biocides [36].

The extensive use of microbicides in a wide range of applications has been questioned with regard to their role in the development of bacterial resistance to antimicrobials. However, for natural isolates, there was no evidence that cross-resistance between cosmetic preservatives and antibiotics could occur [30]. The aim of this study was to determine the mechanisms of tolerance to formaldehyde-donors shown by bacterial strains belonging to species of *Enterobacter gergoviae*, *Pseudomonas putida* and *Burkholderia cepacia* isolated from cosmetics products, and, because of human health concerns, to search for possible cross-resistance to antibiotics.

Materials and methods

Bacterial strains. A total of 46 strains belonging to *Enterobacter gergoviae*, 22 to *Pseudomonas putida* and 44 to *Burkholderia cepacia* were obtained from contaminated cosmetic formulations (shampoos, lotions, conditioners) preserved with parabens and formaldehyde-donors. Strains were identified by the API system 20NE (Biomeriéux, Marcy l'Etoile, France). Reference strains from the American Type Culture Collection (ATCC) were also included in the study. Strains were cultured on trypticase soy agar (Difco, Detroit, MI, USA) for *E. gergoviae* and *P. putida*, and on plate count agar (Difco) for *B. cepacia*.

Minimal inhibitory concentration (MIC) in solid medium. Susceptibility tests to 16 preservatives and 12 antibiotics were performed by serial dilutions in Mueller-Hinton agar (Difco). The inoculum consisted of 104 colony forming units (CFU) per spot and the MIC was defined as the lowest concentration that prevented visible growth after incubation for 18 h at 35ºC. The following preservatives were studied: methylchloroisothiazolinone/methylisothiazolinone and polyaminopropyl biguanide (Thor, Speyer, Germany), DMDM hydantoin (McIntyre, Halifax, Canada), Quaternium-15 (Evonik Degussa Ibérica, Barcelona, Spain), diazolidinyl urea, imidazolidinyl urea and sodium hydroxymethylglycinate (International Specialty Products, West Milford, NJ, USA), methylparaben and propylparaben (Sharon Labs., Ashdod, Israel), methyldibromo glutaronitrile (Shülke, Nordersted, Germany), phenoxyethanol (Seppic, Paris, France), hexamidine isethionate (Laboratoires Sérobiologiques, Pulnoy, France), chlorphenesin (Arnaud, Paris, France), benzalkonium chloride (Comercial Química Massó, Barcelona, Spain), bronopol (Basf, Ludwigshafen, Germany), and triclosan (Ciba Specialty Chemicals, Basel, Switzerland). Antibiotics were purchased from Sigma (Madrid, Spain). Working solutions were prepared daily in suitable sterile diluents. Antibiotics used were: cefotaxime, ceftazidime, ceftriaxone, kanamycin, streptomycin, tetracycline, erythromycin, ciprofloxacin, penicillin, ampicillin, chloramphenicol, and novobiocin.

Outer membrane isolation and SDS-PAGE. Outer membrane proteins were obtained from cells cultivated in Luria Bertani broth (Difco), as described previously [20]. The inner membrane fraction was solubilized from disrupted cells by direct extraction with 2% sodium dodecyl sulfate and the outer membrane was separated by centrifugation (12,000 rpm, 60 min). The proteins obtained were analyzed by sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis (SDS-PAGE). In order to enhance the resolution of protein bands, 4 M urea was added to the resolving gel.

Microbial affinity to solvents (MATS) for selected strains.
The MATS assay was carried out as previously described [11]. After over-night culture, cells were harvested by centrifugation (7500 rpm, 10 min), washed twice with PBS (phosphate buffered saline) at pH 7.0, and resuspended in the same solution at a final optical density (OD_{600}) of 0.8. Each bacterial suspension (2.4 ml) was mixed for 60 s at maximum intensity on a vortex-type agitator with 0.4 ml of chloroform, hexadecane, diethyl ether, or hexane (Panreac, Barcelona, Spain). The mixtures were allowed to stand for 60 min to ensure complete separation of the two phases. A 1-ml sample was then carefully removed from the aqueous phase and its optical density was measured at 600 nm. The microbial affinity for each solvent was calculated using the formula:

$$\% \text{ Affinity} = (OD_0 - OD_f / OD_0) \times 100$$

where OD_0 is the optical density of the bacterial suspension before mixing with the solvent and OD_f the absorbance after mixing and phase separation. Each measurement was performed in duplicate and the experiment was repeated three times with independent bacterial cultures. Two solvent pairs were assayed: (i) chloroform (an acidic solvent) and hexadecane (an apolar solvent), and (ii) diethyl ether (a strong basic solvent) and hexane (an apolar solvent).

Ciprofloxacin accumulation and efflux assays. Ciprofloxacin accumulation and efflux assays were performed by a previously described fluorimetric method [3]. Briefly, isolates were incubated at 37°C until OD_{600} = 0.5–0.7. Bacteria were harvested by centrifugation (7000 rpm) at room temperature, washed, and concentrated 10-fold in PBS pH 7.5. Ciprofloxacin was added to a final concentration of 10 μg/ ml. At timed intervals of 30 s, 1.5 min, 3 min, 6 min, and 9 min, samples were centrifuged in a microfuge at 10,000 rpm at 4°C for 15 s. Pellets were washed in 1 ml of chilled PBS at pH 7.5, and suspended in 1 ml 0.1 M glycine–HCl buffer at pH 3.0, and finally incubated at room temperature overnight to allow bacterial lysis. The suspensions were centrifuged at 20°C for 25 min in order to remove bacterial debris. The concentration of the antibiotic in the supernatants was determined fluorometrically using an SLM Aminco 8100 spectrofluorometer. For the efflux assay, cells were incubated for 3 min with antibiotic before addition of the metabolic inhibitor CCCP (carbonyl cyanide m-chlorophenylhydrazone) at a final concentration of 100 μM, and samples were manipulated in the same way as for quinolone accumulation. The specific extinction and emission wavelengths used to identify ciprofloxacin were 279 and 447, respectively, and they were determined in 0.1 M glycine-HCl at pH 3.0.

Results

Susceptibility of natural preservative-tolerant strains isolated from cosmetic products. The MICs of different preservatives commonly used in the cosmetics industry were determined for *Enterobacter gergoviae*, *Pseudomonas putida* and *Burkholderia cepacia* strains. Results are shown in Table 1. Tolerance to preservatives was defined as more than eight-fold the MIC compared to susceptible strains [24]. Regarding *E. gergoviae*, some strains showed tolerance to formaldehyde-donors, such as diazolidinyl urea, imidazolidinyl urea, Quaternium-15, sodium hydroxymethylglycinate and DMDM hydantoin. No significant changes in

susceptibility were found when other preservatives were tested, such as parabens, isothiazolinone, bronopol, biguanide, methyldibromo glutaronitrile, chlorphenesin, phenoxyethanol, benzalkonium chloride, triclosan or hexamidine. On the other hand, the strains with decreased susceptibility to formaldehyde-donors showed reduced susceptibility to β-lactam antibiotics (penicillin and cephalosporin), tetracycline and ciprofloxacin when compared to the preservative-susceptible *Enterobacter* strains. No increased resistance to novobiocin, macrolides (erythromycin), aminoglycosides (kanamycin, streptomycin) or chloramphenicol was found (Table 2). Similar results were observed for *P. putida* and *B. cepacia* (Table 1). Regarding antibiotics, reduced susceptibility was found in nearly all the drugs tested (Table 2).

Surface characterization and permeability. To exert an antibacterial action, antimicrobial (preservatives/antibiotics) must penetrate the cell envelope or accumulate therein at a sufficiently high concentration. Adaptation of the microbial cell envelope may contribute to the mechanism responsible for resistance to antimicrobial agents. As bacterial species differ in their envelope structures and, hence, in their intrinsic resistance to antibiotics [4,6,19,39], we studied outer membrane proteins, physicochemical characteristics of the cell surface and permeability that may influence cell susceptibility to preservatives/antibiotics in three selected tolerant strains (*E. gergoviae* EU36, *P. putida* EU34 and *B. cepacia* EU67).

The initial reaction of a biocide with a microbial cell involves initial binding to the cell surface, although target sites might be found within the cell. The first reaction of any antibacterial agent involves interaction with the outer cell membrane in the case of Gram-negative bacteria and subsequent passage of the agent to the target site. Outer membrane proteins from *Enterobacter* contain three porins, named OmpF, OmpC and OmpD [19,20]. *E. gergoviae* EU36 did not express the OmpF porin. Therefore, it seems that formaldehyde-donors must enter into the bacterial cell through this porin, since the cell became tolerant when OmpF was not expressed in the outer membrane (Fig. 1). It has been described that hydrophilic molecules pass through the outer membrane by porins [19,26]. Accordingly, porin-deficient strains did not show either tolerance to hydrophobic preservatives (parabens, chlorphenesin, triclosan), or resistance to hydrophobic and higher-molecular weight antibiotics, such as erythromycin. *Pseudomonas putida* EU34 and *B. cepacia* EU67 were tolerant to preservatives and did not show any changes in porin composition with respect to the susceptible strains (Fig. 1). Therefore,

Table 1. Minimal inhitory concenctratio (MIC) for the three species tested for preservatives. Results are expressed in µg/ml

	MIC					
	Enterobacter gergoviae		*Pseudomonas putida*		*Burkholderia cepacia*	
Preservative	No. strains[a]	MIC_{90}[b]	No. strains	MIC_{90}	No. strains	MIC_{90}
DMDM hydantoin	S (26)	1500	S (4)	412.5	S (16)	1650
	R (20)	6000	R (18)	3850	R (28)	16500
Quaternium-15	S (26)	600	S (4)	300	S (16)	300
	R (20)	2500	R (18)	7500	R (28)	2500
Imidazolidinyl urea	S (26)	1500	S (4)	5000	S (16)	300
	R (20)	>7500	R (18)	17500	R (28)	6000
Diazolidinyl urea	S (26)	300	S (4)	300	S (16)	600
	R (20)	5000	R (18)	2500	R (28)	12500
Sodium hydroxymethylglycinate	S (26)	625	S (4)	625	S (16)	1250
	R (20)	1250	R (18)	3500	R (28)	2500
Methylparaben	S (46)	2500	S (22)	2500	S (44)	2500
	R (0)	–	R (0)	–	R (0)	–
Propylparaben	S (46)	500	S (22)	500	S (44)	500
	R (0)	–	R (0)	–	R (0)	–
MCI/MI[c]	S (46)	15	S (22)	10	S (44)	12.5
	R (0)	–	R (0)	–	R (0)	–
Methyldibromo glutaronitrile	S (46)	120	S (22)	100	S (44)	120
	R (0)	–	R (0)	–	R (0)	–
Phenoxyethanol	S (46)	5000	S (22)	5000	S (44)	2500
	R (0)	–	R (0)	–	R (0)	–
Hexamidine isethionate	S (46)	12.5	S (22)	25	S (44)	300
	R (0)	–	R (0)	–	R (0)	–
Chlorphenesin	S (46)	3000	S ()	3000	S (44)	1500
	R (0)	–	R (0)	–	R (0)	–
Benzalkonium chloride	S (46)	125	S (22)	250	S (44)	100
	R (0)	–	R (0)	–	R (0)	–
Bronopol	S (46)	15	S (22)	30	S (44)	25
	R (0)	–	R (0)	–	R (0)	–
Polyaminopropyl biguanide	S (46)	25	S (22)	2500	S (44)	180
	R (0)	–	R (0)	–	R (0)	–
Triclosan	S (46)	<2.5	S (22)	50	S (44)	5
	R (0)	–	R (0)	–	R (0)	–

[a]S: susceptible strains; R: resistant strains.
[b]MIC_{90}: MIC at which 90% of the isolates are inhibited.
[c]MCI/MI: methylchloroisothiazolinone/methylisothiazolinone.

Pseudomonas and *Burkholderia* must have another mechanism implicated in tolerance to formaldehyde-donor preservatives, as well as for increasing antibiotic resistance (this pattern of porins was also observed in all the preservative-tolerant strains in this study).

The charge and cell surface hydrophobicity may influence the interaction with antimicrobial agents [2]. Cationic compounds, such as chlorhexidine and benzalkonium chloride, are thought to interact with negative charges in the bacterial cell wall and outer membrane [12]. The Lewis acid/base and

Table 2. MIC results of antibiotics for the three species tested. Results are expressed in µg/ml

| Antibiotic | Enterobacter gergoviae | | Pseudomonas putida | | Burkholderia cepacia | |
	Strains[a]	MIC$_{90}$[b]	Strains	MIC$_{90}$	Strains	MIC$_{90}$
Cefotaxime	S	0.075	S	6	S	5
	R	0.30	R	15	R	20
Ceftazidime	S	0.0125	S	0.5	S	1
	R	0.5	R	2.75	R	3.5
Ceftriaxone	S	0.025	S	2.75	S	7.5
	R	0.15	R	12.5	R	17.5
Kanamycin	S	0.3	S	0.25	S	15
	R	0.6	R	0.75	R	75
Streptomycin	S	0.5	S	2	S	10
	R	0.5	R	6.5	R	100
Tetracycline	S	1.25	S	2	S	35
	R	4	R	8	R	35
Erythromycin	S	10	S	10	S	70
	R	10	R	100	R	125
Ciprofloxacin	S	0.0025	S	0.0125	S	0.125
	R	0.01	R	0.05	R	1
Penicillin	S	20	S	180	S	100
	R	50	R	250	R	550
Ampicillin	S	6	S	100	S	350
	R	12.5	R	100	R	850
Chloramphenicol	S	10	S	75	S	17.5
	R	25	R	125	R	125
Novobiocin	S	350	S	700	S	3.1
	R	350	R	700	R	6.2

[a]S/R: susceptible/resistant to formaldehyde donors (DMDM hydantoin, Quarternium-15, imidazolidinyl urea, diazolidinyl urea, sodium hydroxymethylglycinate), respectively. For *E. gergoviae*, n (S) = 26, n (R) = 20.
For *P. putida,* n (S) = 4, n (R) = 18 and for *B. cepacia* n (S) = 16, n (R) = 28.
[b]MIC$_{90}$: MIC at which 90% of the isolates are inhibited.

hydrophobicity of cell envelopes can be assessed by microbial affinity to solvents (MATS) [8]. Monopolar solvents (i.e., chloroform and diethyl ether) were selected for the estimation of the Lewis acid/base character, and apolar solvents (hexane and hexadecane) were used to estimate the hydrophobicity properties. *B. cepacia* EU67 showed the most affinity to chloroform (Lewis acid character or electron acceptor). Affinity for chloroform implies an increased number of protonated groups, such as NH$_3$ and/or OH groups, on the bacterial surface [15]. Affinity to diethyl ether (Lewis base or electron donor) was observed in *E. gergoviae* EU36. *Pseudomonas putida* EU34 had a moderate affinity to both chloroform (Lewis acid) and diethyl ether (Lewis base). In general, all strains

showed a hydrophilic character due to the low affinity for apolar solvents such as hexadecane and hexane. However, *Burkholderia* displayed slowly increasing hydrophobicity compared to *Enterobacter* and *Pseudomonas* (Table 3).

Figure 2 shows the results of experiments comparing ciprofloxacin accumulation with and without inhibition by CCCP. After 6 min of bacterial exposure to ciprofloxacin, a species-specific steady-state concentration was achieved, confirming previously published data [3]. Ciprofloxacin accumulation in *E. gergoviae* EU36 was lower than in the susceptible type strain ATTCC 12833 because of deficiency of the OmpF porin. Denergized cells with CCCP had similar steady-state accumulation. A slower entry of ciprofloxacin and a similarly
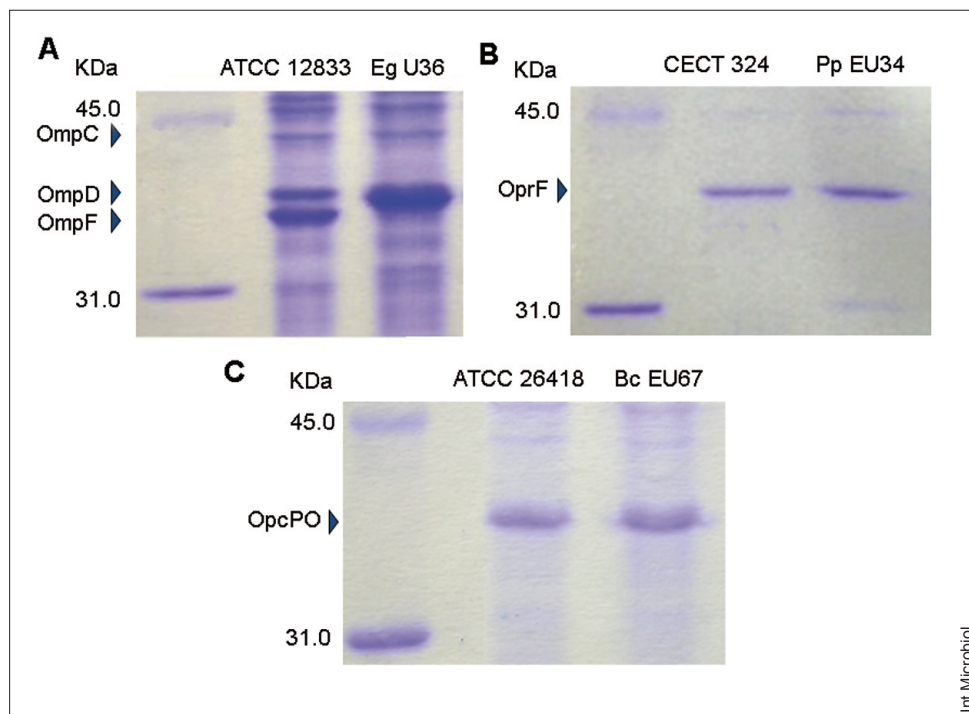
Fig. 1. SDS-PAGE of outer membrane proteins from *Enterobacter gergoviae* (**A**), *Pseudomonas putida* (**B**), and *Burkholderia cepacia* (**C**).

efficient efflux pump compared to the susceptible type strain may be sufficient to explain the resistance observed in *E. gergoviae* EU36. *Pseudomonas putida* EU34 accumulated slightly less than *E. gergoviae* EU36, but denergizated cells that accumulated ciprofloxacin had similar values to denergized *Enterobacter* cells. In this case, the low entry for *Pseudomonas* and effective efflux pumps could explain the relative resistance observed, since the susceptible *Pseudomonas* type strain had a similar behavior to its preservative-tolerant counterpart. *Burkholderia* strains accumulated less ciprofloxacin than *Pseudomonas*, especially *B. cepacia* EU67, but *Burkholderia* had a very effective efflux system that was higher than those observed in *Pseudomonas* and *Enterobacter*. *Burkholderia* preservative-tolerant strains had a dramatically low entry, but no porin suppression was observed. Probably, surface characteristics, such as hydrophobicity and the Lewis acid character, may have influenced the uptake of ciprofloxacin.

Discussion

Preservatives are added to cosmetic formulations in order to inhibit the growth of microorganisms. They are normally used at high concentrations, which are rapidly bactericidal, but, in some circumstances, sublethal concentrations can occur in the products and this exerts selective pressure on the bacteria leading to reduced susceptibility to these preservatives.

It has been proposed that antibiotics/biocides can penetrate the envelopes of Gram-negative bacteria by three routes: (i) the hydrophilic pathway, through water-filled porin channels; (ii) the hydrophobic pathway, through the lipid bilayer; and (iii) self-promoted, which involves the displacement of divalent cations that bridge adjacent lipopolysaccharide (LPS) molecules, thereby disrupting the structure of the outer membrane and exposing areas of the phospholipid bilayer [27].

Table 3. Lewis acid-base and hydrophobicity surface characteristics

	Microbial affinity to solvents (MATS)			
Strains	Chloroform	Hexadecane	Diethyl ether	Hexane
Enterobacter gergoviae (EU36)	33.80 ± 0.7	7.17 ± 0.36	50.54 ± 3.11	13.89 ± 0.77
Pseudomonas putida (EU34)	68.77 ± 7.62	14.14 ± 5.6	66.38 ± 2.6	33.24 ± 3.86
Burkholderia cepacia (EU67)	86.60 ± 6.74	29.89 ± 3.59	69.62 ± 6.62	51.24 ± 5.64
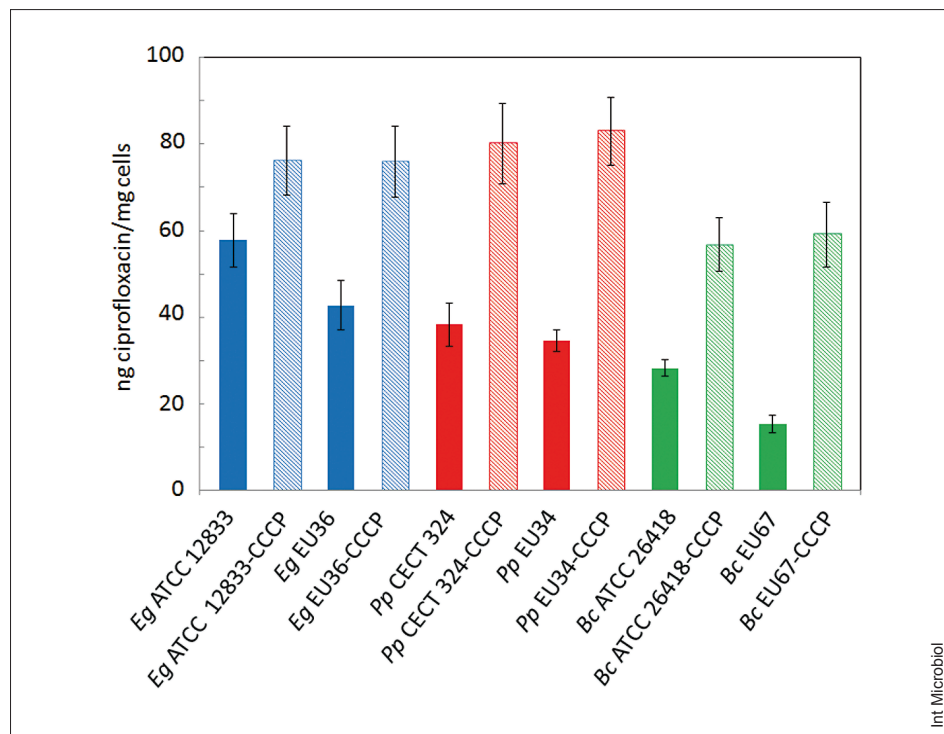
Fig. 2. Steady-state accumulation of ciprofloxacin (cip) after 6 min exposure. The height of each column represents the mean of the results of three independent experiments. The standard deviations are indicated by the bars. *Eg, Enterobacter gergoviae; Pp, Pseudomonas putida; Bc, Burkholderia cepacia*.

Permeability (reduced entry and/or an overexpressed efflux system) may be considered as a common and basic mechanism of resistance, which is perhaps even more frequent than target modification or production of antibiotic-inactivating enzymes.

Multidrug efflux pumps, especially those belonging to the resistance-nodulation-division family, play a major role in establishing the "intrinsic or acquired" resistance of Gram-negative bacteria to a wide range of toxic compounds, including antibiotics [16,31]. Efflux through RND-family pumps (e.g. AcrAB, MexAB–OprM) is driven by the proton motive force, an electrochemical gradient in which the movement of hydrogen ions drives the transport of the substrate that can be inhibited by CCCP. AcrAB-TolC multidrug resistance confers resistance to a wide variety of lipophilic and amphiphilic compounds, such as dyes, detergents, and antimicrobial agents (ethidium bromide, crystal violet, sodium dodecyl sulfate, bile acids, tetracycline, chloramphenicol, fluoroquinolones, β-lactams, erythromycin) [31]. The presence of similar systems has been reported for *Enterobacteriaceae*, and a strain of *Enterobacter cloacae* overexpressing the AcrAB system exhibited a reduction of porin gene expression [29]. A similar mechanism was probably responsible for the natural *E. gergoviae* isolates

that were tolerant to preservatives and had increasing resistance to several antibiotics used in this study. In previous studies using a series of *E. gergoviae* derivatives isolated with increasing methylisothiazolinone–chloromethylisothiazolinone (MIT-CMIT) and triclosan concentrations, antibiotic susceptibility has not been altered and a different mechanism of tolerance has been described [30]. Note that, no alteration in porin expression was observed in *Pseudomonas* nor in *Burkholderia*; overexpression of the efflux system probably belongs to the RND (resistance-nodulation-cell division) family of transporters [38]. Most of the efflux systems characterized in pseudomonads export both antibiotics and biocides. Resistance to formaldehyde-donors both in pseudomonads and enterobacteria has been described by the action of formaldehyde-dehydrogenases [18,21]; however, no specific assays were carried out to determine this enzymatic activity, since a large difference in the MIC of formaldehyde would be expected.

To date, there have been several reports of cross-resistance between antibiotics and disinfectants used in the food industry and hospital environment [5,7,9,39], but, as far as we know, there has been no evidence that this could be possible for preservatives in cosmetics, except for laboratory selected bacterial strains [30]. In our work, increasing resistance to an-

tibiotics in natural preservative-tolerant isolates was more than eight-fold the MIC with respect to susceptible strains. In some cases, such as ciprofloxacin, this increasing resistance was insufficient to encourage clinical resistance because the serum concentration of ciprofloxacin was still high. However, surviving cells would have the potential to mutate spontaneously to higher, more clinically relevant levels of resistance to quinolones (such as ciprofloxacin) or to other antibiotics [1,14]. Antibacterial agents and antibiotics share the same resistance problem: resistance will certainly increase as the drug persists, especially at low levels (e.g., residues) for long periods of time. However, this concern is irrelevant with substances that do not leave residues (alcohols, bleaches, peroxides), although it could be possible with preservatives.

It is claimed that all formaldehyde-donors/releasers are microbicidal on account of the formaldehyde released. However, in some cases, the low quantity of released formaldehyde may not be sufficient to create a biocidal action. We have shown that mechanisms other than formaldehyde-dehydrogenase activity could be responsible for a moderate tolerance to these preservatives. Also, we showed that formaldehyde-donors must penetrate into the cell through a hydrophilic pathway (porins) like some antibiotics and they could be substrates for efflux pumps.

As in clinical or veterinary practice, the development of antibiotic resistant strains, due in this case to the selective pressure from preservatives included in consumer products, could be a risk for human health. Nevertheless, the great contribution of disinfection, preservation and acceptance of hygienic measures that have supported advances in public health over the last century cannot be denied. Indeed, if reductions in the number of infections requiring antibiotic treatment can be achieved through the use of biocidal products, then this is likely to decrease rather than increase the incidence of antibiotic resistance. However, in order to preserve the role of biocides in hygiene, it is paramount to prevent the emergence of bacterial resistance and cross-resistance through their appropriate and prudent use.

Competing interests. None declared.

References

1. Berlanga M, Viñas M (2000) Salicylate induction of phenotypic resistance to quinolones in *Serratia marcescens*. 46:279-282
2. Berlanga M, Ruiz N, Hernandez-Borrell J, Montero T, Viñas M (2000) Role of the outer membrane in the accumulation of quinolones by *Serratia marcescens*. Can J Microbiol 46:716-722
3. Berlanga M, Montero MT, Hernández-Borrell J, Viñas M (2004) Influence of the cell wall on ciprofloxacin susceptibility in selected wild-type Gram-negative and Gram-positive bacteria. Int J Antimicrob Agents 23:627-630
4. Bruchmann S, Dötsch A, Nouri B, Chaberny IF, Häussler S (2013) Quantitative contributions of target alteration and decreased drug accumulation to *Pseudomonas aeruginosa* fluoroquinolone resistance. Antimicrob Agents Chemother 57:1361-1368
5. Capita R, Riesco-Peláez F, Alonso-Hernando A, Alonso-Calleja C (2014) Exposure of *Escherichia coli* ATTCC 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
6. Choi C-W, Park EC, Yun SH, Lee S-Y, Lee YG et al. (2014) Proteomic characterization of the outer membrane vesicle of *Pseudomonas putida* KT2440. J Proteome Res 13:4298-4309
7. Condell O, Iversen C, Cooney S, Power KA, Walsh C, Burgess C, 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 doi:10.1128/AEM.075334-11
8. Djeribi R, Boucherit Z, Bouchloukh W, Zouaoui W, Latrache H, Hamadi F, Menaa B (2013). A study of pH effects on the bacterial surface physicochemical properties of *Acinetobacter baumannii*. Colloids Surface B 102:540-545
9. Furi L, Ciusa ML, Knight D, Di Lorenzo V, Tocci N et al. (2013) Evaluation of reduced susceptibility to quaternary ammonium compounds and bisbiguanides in clinical isolates and laboratory-generated mutants of *Staphylococcus aureus*. Antimicrob Agents Chemother 57:3488-3497
10. Giacomel CB, Dartora G, Dienfethaeler HS, Haas SE (2013) Investigation on the use of expired make-up and microbiological contamination of mascaras. Int J Cosmetic Sci doi:10.1111/ics12053
11. Giaouris E, Chapot-Chartier M-P, Briandet R (2009) Surface physicochemical analysis of natural *Lactococcus lactis* strains reveals the existence of hydrophobic and low charged strains with altered adhesive properties. J Food Microbiol 131:2-9
12. Gilber P, Moore LE (2005) Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol 99:703-715
13. Gomez-Escalada M, Russell AD, Maillard J-Y, Ochs D (2005) Triclosan–bacteria interactions: single or multiple target sites? Lett Appl Microbiol 41:476-481
14. González-Zorn B, Escudero JA (2012) Ecology of antimicrobial resistance: humans, animals, food and environment. Int Microbiol 15:101-109
15. Hamadi F, Latrache H, Zahir H, El Abed S, Ellouali M, Saad IK (2012) The relation between the surface chemical composition of *Escherichia coli* and their electron donor/electron acceptor (acid-base) properties. Res J Microbiol 7:32-40
16. Hinchliffe P, Symmons MF, Hughes C, Koronakis V (2013) Structure and operation of bacterial tripartite pumps. Annu Rev Microbiol 67:221-242
17. Hiom SJ (2013) Preservation of medicines and cosmetics. In: Fraise AP, Maillard J-Y, Sattar SA (eds.) Principles and practice of disinfection, preservation and sterilization, John Wiley, Oxford, pp 388-407
18. Ito K, Takahashi M, Yoshimoto T, Tsuru D (1994) Cloning and high-level expression of the glutathione-independent formaldehyde dehydrogenase gene from *Pseudomonas putida*. J Bacteriol 176:2483-2491
19. Jaskulski MR, Medeiros BC, Borges JV, Zalewsky R, Fonseca MEC et al. (2013) Assessment of extended-spectrum β-lactamase, KPC carbapenemase and porin resistance mechanisms in clinical samples of *Klebsiella pneumonia* and *Enterobacter* spp. Int Antimicrob Agents 42:76-79

20. Kaneko M, Yamaguchi A, Sawai T (1984) Purification and characterization of two kinds of porins from the *Enterobacter cloacae* outer membrane. J Bacteriol 158:1179-1181

21. Kaulfers PM, Marquardt A (1991) Demonstration of formaldehyde dehydrogenase activity in formaldehyde-resistant *Enterobacteriaceae*. FEMS Microbiol Lett 79:335-338

22. Kohanski MA, Dwyer DJ, Collins JJ (2010) How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol doi:10.1038/nrmicro2333

23. Maillard JY (2002) Bacterial target sites for biocide action. J Appl Microbiol 92:16S-27S

24. McDonell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12:147-179

25. Molina-Cabrillana J, Bolaños-Rivero M, Álvarez-León EE, Martín Sánchez AM, Sánchez-Palacios M, Álvarez D, Sáez-Nieto JA (2006) Intrinsically contaminated alcohol-free mouthwash implicated in a nosocomial outbreak of *Burkholderia cepacia* colonization and infection. Infect Control Hosp Epidemiol 27:1281-1282

26. Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593-656

27. Ortega Morente E, Fernández-Fuentes MA, Grande Burgos MJ, Abriouel H, Pérez Pulido R, Gálvez A (2013) Biocide tolerance in bacteria. Int J Food Microbiol doi:10.1016/j.ijfoodmicro.2012.12.028

28. Orús P, Leranoz S (2005) Current trends in cosmetic microbiology. Int Microbiol 8:77-79

29. Pérez A, Canle D, Latasa C, Poza M, Beceiro A, et al., (2007) Cloning, nucleotide sequencing, and analysis of the AcrAB-TolC eflux pump of *Enterobacter cloacae* and determination of its involvement in antibiotic resistance in a clinical isolate. Antimicrob Agents Chemother 51:3247-3253

30. Périamé M, Pagès J-M, Davin-Regli (2014) *Enterobacter gergoviae* membrane modifications are involved in the adaptive response to preservatives used in cosmetic industry. J Appl Microbiol 118:49-61

31. Piddock LJV (2006) Multidrug-resistance efflux pumps—not just for resistance. Nat Rev Microbiol 4:629-636

32. Russell AD, McDonnell G (2000) Concentration: a major factor in studying biocidal action. J Hosp Infect 44:1-3

33. Saeb-Lima M, Solis-Arreola GV, Fernandez-Flores A (2015) Mycobacterial infection after cosmetic procedure with botulinum toxin A. J Clin Diagn Res doi:10.7860/JCDR/2015/11741.5756

34. Sheldon ATJr (2005) Antiseptic "resistance": real or perceived threat? Clin Infect Dis 40:1650-1656

35. Warburton KL, Wilkinson M (2015) Contact allergy to methylisothiazolinone: Has there been any change? Experience of a UK centre. Contact Derm doi:10.1111/cod.12403

36. Webber MA, Whitehead RN, Mount M, Loman NJ, Pallen MJ, Piddock LJV (2015) Parallel evolutionary pathways to antibiotic resistance selected by biocide exposure. J Antimicrob Chemother. doi:10.1093/jac/dkv109

37. Whitehead RN, Overton TW, Kemp CL, Webber MA (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

38. Zhang L, Li XZ, Poole K (2001) Fluoroquinolone susceptibilities of efflux-mediated multidrug-resistant *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. J Antimicrob Chemother 48:549-552

39. Zhou G, Shi Q-S, Ouyang Y-S, Chen Y-B (2014) Involvement of outer membrane proteins and peroxide-sensor genes in *Burkholderia cepacia* resistance to isothiazolone. World J Microbiol Biotechnol 30:1251-1260

XXIII CONGRESO LATINOAMERICANO DE MICROBIOLOGÍA
XIV CONGRESO ARGENTINO DE MICROBIOLOGÍA 2016

asociación argentina de microbiología

ALAM
ASOCIACIÓN LATINOAMERICANA DE MICROBIOLOGÍA

26 al 30 de septiembre de 2016
Salón Metropolitano. Rosario, Santa Fe, Argentina

XXIII Congreso Latinoamericano de Microbiología

XIV Congreso Argentino de Microbiología

IV Congreso Latinoamericano de Microbiología de Medicamentos y Cosméticos - CLAMME

Reunión de la Sociedad Latinoamericana de Tuberculosis y otras Micobacteriosis (SLAMTB)

CLAMME 2016

SLAMTB
Sociedad
Latinoamericana de
Tuberculosis y otras
Micobacteriosis

Presidente: Marcelo Galas
Secretarios Científicos: Manuel Gómez Carrillo
Marisa Almuzara
Representantes ALAM:
Howard Junca (Presidente Congreso ALAM 2014, Colombia)
Nicolás Guiliani (Presidente congreso ALAM 2018, Chile)

Temario científico preliminar

Infecciones de impacto en Latinoamérica.
Nuevos desarrollos en vacunas.
Evasión de la respuesta inmune de los microorganismos.
Evolución microbiana.
Viejas epidemias, emergencias y re emergencias microbianas.
Enfoques y estrategias para control de la resistencia a antimicrobianos.
Nuevas herramientas en el diagnóstico microbiológico.
Patogenia celular y molecular de infecciones microbianas.
Los microorganismos en el modelo de agricultura sustentable.
Estrategias para la protección y remediación de ambientes contaminados. Biodegradación de contaminantes ambientales.
Bioseguridad y biocustodia.
Tendencias en la detección molecular y caracterización de patógenos emergentes en alimentos
Genómica aplicada a la vigilancia de los patógenos asociados a ETA y al diagnóstico y vigilancia de las enfermedades infecciosas

Información: http://www.alam-cam2016.aam.org.ar/

ORGANIZACIÓN
asociación argentina de microbiología
ALAM
ASOCIACIÓN LATINOAMERICANA DE MICROBIOLOGÍA

ORGANIZACIÓN LOCAL
GSF
Gobierno de Santa Fe
MR
MUNICIPALIDAD DE ROSARIO