Effect of Quaternary Ammonium Salts and Amine Oxides on the Surface Hydrophobicity of *Enterobacter cloacae*

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The effect of quaternary ammonium salts (QAS) and amine oxides (AO) on the surface hydrophobicity of a clinical isolate of *Enterobacter cloacae* was studied. The surface hydrophobicity was evaluated by the method of bacterial adherence to hydrocarbon – xylene and on the basis of salt aggregation test of ammonium sulfate. The efficacy of amphiphilic compounds tested depended on the length of the carbon chain in their molecule. A strong inhibition of adherence of the studied strain to xylene was caused by ATDBr (QAS with the carbon chain) in the whole concentration range. At highest concentration tested, 50 μg cm^{-1}, it decreased the adherence to 18.1 % against the control. The amine oxide TMABr with the side carbon chain inhibited the adherence, but not so strong as ATDBr. Both types of amphiphilic compounds without carbon chain (TMABr and TMANO) were ineffective in the influencing of the surface hydrophobicity of tested strain, or caused moderate stimulation (TMABr, $P < 0.05$) or inhibition (TMANO) of adherence, respectively, which was statistically insignificant.

Amphiphilic compounds to which the quaternary ammonium salts (QAS) and amine oxides (AO) belong have an antimicrobial effect by damaging the cytoplasmic membrane whereas the condition for their effect is the presence of the alkyl chain and polar head in their molecule. In the homologous series of QAS the antimicrobial activity increases with the prolonging alkyl substituent up to the certain length and decreases with its further elongation [1]. This so-called "cut-off" effect was explained by *Devínsky et al.* [2] as the consequence of membrane perturbation and was observed in studies of antimicrobial efficiency of homologous series of QAS on *Pseudomonas aeruginosa* and *Salmonella typhimurium* isolated from patients with hospital infections [1, 3]. Another demonstration of the membrane integrity damage is the inhibition of respiration and interference with biosynthetic processes of the bacterial cell [4—6]. Disinfectant substances based on QAS and AO are widely used in both hygienic and epidemiologic practice. In addition it was found that the compounds of this type significantly influence the production of virulence factors of the gram-negative bacteria [5, 6].

Recently we tested the antimicrobial activity of compounds with long alkyl chain as well as their analogues lacking the alkyl chain on *Pseudomonas aeruginosa*. Only those compounds with long alkyl chain in their molecule showed antimicrobial efficacy [5]. Therefore we were interested in the influence of this type amphiphilic compounds on the surface hydrophobicity of an opportunistic nosocomial pathogen *Enterobacter cloacae.* The surface hydrophobicity of bacteria is the primary modulator of adhesion for hydrophobic organisms, which is an important factor of their virulence.

EXPERIMENTAL

Enterobacter cloacae 6297 strain was isolated from clinical material of a patient suffering from nosocomial infection and in the previous screening it exhibited a high cell-surface hydrophobicity. The cultivation and bacterial hydrophobicity determination were done in the synthetic medium Davis [7].

The chromatographically pure quaternary ammonium salts (see structure formulas) (1-methyldodecyl)-trimethylammonium bromide (ATDBr) and tetramethylammonium bromide (TMABr) as well as the amine oxides (1-methyldodecyl)dimethylamine oxide (ATDNO) and trimethylamine oxide (TMANO) were laboratory preparations.

Surface hydrophobicity of bacterial cells treated with concentrations of QAS and AO (ρ/(μg cm^{-1}): 6.25; 12.5; 25; 50) and untreated cells (controls) was assessed according to the two techniques: bacterial adhesion to hydrocarbons (BATH) and the salt aggregation test (SAT).

Bacterial adhesion to hydrocarbon (xylene) was performed as originally proposed by *Rosenberg et al.* [8]. Bacteria were harvested after 24 h incubation, washed twice and resuspended in phosphate-urea-magnesium buffer, pH 7.0 (16.9 g K_2HPO_4, 7.3

g KH_2PO_4, 1.8 g urea, 0.2 g $MgSO_4 \cdot 7H_2O$, distilled water to 1 dm^3). The cells were resuspended in BATH buffer to an absorbance of $A_{400} = 1.0$ in a total volume of 10 cm^3 × 1 cm^3; xylene was added to each test tube containing 4 cm^3 of bacterial suspension. The tubes were then shaken for 60 s on a vortex mixer and afterwards left for 30 min at room temperature. After the samples were separated into two layers, the aqueous layer was removed and the absorbance at $\lambda =$ 400 nm was measured. The results were expressed as fractions of OD (optical density) of the aqueous layer with reference to the cell suspension without xylene. The exposed strain was considered hydrophobic when it exhibited a fraction of adsorption to hydrocarbon $\geqq$ 35 %.

Salt aggregation test was performed according to the method described by *Blanco et al.* [9] with ammonium sulfate solutions (c/(mol dm^{-3}): 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 2.0) in 0.2 M phosphate buffer, pH 6.8. 25 μg cm^{-1} of the bacterial cell suspension and 25 μg cm^{-1} of an ammonium sulfate solution were mixed on a glass slide and the results were scored after 2 min. The exposed strain was considered hydrophobic when it aggregated at lower ammonium sulfate concentrations than 1.4 mol dm^{-3}.

Microsoft Excel software was used to perform Student's t-tests. P values of less than 0.05 were defined as statistically significant differences.

RESULTS AND DISCUSSION

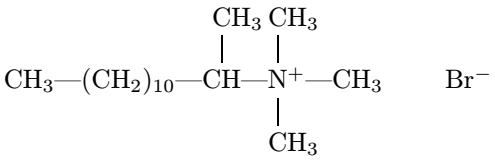
The data in Table 1 show that the substances studied in a number of tested concentrations influenced the surface hydrophobicity of *E. cloacae*. A strong inhibition of adherence of the studied strain to xylene was caused by ATDBr (see formulas) in the whole concentration range ($P < 0.05$). The highest concentration tested, 50 μg cm^{-3}, decreased the adherence to 18.1 % compared to the control. On the other hand, TMABr was ineffective or caused a moderate stimulation of adherence. The amine oxide ATDNO with the side carbon chain inhibited the adherence, but not so strong as ATDBr. However, the results of adherence inhibition were statistically significant ($P < 0.05$). The concentration of 50 μg cm^{-3} evoked an inhibition of 62.1 %. TMANO was ineffective or caused a very poor inhibition, which was statistically insignificant. It caused a stimulation at a concentration of 50 μg cm^{-3}. The values of *E. cloacae* cells adherence on xylene were in correlation with the results of the salt-aggregative ability of the tested strain in SAT test.

The influence of the surface hydrophobicity, which represents an adherence marker as an important virulence factor of *E. cloacae*, depends on the length of the carbon chain in the molecule of quaternary ammonium salts as well as of amine oxides. This fact was especially expressive in the case of both quaternary ammo-

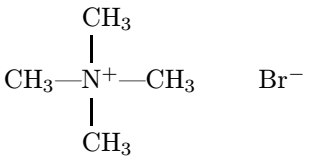
Table 1. Effect of QAS and AO on the Surface Hydrophobicity of *Enterobacter cloacae*

Compound	ρ	Adherence to xylene (mean ± SD)/%[a]	SAT
	μg cm^{-3}		c/(mol cm^{-3})
ATDBr	0	(63.1 ± 1.7)/100	0.8
	6.25	(50.2 ± 2.8)[b]/79.5	1.0
	12.5	(37.2 ± 2.3)[b]/58.9	1.0
	25	(20.9 ± 2.0)[b]/33.1	1.4
	50	(11.4 ± 0.2)[b]/18.1	1.4
TMABr	0	(64.5 ± 0.3)/100	0.8
	6.25	(66.0 ± 3.5)/102.3	0.8
	12.5	(71.0 ± 1.5)[b]/110.0	0.8
	25	(70.3 ± 1.0)[b]/108.9	0.8
	50	(71.3 ± 0.4)[b]/110.0	1.0
ATDNO	0	(68.9 ± 1.0)/100	0.8
	6.25	(67.5 ± 2.4)[b]/98.0	1.0
	12.5	(59.8 ± 5.0)[b]/86.6	1.0
	25	(60.2 ± 4.0)[b]/87.2	1.4
	50	(42.9 ± 1.6)[b]/62.1	1.4
TMANO	0	(71.3 ± 2.2)/100	0.8
	6.25	(69.7 ± 0.7)/97.7	0.6
	12.5	(71.1 ± 1.9)/99.7	1.0
	25	(65.5 ± 4.6)/91.8	0.8
	50	(75.4 ± 0.2)[b]/105.7	0.6

a) Percentage of inhibition of adherence compared with control; *b*) $P < 0.05$ compared with the hydrophobicity in the corresponding control.



ATDBr



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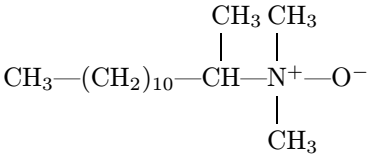

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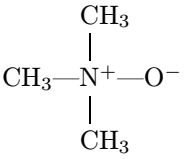

TMANO

Structural formulas of tested compounds

nium salts, ATDBr and TMABr. These findings are in agreement with our previous results concerning the effect of amphiphilic compounds on the virulence factors of *P. aeruginosa* [5] and the biological properties of *S. typhimurium* [6]. It is known that the bacterial adherence as a phenomenon contributing to the development of infection is associated with the hydrophobicity of the bacterial surface [10]. In the interpretation of bacterial adherence, the term hydrophobicity is often used because it has been observed that bacterial adherence increased with increasing bacterial hydrophobicity and decreased with decreasing hydrophobicity [11—13]. It has been observed that the presence of surface appendages, such as fimbriae and/or pili or fibrils, renders microbial cells more hydrophobic and more adhesive than nonfimbriate cells [14—16]. This is because fimbriae are proteins consisting mainly of amino acids with a nonpolar group, so fimbriate bacteria should therefore have a lower negative surface charge than the nonfimbriate bacteria [14]. This is probably due to displacement of water from the interacting surfaces of procaryotic-eucaryotic cells and the formation of an adhesive bond through a gain entropy [14]. Our findings indicate that amphiphilic compounds QAS and AO, mainly those with long carbon chain, are able to interfere with the membrane integrity and perhaps also with the synthesis or expression of surface appendages. The results attained in the submitted paper extend the fact known from extensive research of both organic ammonium salts and amine oxides that for their efficacy, the length of the aliphatic chain, which has a fundamental effect on the hydrophilic-lipophilic balance of the molecule, is decisive. The influence of both QAS and AO with the long carbon chain on the surface hydrophobicity can interfere with the biological properties of *E. cloacae*.

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