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Laboratory note

Synthesis and antimicrobial activities of new quats

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Summary — (Alkoxymethyl)dodecyldimethylammonium, (cycloalkoxymethyl)dodecyldimethylammonium, (alkylthiomethyl)-dodecyldimethylammonium, (alkoxymethyl)dimethyloctylammonium, (cycloalkoxymethyl)dimethyloctylammonium and (alkylthiomethyl)dimethyloctylammonium chlorides were prepared in high yield. All the chlorides studied showed antimicrobial activity. The relationship between the chemical structure and antimicrobial activity was analyzed using the rough sets method.

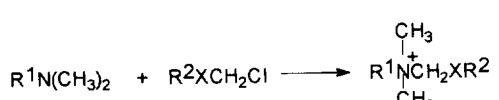
(alkoxymethyl)alkyldimethylammonium chloride / (cycloalkoxymethyl)alkyldimethylammonium chloride / (alkylthiomethyl)-alkyldimethylammonium chloride / antimicrobial activity / rough sets method

Introduction

Quaternary ammonium compounds (quats) are the essential compounds for many antimicrobials. After Domagk's discovery in 1935 [1] of the biocidal properties of quats, several generations of structurally variable quats were developed. The fact that quats are cationic surfactants allows the user to apply them in a variety of ways. We decided to look for new quats that might prove more effective in the antimicrobial field.

Chemistry

The new quats **1–72** (tables I, II) were prepared by the reaction of dodecyldimethylamine or dimethyloctylamine with chloromethylalkyl ethers or sulfides or chloromethylcycloalkyl ethers.



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The yield from this reaction was very good, ie, between 95 to 80%. Dodecyldimethylamine and dimethyloctylamine were commercially available. Chloromethylalkyl ethers or sulfides or chloromethylcycloalkyl ethers were synthesized from alcohols, thiols or cycloalkohols.

Antimicrobial activity

All chlorides **1–72** were tested for antibacterial activity against *Staphylococcus aureus* ATCC 6538. The most active chlorides resulting from the rough sets method were tested for the antimicrobial activities of the 12 organisms presented in table IX.

Result and discussion

All synthesized chlorides were hygroscopic and had to be kept over phosphorus pentoxide. They are active against *Staphylococcus aureus* ATCC 6538. Their activity depends on the length and kind of substituent at the quaternary nitrogen atom. The relationship between the chemical structure and antibacterial activity was analyzed via the rough sets method [2, 3]. This method was successfully used in the analysis of relationships between the structure and the anti-

Table I. (Alkoxymethyl)-, (cycloalkoxymethyl)- and (alkylthiomethyl)dimethyldodecylammonium chlorides.

Chloride	*XR²*	*Yield (%)*	*Chloride*	*XR²*	*Yield (%)*	*Chloride*	*XR²*	*Yield (%)*
1	OC_2H_5	90	**13**	$OCH_2CH(CH_3)_2$	91	**25**	OC_6H_{11}	91
2	OC_4H_9	92	**14**	$O(CH_2)_2CH(CH_3)_2$	93	**26**	OC_6H_{10}-4-CH_3	89
3	OC_6H_{13}	93	**15**	$OCH_2CH(CH_3)CH_2CH_3$	92	**27**	OC_7H_{13}	92
4	OC_8H_{17}	94	**16**	$OCH(CH_3)(CH_2)_2CH_3$	90	**28**	OC_8H_{15}	91
5	$OC_{10}H_{21}$	92	**17**	$OCH_2CH(CH_2CH_3)_2$	91	**29**	$OC_{12}H_{23}$	90
6	$OC_{12}H_{25}$	90	**18**	$OCH_2CH(CH_3)(CH_2)_2CH_3$	91	**30**	SC_4H_9	82
7	OC_3H_7	91	**19**	$OCH(CH_2CH_2CH_3)_2$	93	**31**	SC_6H_{13}	82
8	OC_5H_{11}	95	**20**	$OCH_2CH(C_2H_5)(CH_2)_3CH_3$	91	**32**	SC_8H_{17}	84
9	OC_7H_{15}	93	**21**	$OCH(CH_3)(CH_2)_5CH_3$	90	**33**	$SC_{10}H_{21}$	83
10	OC_9H_{19}	91	**22**	$OCH(C_2H_5)(CH_2)_4CH_3$	92	**34**	$SC_{12}H_{25}$	80
11	$OC_{11}H_{23}$	90	**23**	$O(CH_2)_2CH(CH_3)(CH_2)_3CH(CH_3)_2$	91	**35**	SC_3H_7	82
12	$OCH(CH_3)_2$	92	**24**	OC_5H_9	94	**36**	SC_5H_{11}	83

Table II. (Alkoxymethyl)-, (cycloalkoxymethyl)- and (alkylthiomethyl)dimethyloctylammonium chlorides.

Chloride	*XR²*	*Yield (%)*	*Chloride*	*XR²*	*Yield (%)*	*Chloride*	*XR²*	*Yield (%)*
37	OC_2H_5	90	**49**	$OCH_2CH(CH_3)_2$	91	**61**	OC_6H_{11}	95
38	OC_4H_9	93	**50**	$O(CH_2)_2CH(CH_3)_2$	92	**62**	OC_6H_{10}-4-CH_3	90
39	OC_6H_{13}	95	**51**	$OCH_2CH(CH_3)CH_2CH_3$	93	**63**	OC_7H_{13}	94
40	OC_8H_{17}	95	**52**	$OCH(CH_3)(CH_2)_2CH_3$	91	**64**	OC_8H_{15}	94
41	$OC_{10}H_{21}$	94	**53**	$OCH_2CH(CH_2CH_3)_2$	91	**65**	$OC_{12}H_{23}$	91
42	$OC_{12}H_{25}$	91	**54**	$OCH_2CH(CH_3)(CH_2)_2CH_3$	93	**66**	SC_4H_9	82
43	OC_3H_7	92	**55**	$OCH(CH_2CH_2CH_3)_2$	94	**67**	SC_6H_{13}	81
44	OC_5H_{11}	94	**56**	$OCH_2CH(C_2H_5)(CH_2)_3CH_3$	90	**68**	SC_8H_{17}	83
45	OC_7H_{15}	95	**57**	$OCH(CH_3)(CH_2)_5CH_3$	91	**69**	$SC_{10}H_{21}$	80
46	OC_9H_{19}	94	**58**	$OCH(C_2H_5)(CH_2)_4CH_3$	92	**70**	$SC_{12}H_{25}$	80
47	$OC_{11}H_{23}$	91	**59**	$OCH_2CH_2CH(CH_3)(CH_2)_3CH(CH_3)_2$	92	**71**	SC_3H_7	84
48	$OCH(CH_3)_2$	90	**60**	OC_5H_9	94	**72**	SC_5H_{11}	85

bacterial activity of quaternary imidazolium [4, 5], pyridinium [6], quinolinium and isoquinolinium compounds [7] and in the analysis of medical data [8].

Synthesized chlorides are divided into three classes of antibacterial activity. The classes correspond to the following ranges of minimum inhibitory concentration (MIC): class 1, highly effective; MIC $\leq$ 27.6 μM/L; class 2, fairly effective; 27.6 < MIC < 100 μM/L; class 3, slightly effective; MIC $\geq$ 100 μM/L.

The attributes describing the structure of 72 chlorides are presented in table III. Table IV presents the information system. The decision rules obtained from the information system upon removal of two insignificant condition attributes, ie, 1 and 3, are shown in table V.

The most active compounds against *Staphylococcus aureus* ATCC 6538 are chlorides with: R^2 = 1-methylbutyl, 3,8-dimethyloctyl, 2-ethylhexyl, *n*-nonyl and cyclododecyl (rules 1–5); R^2 = ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-heptyl, *n*-octyl, *n*-decyl, *n*-undecyl, cyclopentyl and cyclohexyl; R^1 = dodecyl (rules 6–15); R^2 = *n*-decyl; R^1 = octyl (rule 16).

These rules clarify which chlorides obtained are the most active. Of the 72 synthesized chlorides, 39 belong to one class of activity. These are the following: **1**, **2**, **4**, **7–25**, **27**, **29–32**, **34–36**, **40–42**, **46**, **52**, **56**, **59**, **65** and **69** for which antimicrobial activities were tested. The MIC and minimum bactericidal or fungicidal concentration (MBC) were measured. The obtained MIC and MBC values for 39 chlorides are given in tables VI–VIII.

In general, chlorides (alkoxymethyl)dodecyldimethylammonium, (cycloalkoxymethyl)dodecyldimethylammonium, (alkythiomethyl)dodecyldimethylammonium, (alkoxymethyl)dimethyloctylammonium, (cycloalkoxymethyl)dimethyloctylammonium and (alkylthiomethyl)-dimethyloctylammonium are highly active against cocci. Activity against bacilli, rods and fungi is a little weaker, but is also at a high level. In comparison with commercially available didecyldimethylammonium chloride **73**, the nine tested chlorides **1**, **4**, **8**, **9**, **18**, **21**, **22**, **31** and **41** had comparable mean values for MIC and MBC (tables VI–VIII) and in one case chloride **10** was more active.

To summarize, 10 new quats, potential substitutes for the well-known didecyldimethylammonium chloride were found, as follows: dodecyl(ethoxymethyl)-dimethylammonium **1**, dodecyldimethyl(octyloxymethyl)ammonium **4**, dodecyldimethyl(pentyloxymethyl)ammonium **8**, dodecyl(heptyloxymethyl) dimethylammonium **9**, dodecyldimethyl(nonyloxymethyl)ammonium **10**, dodecyldimethyl(2-methylpentyloxymethyl)ammonium **18**, dodecyldimethyl(1-methylheptyloxymethyl)ammonium **21**, dodecyl(2-ethylhexyloxymethyl)dimethylammonium **22**, dodecyl-(hexylthiomethyl)dimethylammonium **31** and (decyloxymethyl)octyldimethylammonium **41**.

Experimental protocols

Chemistry

NMR spectra were recorded on a Varian Model XL 300 spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C in $CDCl_3$ at 20 °C with tetramethylsilane as internal reference. Satisfactory elemental analyses were obtained; ie: C ± 0.36, H ± 0.31 and N ± 0.29.

Table III. Domains of condition attributes.

No	*Attribute*	*Code value* 0	1	2	3	4	5	6	7	8	9	10	11	12
1	Type of X	Oxygen	Sulphur											
2	Type of R^1	C_8H_{17}	$C_{12}H_{25}$											
3	Type of R^2	*n*-Alkyl	Alkyl	Cykloalkyl										
4	Type of *n*-alkyl	Without	–	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_6H_{13}	C_7H_{15}	C_8H_{17}	C_9H_{19}	$C_{10}H_{21}$	$C_{11}H_{23}$	$C_{12}H_{25}$
5	Type of alkyl	Without	C_3H_7[a]	C_4H_9[b]	C_5H_{11}[c]	C_5H_{11}[d]	C_5H_{11}[e]	C_6H_{13}[f]	C_6H_{13}[g]	C_7H_{15}[h]	C_8H_{17}[i]	C_8H_{17}[j]	C_8H_{17}[k]	$C_{10}H_{21}$[l]
6	Type of cykloalkyl	Without	C_5H_9	C_6H_{11}	C_7H_{13}[m]	C_7H_{13}	C_8H_{15}	$C_{12}H_{23}$						

[a]$CH(CH_3)_2$; [b]$CH_2CH(CH_3)_2$; [c]$(CH_2)_2CH(CH_3)_2$; [d]$CH_2CH(CH_3)_3CH_2CH_3$; [e]$CH(CH_3)(CH_2)_2CH_3$; [f]$CH_2CH(CH_2CH_3)_2$; [g]$CH_2CH(CH_3)(CH_2)_2CH_3$; [h]$CH(CH_2CH_2CH_3)_2$; [i]$CH_2CH(C_2H_5)(CH_2)_3CH_3$; [j]$CH(CH_3)(CH_2)_5CH_3$; [k]$CH(C_2H_5)(CH_2)_4CH_3$; [l]$(CH_2)_2CH(CH_3)(CH_2)_3CH(CH_3)_2$; [m]4-$CH_3C_6H_{10}$.

Table IV. Information system.

Chloride	Condition attributes						Class
	1	*2*	*3*	*4*	*5*	*6*	
1	0	1	0	2	0	0	1
2	0	1	0	4	0	0	1
3	0	1	0	6	0	0	3
4	0	1	0	8	0	0	1
5	0	1	0	10	0	0	3
6	0	1	0	12	0	0	3
7	0	1	0	3	0	0	1
8	0	1	0	5	0	0	1
9	0	1	0	7	0	0	1
10	0	1	0	9	0	0	1
11	0	1	0	11	0	0	1
12	0	1	1	0	1	0	1
13	0	1	1	0	2	0	1
14	0	1	1	0	3	0	1
15	0	1	1	0	4	0	1
16	0	1	1	0	5	0	1
17	0	1	1	0	6	0	1
18	0	1	1	0	7	0	1
19	0	1	1	0	8	0	1
20	0	1	1	0	9	0	1
21	0	1	1	0	10	0	1
22	0	1	1	0	11	0	1
23	0	1	1	0	12	0	1
24	0	1	2	0	0	1	1
25	0	1	2	0	0	2	1
26	0	1	2	0	0	3	3
27	0	1	2	0	0	4	1
28	0	1	2	0	0	5	3
29	0	1	2	0	0	6	1
30	1	1	0	4	0	0	1
31	1	1	0	6	0	0	1
32	1	1	0	8	0	0	1
33	1	1	0	10	0	0	3
34	1	1	0	12	0	0	1
35	1	1	0	3	0	0	1
36	1	1	0	5	0	0	1
37	0	0	0	2	0	0	3
38	0	0	0	4	0	0	3
39	0	0	0	6	0	0	2
40	0	0	0	8	0	0	1
41	0	0	0	10	0	0	1
42	0	0	0	12	0	0	1
43	0	0	0	3	0	0	3
44	0	0	0	5	0	0	3
45	0	0	0	7	0	0	3
46	0	0	0	9	0	0	1
47	0	0	0	11	0	0	3
48	0	0	1	0	1	0	3
49	0	0	1	0	2	0	3
50	0	0	1	0	3	0	3
51	0	0	1	0	4	0	2
52	0	0	1	0	5	0	1
53	0	0	1	0	6	0	3
54	0	0	1	0	7	0	3
55	0	0	1	0	8	0	3
56	0	0	1	0	9	0	1
57	0	0	1	0	10	0	2
58	0	0	1	0	11	0	3
59	0	0	1	0	12	0	1
60	0	0	2	0	0	1	3
61	0	0	2	0	0	2	3
62	0	0	2	0	0	3	3
63	0	0	2	0	0	4	2
64	0	0	2	0	0	5	3
65	0	0	2	0	0	6	1
66	1	0	0	4	0	0	3
67	1	0	0	6	0	0	3
68	1	0	0	8	0	0	3
69	1	0	0	10	0	0	1
70	1	0	0	12	0	0	3
71	1	0	0	3	0	0	3
72	1	0	0	5	0	0	3

Table V. Decision algorithm for classification of examined chlorides.

Rules	Attribute				Class
	2	*4*	*5*	*6*	
1			5		1
2			12		1
3			9		1
4		9			1
5				6	1
6	1	4			1
7	1	8			1
8	1	3			1
9	1	5			1
10	1			1	1
11	1	2			1
12	1			2	1
13	1	7			1
14	1			4	1
15	1	11			1
16	0	10			1
17	0		4		2
18	0		10		2
19	0			4	2
20				3	3
21				5	3
22	0	4			3
23	0		1		3
24	0	3			3
25	0		2		3
26	0		3		3
27	0	5			3
28	0			1	3
29	0		6		3
30	0			2	3
31	0	2			3
32	1	10			3
33	0		7		3
34	0		8		3
35	0	7			3
36	0	11			3
37	0		11		3
38	1	6			1 or 3
39	0	8			1 or 3
40		12			1 or 3
41	0	6			2 or 3

Chloromethylalkyl ethers and sulfides and chloromethylcycloalkyl ethers were prepared via the procedures which have been reported earlier [9]. The percentage of ether or sulfide in a crude product was determined by an alkalimetric method: 1 g crude product was added to 10 mL acetone at –40 °C. Free HCl (substrate) was quickly neutralized with 1% KOH in EtOH and 3 mL water was added. The mixture was stirred at 40 °C for 15 min. HCl as a product of hydrolysis of ether or sulfide was neutralized with 2% KOH in EtOH. The crude product contained 96–86% chloromethylalkyl ether, 79–65% chloromethylalkyl sulfide and 95–82% chloromethylcycloalkyl ether.

Table VI. MIC and MBC[a] of examined chlorides.

Strains[b]		*Chlorides*												
		1	*2*	*4*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
I	MIC	2.27	0.89	0.76	3.10	3.57	0.26	1.72	2.3	2.17	2.98	7.14	0.86	3.43
	MBC	2.27	0.89	0.76	3.10	7.14	1.32	3.69	2.3	4.66	2.98	7.14	2.00	3.43
II	MIC	9.74	0.89	6.38	1.55	3.57	1.32	3.69	2.3	9.32	14.9	7.14	0.86	7.14
	MBC	40.6	0.89	12.8	1.55	14.3	1.32	7.39	2.3	18.6	149	7.14	4.29	7.14
III	MIC	4.87	4.46	1.53	1.55	3.57	1.32	3.69	11.5	9.32	2.98	3.57	4.29	7.14
	MBC	9.74	4.46	6.38	1.55	3.57	2.64	3.69	23.0	9.32	14.9	7.14	4.29	7.14
IV	MIC	4.87	4.46	0.77	1.55	3.43	1.32	3.69	11.5	9.32	14.9	7.14	2.00	7.14
	MBC	9.74	4.46	3.82	3.10	7.14	2.64	14.8	115	18.6	29.8	7.14	8.57	14.3
V	MIC	4.87	0.89	1.53	1.55	3.57	1.32	7.39	2.3	9.32	2.98	7.14	4.29	1.71
	MBC	9.74	17.9	25.5	1.55	14.3	1.32	7.39	230	18.6	149	7.14	4.29	3.43
VI	MIC	325	298	128	1550	143	132	29.5	115	1550	1490	1430	143	1430
	MBC	325	298	128	1550	143	132	29.5	2300	1550	1490	1430	1430	1430
VII	MIC	40.6	74	25.5	155	143	132	29.5	1150	311	149	143	71	143
	MBC	40.6	149	128	155	143	132	123	2300	311	149	143	71	143
VIII	MIC	19.5	179	12.8	155	143	13.2	7.39	115	78	149	143	171	143
	MBC	40.6	179	12.8	310	143	13.2	29.5	230	155	149	143	71.4	143
IX	MIC	40.6	74	25.5	31.0	7.14	13.2	62	230	78	29.8	143	35.7	143
	MBC	40.6	74	25.5	155	14.3	24.4	62	230	155	29.8	143	35.7	143
X	MIC	162	74	255	310	143	132	123	2300	311	298	143	35.7	143
	MBC	162	298	255	310	143	132	123	5760	311	298	143	71.4	143
XI	MIC	81	74	128	155	28.6	13.2	62	115	311	298	28.6	71.4	143
	MBC	162	74	128	155	28.6	26.4	62	115	311	298	28.6	71.4	143
XII	MIC	19.5	8.93	12.8	31.1	7.14	13.2	14.7	23.0	78	149	28.6	17.1	28.6
	MBC	19.5	37.2	12.8	155	7.14	13.2	29.5	1150	155	149	28.6	35.7	57.1
Mean values	MIC	59.6	66.1	49.9	200	52.7	37.9	29.0	340	230	217	174	33.6	183
	MBC	71.9	94.8	61.6	233	55.7	40.4	41.3	1038	251	242	175	151	186

[a]MIC and MBC in μM/L; [b]the number of microorganisms in mL ranged from 10^4 to 10^5.

Synthesis

The ammonium chlorides were prepared by dissolving dimethyldodecylamine or dimethyloctylamine in heptane and adding equimolar amounts of the appropriate chloromethylalkyl ether or sulfide or chloromethylcycloalkyl ether. The mixture was stirred and heated under reflux for 10 min when ether was used and for 6 h when sulfide was used. After cooling the solution to room temperature the crude product was separated, extracted and the residue dried in vacuo to give the pure product. The yields were 95–86% for chlorides **1–29** and **37–65**; 89–80% for chlorides **30–36** and **66–72**. (Cyclododecyloxymethyl)dodecyldimethylammonium chloride **29** (oil): ^{1}H-NMR δ ppm: 4.99 (s, 2H), 3.95 (m, 1H), 3.50 (m, 2H), 3.33 (s, 6H), 1.72 (m, 6H), 1.33 (m, 26H), 0.90 (t, J = 6 Hz, 3H); ^{13}C-NMR δ ppm: 87.5, 80.7, 60.4, 46.9, 31.3, 29.2, 29.0, 28.9, 28.8, 28.7, 28.6, 28.1, 25.9, 24.6, 24.3, 22.3, 22.1, 21.9, 19.5, 13.6. (Dodecylthiomethyl)dimethyloctylammonium chloride **70** (oil): ^{1}H-NMR δ ppm: 5.13 (s, 2H), 3.65 (m, 2H), 3.38 (s, 6H), 3.01 (t, J = 7 Hz, 2H), 1.72 (m, 4H), 1.36 (m, 28H), 0.90 (t, J = 7 Hz, 6H); ^{13}C-NMR δ ppm: 48.4, 42.2, 35.1, 31.2, 31.0, 29.2, 29.0, 28.8, 28.7, 28.6, 28.5, 28.4, 27.9, 25.8, 22.0, 21.9, 13.5, 13.4.

Table VII. MIC and MBC[a] of examined chlorides.

Strains[b]		*Chlorides*												
		17	***18***	***19***	***20***	***21***	***22***	***23***	***24***	***25***	***27***	***29***	***30***	***31***
I	MIC	1.37	1.65	0.40	2.55	0.77	2.55	2.38	1.44	11.0	13.3	1.57	2.84	13.1
	MBC	13.7	3.30	0.79	12.8	1.53	12.8	2.38	1.44	11.0	13.3	3.36	2.84	131
II	MIC	1.37	6.87	1.59	1.28	12.8	1.28	11.9	2.87	13.8	13.3	3.36	1.42	13.1
	MBC	2.75	13.7	1.59	1.28	12.8	1.28	23.9	14.4	138	13.3	3.36	2.84	26.3
III	MIC	2.75	1.65	1.59	12.8	1.53	2.55	11.9	1.44	1.38	13.3	3.36	2.84	13.1
	MBC	13.7	3.43	3.17	25.5	3.06	25.5	23.8	14.4	27.6	26.6	3.36	14.2	131
IV	MIC	2.75	1.65	6.61	12.8	1.53	1.28	11.9	2.87	2.76	13.3	13.4	14.2	13.1
	MBC	27.5	3.30	6.61	12.8	3.06	25.5	23.8	14.4	13.8	26.6	26.9	14.2	26.3
V	MIC	2.75	1.65	3.31	1.28	3.06	1.28	11.9	2.87	2.76	2.66	1.57	2.84	13.1
	MBC	27.5	1.65	6.61	0.77	2.55	7.65	23.8	28.7	13.8	2.66	6.72	28.4	13.1
VI	MIC	1370	137	2650	255	255	128	2380	1440	276	1330	1120	142	131
	MBC	1370	137	2650	1280	255	255	11900	1440	276	1330	2240	142	131
VII	MIC	27.5	27.5	132	128	25.5	25.5	238	144	138	13.3	112	142	26.3
	MBC	137	27.5	132	128	25.5	128	238	144	138	133	224	142	131
VIII	MIC	27.5	13.7	26.4	25.5	25.5	12.8	119	144	138	26.6	26.9	142	26.3
	MBC	27.5	13.7	26.4	25.5	25.5	25.5	238	287	138	133	112	142	26.3
IX	MIC	13.7	137	132	25.5	128	12.8	238	28.7	13.8	26.6	11.2	14.2	2.63
	MBC	27.5	137	132	128	128	12.8	238	144	13.8	26.6	22.4	14.2	2.63
X	MIC	137	137	132	255	255	128	1190	144	138	133	1120	142	131
	MBC	275	137	132	2550	255	255	1190	287	138	133	1120	142	131
XI	MIC	27.5	27.5	26.4	12.8	25.5	12.8	238	144	138	26.6	26.9	142	26.3
	MBC	27.5	27.5	26.4	25.5	128	12.8	1190	144	138	26.6	26.9	1420	263
XII	MIC	13.7	6.87	6.61	12.8	12.8	2.55	119	14.4	13.8	13.3	13.4	14.2	13.1
	MBC	13.7	13.7	13.2	12.8	128	128	119	14.4	13.8	13.3	112	28.4	13.1
Mean	MIC	136	41.7	260	62	62	27.6	381	173	73.9	135	204	63.5	35.2
values	MBC	164	43.2	261	350	91	74.2	1277	211	88.3	156	325	174	85.5

[a]MIC and MBC in μM/L; [b]the number of microorganisms in mL ranged from 10^4 to 10^5.

Table VIII. MIC and MBC[a] of examined chlorides and didecyldimethylammonium chloride.

Strains[b]		*Chlorides*													
		32	***34***	***35***	***36***	***40***	***41***	***42***	***46***	***52***	***56***	***59***	***65***	***69***	***73***[c]
I	MIC	12.1	0.54	0.15	0.14	14.9	13.7	1.28	2.86	34	14.9	1.37	12.8	1.32	1.38
	MBC	12.1	5.38	2.96	0.27	14.9	137	12.8	14.3	34	14.9	2.75	128	2.63	27.6
II	MIC	24.5	1.08	1.5	13.7	29.8	2.75	1.28	2.86	34	7.4	1.37	1.28	2.63	2.76
	MBC	24.5	10.8	2.96	13.7	29.8	13.7	12.8	2.86	34	7.4	13.7	12.8	13.2	2.76
III	MIC	12.1	5.38	15	13.7	14.9	2.75	1.28	2.86	0.03	7.4	13.7	2.56	26.3	27.6
	MBC	12.1	5.38	15	13.7	14.9	27.5	25.5	2.86	17.0	7.4	13.7	12.8	26.3	27.6
IV	MIC	12.1	10.8	15	13.7	149	13.7	12.8	14.3	17.0	7.4	27.5	2.56	13.2	1.38
	MBC	24.4	53.8	29.6	13.7	149	13.7	25.5	143	17.0	74	137	128	26.3	138
V	MIC	12.1	10.8	2.96	2.73	2.98	2.75	1.28	14.3	34	7.4	2.75	2.56	13.2	0.28
	MBC	24.5	10.8	150	13.7	14.9	2.75	25.5	143	170	74	13.7	12.8	26.3	27.6
VI	MIC	245	10800	1500	1370	1490	137	128	1430	3400	1490	2750	1280	1320	13.8
	MBC	1225	10800	1500	1370	1490	137	255	2860	3400	1490	2750	1280	1320	13.8
VII	MIC	123	10800	296	137	149	27.5	128	28.6	1700	149	27.5	12.8	132	13.8
	MBC	245	10800	296	137	149	27.5	128	286	1700	149	27.5	12.8	132	13.8
VIII	MIC	123	215	148	27.3	149	2.75	12.8	149	170	298	1370	25.6	132	13.8
	MBC	245	215	296	137	149	13.7	15.5	286	1700	298	1370	25.6	132	13.8
IX	MIC	24.5	108	148	137	29.8	27.5	25.5	14.3	340	149	27.5	12.8	13.2	2.76
	MBC	245	108	296	137	29.8	27.5	25.5	143	1700	149	27.5	12.8	13.2	2.76
X	MIC	2450	10800	296	137	298	275	255	143	1700	149	1370	12.8	2630	276
	MBC	2450	10800	296	137	298	275	1280	143	1700	298	1370	25.6	2630	276
XI	MIC	24.5	215	296	27.3	298	137	12.8	28.6	1700	149	13.7	25.6	132	13.8
	MBC	24.5	1080	296	137	298	137	25.5	286	3400	149	1370	256	132	13.8
XII	MIC	12.2	215	29.6	13.7	29.8	27.5	12.8	143	170	29.8	13.7	12.8	26.3	13.8
	MBC	122	1080	29.6	13.7	149	27.5	12.8	143	1700	149	13.7	25.6	132	13.8
Mean values	MIC	257	2783	231	159	224	58.8	49.4	164	789	207	469	118	372	31.8
	MBC	388	2914	268	177	232	70.0	155	350	1298	238	592	161	382	47.6

[a]MIC and MBC in μM/L; [b]the number of microorganisms in mL ranged from 10^4 to 10^5; [c]didecyldimethylammonium chloride.

Table IX. Strains of microorganisms.

Type no	*Strain*
	Cocci
I	*Micrococcus luteus* ATCC 9341
II	*Staphylococcus epidermidis* ATCC 12228
III	*Staphylococcus aureus* ATCC 6538
IV	*Staphylococcus aureus* (MRSA) ATCC 33592
	Bacilli
V	*Bacillus subtilis* NCTC 10452
	Rods
VI	*Serratia marcescens* ATCC 8100
VII	*Klebsiella pneumoniae* ATCC 4352
VIII	*Proteus vulgaris* NCTC 4635
IX	*Escherichia coli* NCTC 8196
X	*Pseudomonas aeruginosa* ATCC 15442
	Fungi
XI	*Candida albicans* ATCC 10231
XII	*Rhodotorula rubra* PhB

Antimicrobial activity

The microorganisms which were used are presented in table IX. Standard strains were supplied by the National Collection of Type Cultures (NCTC) London and American Type Culture Collection (ATCC). The *Rhodotorula rubra* (PhB) strain was taken from the Department of Pharmaceutical Bacteriology, University School of Medical Sciences, Poznań.

MIC was determined by the tube dilution method. A series of chloride dilutions were prepared on Müller–Hinton broth medium (bacteria) or Sabouraud broth medium (fungi). Microorganism growth was determined visually and the lowest chloride concentration which inhibited the multiplication of cells for 24 h at 37 °C was taken as the MIC.

MBCs of the tested chlorides were interpreted as follows: from each tube a sample was cultured on a solid medium with inactivator (2.5% lecitin, 5% lubrol W and 5% polysorbate 80) and after incubation for 48 h at 37 °C (bacteria) or at 28 °C (fungi) results were read out. For the lowest concentration no colony formation was defined as the MBC.

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