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Research article

USE OF AQUEOUS COMPOSITIONS OF POLYACRYLAMIDE WITH ZINC AND COPPER CATIONS AS A POSSIBLE WAY TO REDUCE THE RISKS OF MICROBIAL CONTAMINATION IN OBJECTS IN THE HOSPITAL ENVIRONMENT

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Microbial contamination means that infectious agents are identified on objects in the hospital environment. This serious issue is the most significant for healthcare organizations. Covering abiotic surfaces with a thin polymer film can be a promising way to fight against microbial adhesion and colonization. This film acts as a depot of an antibacterial substance.

In this study, our aim was to investigate antimicrobial effects of new water compositions of polyacrylamides (PAM) with $CuSO_4$ and $ZnSO_4$.

We examined antibacterial activity of 5%-solutions of $CuSO_4$ and $ZnSO_4$ and their compositions with various PAM types in a concentration equal to 0.075 % against such reference cultures as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. We estimated use of PAM as a growth substrate as well as antimicrobial activity of the analyzed solutions and compositions in agar and liquid nutrient media.

As a result, we established that bacterial cultures did not use PAM as sole nutrition source when growing in a liquid mineral medium and on PAM-films covering glass and plastic surfaces. More apparent inhibitory effects were produced on microorganisms cultivated on solid and liquid nutrient media by 5%-solution of $ZnSO_4$. When PAM Praestol 857 and PAM Praestol were added to solutions of Cu^{2+} and Zn^{2+} cations, it resulted in an authentic increase in a diameter of a zone with inhibited bacterial growth in the agar medium. In the liquid medium, salts of both metals inhibited the growth and viability of all the analyzed microorganisms already in a concentration equal to 0.16 % or lower. Adding PAM Praestol 2530 led to a slight decrease in antibacterial efficiency of the examined metal salts whereas PAM Praestol 857 had practically no influence on bacteriostatic and bactericidal effects produced by them.

Therefore, use of the obtained composite solutions where $CuSO_4$ or $ZnSO_4$, immobilized on a PAM matrix act as an antibacterial component seems a promising way to disinfect objects in the hospital environment. This can significantly reduce risks of hospital-acquired infections.

Keywords: *risks of microbial contamination, $CuSO_4$, $ZnSO_4$, polyacrylamides (PAM), antimicrobial solutions, antimicrobial activity, hospital environment.*

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Contamination of abiotic surfaces with pathogens and opportunistic pathogens is a substantial threat for human health and health of farm animals [1]. Microbial contamination or, in other words, infectious agents occurring on various environmental objects has specific significance when it comes to healthcare organizations, first of all, intensive care units, emergency surgery and burn units [2, 3]. It creates high risks of healthcare-associated (nosocomial) infections. The official multicentered study on prevalence of nosocomial infections was accomplished by the World Health Organization experts in 50 clinics located in 14 different countries. It established that 8.7 % of all hospitalized patients or more than 1.4 million people worldwide had infectious complications [4]. Their development requires additional diagnostic and treatment procedures thereby extending a period a person has to spend in hospital and resulting in substantial expenses. In addition, these developing complications deteriorate a patient's quality of life considerably and create elevated risks that a primary disease would have an adverse outcome [2].

M. Robakowska with colleagues (2017) detected microbial contamination in 20 % of the swabs taken off various objects in the hospital environment when performing bacteriological control in a multi-field in-patient hospital [5]. Healthcare organizations use varied disinfectants to prevent infections caused by microbe circulation and persistence on objects and equipment [3, 5]. Most disinfectants produce their effects directly at the moment when a surface is being treated with them or during a very short time. Moreover, at present most clinically significant bacteria are developing greater resistance to disinfectants that are commonly used in hospitals [6]. Covering abiotic surfaces with a thin polymer film containing antibacterial substances can be a promising way to fight against microbial adhesion and colonization. Such disinfectants are more effective since their effects last longer due to a polymer basis provided for a material that acts as a depot of a biocide.

Inorganic salts, salts of Cu and Zn in particular, are known to produce a wide range of antibacterial effects [7–10]; polyacrylamides (PAM) play a significant role in compositions of water soluble polymer with metallic cations acting as reducers and / or being a matrix for aggregation of metal ions or nanoparticles [11]. Properties of these compositions and their application as antibacterial materials are being studied actively now [12]. Nevertheless, as far as we know, experts have not yet made an attempt to compare antimicrobial effects produced on certain pathogens and opportunistic pathogens by water solutions of PAMs with Zn^{2+} and Cu^{2+} cations with different physical and chemical properties.

In this study, our aim was to investigate antimicrobial effects of new compositions of water soluble polyacrylamides (PAM) with $CuSO_4$ and $ZnSO_4$.

Methods and materials. ***Analyzed solutions and compositions***. In this study, we analyzed a 5%-solution of $CuSO_4$, 5%-solution of $ZnSO_4$, various types of polyacrylamides (PAM) in a concentration equal to 0.075 %: PAM Praestol 806, PAM Praestol 857, PAM Praestol 2510, PAM Praestol 2530, as well as a 5%-solution of $CuSO_4$ in PAM Praestol 857, 5%-solution of $ZnSO_4$ in PAM Praestol 857, 5%-solution of $ZnSO_4$ in PAM Praestol 2530 (provided by the Institute of Technical Chemistry of Ural Branch of RAS, Perm). PAM are acrylamide polymers, which, when being solved in water, are used for gelation of liquids and creation of film coatings. Their approximate molecular mass varies between 8 and 14 million. PAM Praestol 806 and PAM Praestol 857 become positively charged when dissolved in water; PAM Praestol 2510 and PAM Praestol 2530 become negatively charged.

Bacterial strains. We selected several strain cultures as our test objects including *Escherichia coli* ATCC®25922, *Klebsiella pneumoniae* ATCC®700603, *Pseudomonas aeruginosa* ATCC®27853, *Staphylococcus aureus* ATCC®25923 (provided by the Scientific Centre for Expert Evaluation of Medicinal Products of the Ministry of Health of the Russian Federation, Moscow).

Using PAM as a growth substrate. Bacteria's capability to use PAM as a sole growth substrate was studied both in a liquid and agar medium as well as on abiotic surfaces. In the first test, 100 µl of a suspension (10^6 cells/ml) of each culture and PAM in a concentration up to 0.075 % were added to penicillin vials containing 2 ml of a Nitrogen-free mineral salts medium (N) with the following composition (g/l): KH_2PO_4 – 1.0; $K_2HPO_4 \times 3H_2O$ – 1.6; NaCl – 0.5; $MgSO_4 \times 7H_2O$ – 0.5, $CaCl_2$ – 0.005; $FeSO_4 \times 7H_2O$ – 0.01; $CoCl_2 \times 6H_2O$ – 0.01 (pH 7.2 ± 0.2). A variant with the N medium without PAM was used as a negative control; a variant with Lysogeny broth (LB) was used as a positive control. The cultures were then incubated under 37 ºC without mixing and in a shaker at a mixing speed equal to 120 turns per minute for 7 days. A film of each PAM type was created out of 1 ml on an agar medium N (the 2nd variant); it was then dried and after that a bacterial suspension (100 µl, 10^6 cells/ml) was deposited on its surface with a spatula. N-agar without PAM was used as a negative control and LB-agar was used as a positive control. Glass and plastic Petri dishes were used as abiotic surfaces; a PAM-film of each type was created on their bottoms out of 1 ml of a solution and a bacterial suspension was deposited as described above. A surface without preliminary treatment with PAM was used a control.

A capability to use PAM as a source of carbon or nitrogen was investigated in immunological flat bottom plates. To do that, we added ammonia chloride to wells with 200 µl of a nitrogen-free mineral medium N until its ultimate concentration reached 5 mM, or glucose up to the ultimate concentration 0.1 % as well as various PAM up to a concentration equal to 0.075 % as a source of carbon or nitrogen accordingly. Then, 10 µl of cell suspensions (10^6 cells/ml) of each bacterial culture were inoculated in the wells. The wells that were added only with PAM were used as control (PAM as a sole nutrition source, similar to the first experiment); the wells without PAM were used as a negative control and the wells with LB-medium were used as a positive control. The plates were incubated under 37 ºC without mixing and in a shaker at a mixing speed equal to 120 turns per minute for 7 days. Bacterial growth was estimated as per optical density (OD) of a cell suspension measured with automated microplate PowerWave X spectrophotometer (Biotech, USA) at λ = 600 nm.

We assessed effects produced by inorganic salts and compositions on bacteria by using agar diffusion tests and twofold serial cultivations in the microplates [13, 14].

Assessment of antimicrobial effects produced by solutions and compositions on a solid medium (disk diffusion test and gel diffusion test). Bacterial cultures were grown in a liquid nutrient LB-medium for 18–24 hours and then standardized to 10^6 cells/ml. The prepared bacterial suspension was inoculated as an 'unbroken' lawn on LB-agar in Petri dishes. Sterile paper disks (d = 6 mm) were placed on the agar surface and saturated with the above listed solutions and compositions (10 µl); also, droplets with the same volume were deposited without a disk. Next, the inoculations were cultivated under 37 °C for 24 hours and antimicrobial effects were then estimated as per a diameter of a zone where the bacterial growth was inhibited (taken in mm).

Assessment of antimicrobial effects produced by solutions and compositions in a liquid medium. Bacterial cultures were grown in the same way as in the previously described test. Bacteriostatic (MIC or minimum inhibitory concentration) and bactericidal (MBC or minimum bactericidal concentration) effects produced by inorganic salts with added PAM and without them were investigated in wells of a polystyrene immunological plate as per the conventional procedure. It involved estimating OD_{600} of the cultures and their growth after inoculation on LB agar from wells without any apparent bacterial growth. Concentrations of inorganic salt solutions in a monovariant and in compositions varied in a range between 0.01 and 5 %.

Statistics. All the experiments were performed not less than threefold, simple means and standard deviations were calculated. Va-

lidity of differences between the samples was estimated as per *t*-test (differences were considered statistically authentic at $p \leq 0.05$). The data were analyzed with standard software packages, Microsoft Office XP Excel and STATISTICA 10.0.

Results. ***Bacteria's capability to use PAM as a growth substrate.*** All the analyzed reference bacterial cultures did not grow in either test variant (absence of any visible growth) during 7-day incubation in a liquid medium N with added PAM (Table 1). This indicates that the analyzed microorganisms did not use PAM as a sole nutrition source. Nevertheless, bacterial cells preserved their viability in PAM under the given conditions up to the 7th day in the experiment. Consequently, colony formation occurred in inoculation on LB (the data are not presented here). Cultures were established to grow on PAM-films created on the agar medium N but only on the 7th day of exposure. We did not detect any visible growth on PAM-films created on glass or plastic. Moreover, gram-negative bacteria cells did not preserve their viability under the given conditions, as opposed to *S. aureus* (excluding PAM Praestol 2530).

Table 1

Growth and viability* of bacteria on PAM as a sole nutrition source in different model systems

A test variant	Strain			
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
PAM Praestol 806				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass*	-	-	-	33 CFU/dish
plastic*	-	-	-	7 CFU/dish
PAM Praestol 857				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass	-	-	-	12 CFU/dish
plastic	-	-	-	No calculation
PAM Praestol 2510				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass	-	-	-	3 CFU/dish
plastic	-	-	-	54 CFU/dish
PAM Praestol 2530				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass	-	-	-	-
plastic	-	-	-	-
Controls (no PAM added)				
liquid medium N	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass **	+/-	+/-	+/-	+/+
plastic **	-/-	-/-	+/-	+/-

Note: The Table provides the results of the 3rd/7th day in the experiment: "-" means absence of visible growth, "+" is visible growth; * is one-day exposure, viability on LBA identified after a swab off a surface; ** is 1/7 day exposure, viability on LBA identified after a swab off a surface.

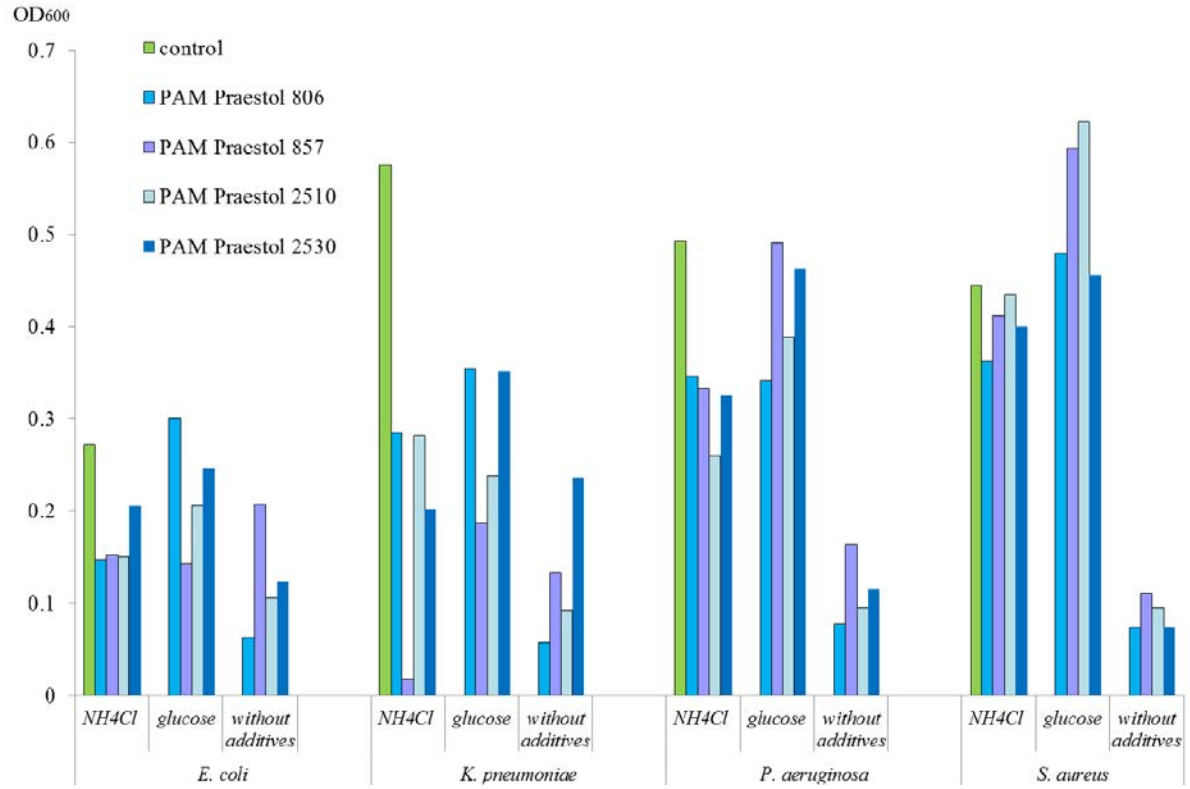

Figure 1. Bacteria growth on PAM as a sole substrate of nutrition, as a sole carbon or sole nitrogen source (without aeration)

Bacteria's capability to use various PAM as a source of carbon or nitrogen nutrition had to be investigated profoundly. To do that, we cultivated the analyzed test-cultures in wells of a polystyrene plate in a liquid medium N with PAM and 0.1-% glucose as a source of carbon or with 5 mM ammonia chloride as a source of nitrogen. A control test variant contained glucose or ammonia without any PAM added. Similar to the previous tests, bacteria growth was not detected in any test variant in a liquid mineral medium with PAM without any additional nutrition sources (Figure 1). Bacteria grew on a medium with PAM and glucose more often; in a smaller number of cases, growth was also detected on a medium with added ammonia chloride. This indicates that the analyzed acryl polymers can be used either solely as sources of nitrogen or sources of carbon. Table 2 provides the results obtained by statistical analysis of the test data.

Regardless of where PAM are used, either in healthcare or agriculture, these polymers can come in contact with various microorganisms and be exposed to biodegradation. Most studies on PAM degradation providing evidence that this polymer could be used by bacteria have been accomplished either in soil or with soil bacterial strains. It has been established that bacteria can use polyacrylamide as a source of energy or nitrogen nutrition due to their amidase activity despite its resistance to microbial degradation [15–19]. Very few studies mention possible microbial PAM degradation without any additional sources of nutrition / energy [20, 21]. Shanker with colleagues (1990) reported that when ammonia sulfate was added as an additional source of nutrition, this enhanced bacteria capabilities to degrade acrylamide [22]. The process was established to develop even more actively in case glucose was added. PAM-degrading bacteria are assumed to largely hydrolyze amide groups in the side chain of the polymer and to a lesser extent affect the main carbon chain [11]. It is noteworthy that microbial amidases (for example, N-acyl-L-amino-acid amidohydrolase [EC 3.5.1.4]) deaminate aliphatic amides down to their carbon acids and ammonia and this reaction is substrate-specific. Amidase production is considered a species

Table 2

Bacteria growth on PAM as a sole substrate or a source of carbon / nitrogen nutrition

A test variant		Absorbance, units (λ = 600)											
		A	*E. coli*		**B**	*K. pneumoniae*		**C**	*P. aeruginosa*		**D**	*S. aureus*	
PAM Praestol 806													
N with aeration	NH_4Cl	1	0.201 ± 0.011	$p^{3,19,25,C,D}$	1	0.248 ± 0.116	$p^{25,D}$	1	0.364 ± 0.026	$p^{3,7,25,A}$	1	0.439 ± 0.045	$p^{3,A,B}$
	glucose	2	0.429 ± 0.201	p^{3}	2	0.439 ± 0.286		2	0.262 ± 0.130	p^{25}	2	0.375 ± 0.065	p^{3}
	without additives	3	0.054 ± 0.017	$p^{1,2,15,25,C}$	3	0.052 ± 0.026	p^{21}	3	0.091 ± 0.002	$p^{1,15,25,A}$	3	0.088 ± 0.005	p–1,2
N without aeration	NH_4Cl	4	0.147 ± 0.056	$p^{5,6,26,D}$	4	0.285 ± 0.236	p^{26}	4	0.346 ± 0.213		4	0.362 ± 0.016	$p^{5,6,16,22,A}$
	glucose	5	0.300 ± 0.096	$p^{4,6,11,26,D}$	5	0.355 ± 0.266		5	0.341 ± 0.247		5	0.480 ± 0.017	$p^{4,6,11,17,26,A}$
	without additives	6	0.063 ± 0.025	$p^{4,5,12,24,26}$	6	0.058 ± 0.029	$p^{18,24,26}$	6	0.078 ± 0.001	$p^{12,26,D}$	6	0.074 ± 0.001	$p^{4,5,18,26,C}$
PAM Praestol 857													
N with aeration	NH_4Cl	7	0.173 ± 0.042	$p^{9,25,D}$	7	0.109 ± .062	$p^{25,C,D}$	7	0.227 ± 0.049	$p^{1,9,25,B,D}$	7	0.379 ± 0.059	$p^{9,A,B,C}$
	glucose	8	0.296 ± 0.226	p^{25}	8	0.246 ± 0.131	p^{25}	8	0.261 ± 0.167		8	0.394 ± 0.027	p^{9}
	without additives	9	0.082 ± 0.025	$p^{7,12,25}$	9	0.063 ± 0.002	$p^{12,25,C,D}$	9	0.093 ± 0.005	$p^{7,12,25,B}$	9	0.092 ± 0.016	$p^{7,8,12,25,B}$
N without aeration	NH_4Cl	10	0.152 ± 0.032	$p^{26,D}$	10	0.0178 ± 0.072	$p^{26,D}$	10	0.333 ± 0.180		10	0.412 ± 0.036	$p^{11,12,A,B}$
	glucose	11	0.143 ± 0.016	$p^{5,26,D}$	11	0.187 ± 0.060	$p^{26,D}$	11	0.491 ± 0.211		11	0.593 ± 0.020	$p^{5,10,26,A,B}$
	without additives	12	0.207 ± 0.055	$p^{6,9,26,D}$	12	0.133 ± 0.063	$p^{9,26}$	12	0.164 ± 0.034	$p^{6,9,26,D}$	12	0.110 ± 0.026	$p^{9,10,11,26,A,C}$
PAM Praestol 2510													
N with aeration	NH_4Cl	13	0.166 ± 0.064	$p^{25,D}$	13	0.144 ± 0.082	$p^{25,C,D}$	13	0.251 ± 0.091	$p^{25,B}$	13	0.381 ± 0.076	$p^{15,A,B}$
	glucose	14	0.453 ± 0.203	$p^{15,17}$	14	0.578 ± 0.213	$p^{15,17,25}$	14	0.275 ± 0.109	$p^{17,25,D}$	14	0.486 ± 0.037	$p^{15,17,25,C}$
	without additives	15	0.099 ± 0.006	$p^{3,14,25}$	15	0.093 ± 0.007	$p^{14,25,C}$	15	0.103 ± 0.006	$p^{3,25,B,D}$	15	0.097 ± 0.007	$p^{13,14,25,C}$
N without aeration	NH_4Cl	16	0.150 ± 0.029	$p^{26,B,D}$	16	0.282 ± 0.025	$p^{18,25,A,D}$	16	0.260 ± 0.203	p^{26}	16	0.435 ± 0.017	$p^{4,26,A,B}$
	glucose	17	0.206 ± 0.077	$p^{14,26,D}$	17	0.238 ± 0.071	$p^{14,18,26,D}$	17	0.389 ± 0.255	p^{14}	17	0.622 ± 0.078	$p^{5,14,16,18,26,A,B}$
	without additives	18	0.106 ± 0.037	p^{26}	18	0.093 ± 0.037	$p^{6,16,17,26}$	18	0.095 ± 0.014	p^{26}	18	0.095 ± 0.006	$p^{6,16,17,26}$
PAM Praestol 2530													
N with aeration	NH_4Cl	19	0.124 ± 0.034	$p^{1,25,D}$	19	0.211 ± 0.094	$p^{25,D}$	19	0.228 ± 0.114	p^{25}	19	0.380 ± 0.028	$p^{21,A,B}$
	glucose	20	0.310 ± 0.265		20	0.374 ± 0.225		20	0.340 ± 0.135		20	0.392 ± 0.023	p^{21}
	without additives	21	0.100 ± 0.041	p^{25}	21	0.104 ± 0.003	$p^{3,25}$	21	0.172 ± 0.061	p^{25}	21	0.108 ± 0.007	$p^{19,20,25}$
N without aeration	NH_4Cl	22	0.206 ± 0.135	p^{26}	22	0.202 ± 0.016	$p^{24,26,C,D}$	22	0.326 ± 0.023	$p^{24,26,B,D}$	22	0.400 ± 0.008	$p^{4,23,24,B,C}$
	glucose	23	0.247 ± 0.166	p^{26}	23	0.352 ± 0.097		23	0.463 ± 0.138	p^{24}	23	0.456 ± 0.016	$p^{22,24}$
	without additives	24	0.123 ± 0.011	$p^{6,26,B,D}$	24	0.236 ± 0.018	$p^{6,22,26,A,C,D}$	24	0.116 ± 0.021	$p^{6,22,23,26,B,D}$	24	0.075 ± 0.001	$p^{22,23,26,A,B,C}$
Controls													
N with aeration	glucose, NH_4Cl	25	0.391 ± 0.008	$p^{1,3,7,8,9,13,15,19,21}$	25	0.462 ± 0.012	$p^{1,3,7,8,9,13,15,19,21}$	25	0.452 ± 0.015	$p^{1,3,7,9,13,14,15,19,21,D}$	25	0.411 ± 0.051	$p^{9,14,15,21,D}$
N without aeration	glucose, NH_4Cl	26	0.272 ± 0.027	$p^{4,5,6,10,11,12,16,17,18,22,23,24,B,C,D}$	26	0.576 ± 0.032	$p^{4,6,10,11,12,16,17,18,22,24,A,C,D}$	26	0.493 ± 0.087	$p^{6,12,18,22,24,A,B,D}$	26	0.445 ± 0.034	$p^{5,6,11,12,16,17,18,24,A,B,C}$

Note: p^{n} means the indicator is authentically different from the variant n (*t*-test).

character of pseudomonades and acetamide is included into selective media for identifying and controlling purity of *P. aeruginosa* culture in clinical practice as well as in environmental studies in the USA (APHA (1995) Standard Methods for the Examination of Water and Wastewater, Washington, DC). There are other bacteria types among amidase producers including those colonizing the human body such as *E. coli*, *K. pneumoniae*, *S. aureus*, *Enterococcus faecalis* and *Helicobacter pylori* [23–25]. However, substrate specificity of their amidases and a possibility to enter a reaction with acrylamide and PAM has not been confirmed since in most cases these bacteria produce only enzymes able to hydrolyze the amide bond between the residue of N-Acetylmuramic acid

in the glycan chain and L-alanine in the peptide segment of peptidoglycan of the cell wall, for example, N-acetylmuramoyl-L-alanine amidase [24]. Though some of them are constitutive, synthesis of *P. aeruginosa* amidase is induced by amides through a regulatory protein coded by *amiR* whereas *amiC* negatively regulates expression of this enzyme [26]. The cellular mechanism of amidase induction by polyacrylamides is not clear since the polymer is too large to be able to penetrate bacterial cells and act as a direct inducer. We can assume that the more intensive growth in test variants with PAM and additional sources of carbon / nitrogen are associated with carbon / nitrogen being released from the side-chain amide (cation PAM) and carboxyl groups (anion PAM). It is interesting that PAM were offered by H.H. Tuson with colleagues (2012) as an alternative to agar-agar in studies that address bacterial growth on solid nutrient media. Thus, most gram-negative (*E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella enterica* serovar Typhimurium and *Serratia marcescens* ATCC) and gram-positive (*B. subtilis*, *S. epidermidis*) bacteria strains grew on PAM-gels so-polymerized with acrylic acid but absorbance of the cultures (OD_{595}) did not exceed 0.3 a.u. [27]. So, the experiment data indicated that clinically significant bacteria did not use PAM as a sole nutrition source and their growth was also rather slow in a medium with other substrates added to it. All this made it possible to move on to the next stage in our research, namely, to assessing antimicrobial effects produced by water solutions of PAMs with Zn^{2+} and Cu^{2+} cations.

Assessment of antimicrobial effects produced by investigeted composition by disk diffusion test and gel diffusion test. We established antibacterial effects produced on the analyzed test-cultures by 5%-solutions of Cu and Zn sulfates combined with water solutions of PAM Praestol 857 and PAM Praestol 2530. As expected, polyacrylamide solutions did not have any influence on bacteria whereas solutions of Cu and Zn sulfate inhibited growth of all the analyzed strains. More apparent effects were identified for 5%-solution of $ZnSO_4$ (*t*-test; $p = 0.019$) (Figures 2 and 3).

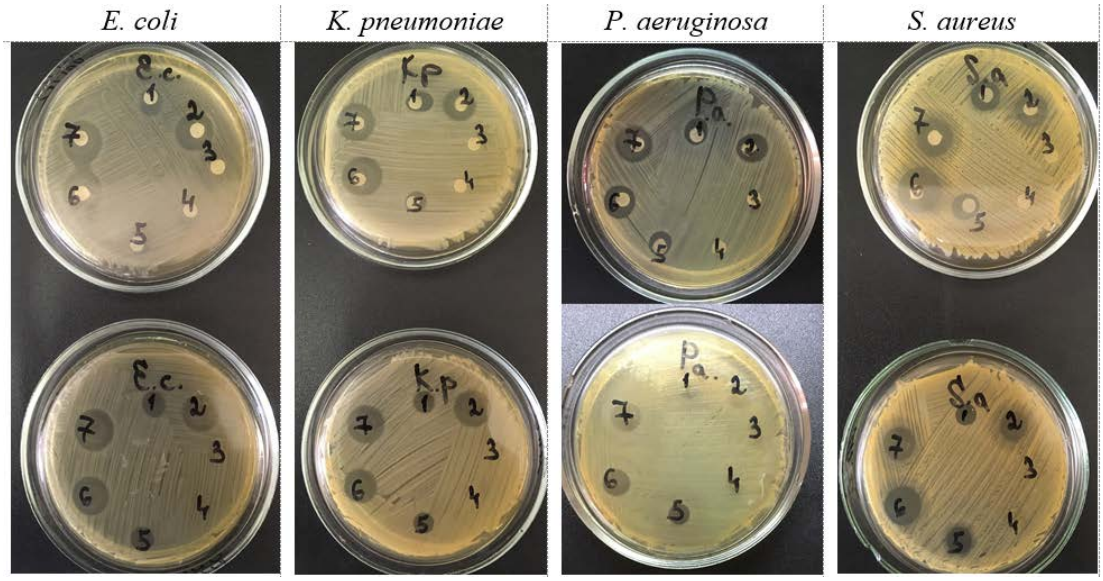

Figure 2. Dishes with the results obtained by a test aimed at identifying antibacterial effects produced by the analyzed solution with disk diffusion test (top) and with droplet deposition (bottom): 1 is 5%-solution of $CuSO_4$; 2 is 5%-solution of $ZnSO_4$; 3 is 0.075%-PAM Praestol 857; 4 is 0.075%-PAM Praestol 2530; 5 is $CuSO_4$ + PAM Praestol 857; 6 is $ZnSO_4$ + PAM Praestol 857; 7 is $ZnSO_4$ + PAM Praestol 2530

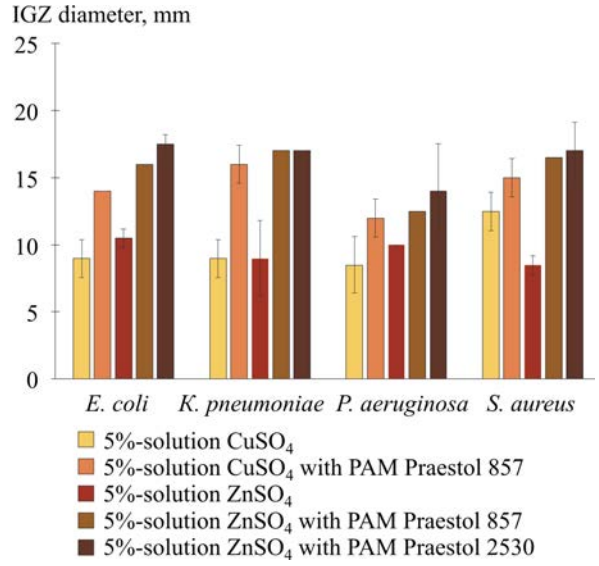

Figure 3. Antimicrobial effects produced by solutions of Cu and Zn salts combined with PAM and without them (IGZ is a diameter of zones with inhibited growth, droplet test)

It is noteworthy that adding polymers to $ZnSO_4$ solution led to an authentic increase in a zone with inhibited bacterial growth (*t*-test; $p = 0.030$ and $p = 0.025$ for PAM Praestol 857 and PAM Praestol 2530 accordingly). Obviously, an increase in a zone with inhibited bacterial growth in the test variants 'inorganic salt + PAM' is associated with a wider area on agar surface being covered with a water composition PAM and cations for both types of deposition. We should note that an antibacterial effect produced by $ZnSO_4$ combined with PAM Praestol 2530 was a bit more apparent than for a combination with PAM Praestol 857 although the difference was not statistically significant.

Assessment of antimicrobial effects in a liquid medium. The next task was to estimate influence exerted by polymers on antibacterial effects of salts that were established to become more intensive in an agar medium. To do that, we identified MICs and MBCs of inorganic salt solutions and four our test-compositions in a liquid nutrient medium (Table 3). As expected, salts of Zn and Cu inhibited growth and viability of all the analyzed microorganisms; in most cases, both MIC and MBC of zinc sulfate were by 1–2 dilutions lower than those of copper sulfate. MICs of metal salts did not exceed 0.16 % in all the test variants. Bacteria viability was mostly inhibited under a concentration of salts being per 1 or 2 dilutions (for *P. aeruginosa*) higher than MIC. We should also highlight apparent bactericidal effects (MBC/MIC = 1) of copper sulfate with respect to *E. coli* and *K. pneumoniae*. PAM addition to the analyzed medium mostly did not have any influence on antibacterial effects produced by salts. We detected a growth in MIC and MBC for some cultures but only in the test variant with added $ZnSO_4$. As for a relationship between antibacterial effects and a type of a polymer under these conditions, MIC and MBC were higher when PAM Praestol 2530 was added to the analyzed medium. In some cases, induction of antibacterial effects was identified for copper salts.

Table 3

MICs and MBCs of $CuSO_4$ and $ZnSO_4$ solutions and their compositions with PAM as regards the analyzed strains

A test variant	Strain							
	E. coli		*K. pneumoniae*		*P. aeruginosa*		*S. aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
$CuSO_4$	0.08	0.16	0.16	0.16	0.16	0.31	0.08	0.16
$ZnSO_4$	0.02	0.04	0.04	0.08	0.08	0.31	0.02	0.08
$CuSO_4$, PAM* Praestol 857	0.08	0.16	0.08	0.16	0.16	0.63	0.08	0.16
$CuSO_4$, PAM Praestol 2530	0.08	0.16	0.08	0.16	0.16	0.63	0.08	0.16
$ZnSO_4$, PAM Praestol 857	0.02	0.08	0.08	0.16	0.08	0.31	0.02	0.04
$ZnSO_4$, PAM Praestol 2530	0.04	0.16	0.04	0.16	0.08	0.31	0.04	0.31

Note: PAM were added in all test variants in a concentration equal to 0.075 %. The Table provides the results of four experiments with similar indicators out of total six.

Microorganisms are known to be sensitive to effects produced by salts of heavy metals. Therefore, the latter are commonly used to treat some communicable human and animal diseases. Inhibition of bacterial growth with metal ions is associated with various metabolic processes in prokaryotic cells including impaired protein functions, production of reactive oxygen species and antioxidant depletion as well as damage to membranes and genotoxicity [28]. Toxicity of different inorganic salts has different mechanisms and hazard levels for representatives of different taxons. Thus, A. Singh with colleagues (2015) showed *E. coli*, *S. aureus* and *K. pneumoniae* to be highly sensitive to zinc salts; the first two cultures were more sensitive than *K. pneumoniae*, and in addition Zn sulfate in a concentration equal to 10 mM completely inhibited the growth of all three strains [13]. These data are in line with observations by D. Chudobova and others (2015) [29] and our research as well: $ZnSO_4$ MICs did not exceed 0.08 % for all the analyzed strains (the figure corresponds to 5 mM). Most studies in the area show that Cu salts are less deleterious for bacteria than Zn salts even when similarly tolerant strains are described in them. Thus, S.B. Cheknev with colleagues (2015) established that inhibiting effects produced by zinc sulfate on S. *aureus* was by 1.3–1.6 times stronger than effects of copper sulfate whereas these antibacterial effects produced by both sulfates were quite similar as regards *P. aeruginosa* culture [7]. Also, similar results were described for staphylococci in the studies by H. Xue with colleagues (2015) where it was reported that MICs of Zn and Cu salts differed by two times for strains isolated from animals and equaled 2 and 4 mM accordingly [30].

Many researchers share the opinion that thorough cleaning and disinfection of surfaces in the hospital environment allow considerable reduction in risks of infection; they are significant elements in effective prevention. However, traditional disinfection in in-patient hospitals is not always optimal and requires improvement including new treatment procedures that make it possible to achieve longer biocide effects [31]. A promising approach is use of film disinfectants, which are compositions of traditional antibacterial substances and a polymer providing longer persistence of a biocide on a surface. In our tests, PAM addition to a medium did not have any substantial influence on bacteriostatic or bactericidal effects produced by metal salts (some decline in them fixed in isolated cases can be due to inaccuracies when dilutions were being prepared in a dense PAM medium) and when PAM were deposited on an agar surface, it led to an authentic increase in a zone with inhibited bacterial growth. Bearing these two findings in mind, we can conclude that the outlined strategy seems promising.

Conclusion. Providing up-to-date and effective sanitary and anti-epidemiological measures in healthcare organizations is an extremely vital task in health protection. A true challenge in the sphere is to develop and test new disinfectants with prolonged effects produced on infectious agents. No wonder that this research trend, namely Medicine and Technologies of Live Systems, Creation of New Drugs, Biomedical Technologies for Life Support and Human Health Protection, is mentioned in the Order by the Head of the Perm Regional Administration issued on November 01, 2010 No. 83 ‘On major trends in the research and technical policy in the Perm region’.

We detected antibacterial effects produced by water solutions of PAMs combined with Cu and Zn sulfates as regards reference strains of the most common infectious agents causing healthcare-associated infections. All the bacterial cultures did not use PAM as a sole nutrition source when growing in a liquid mineral medium and on PAM-films created on glass and plastic. PAM and products of their biotransformation are highly toxic and this might be a reason for the situation outlined above; also, another reason might be that the analyzed clinically significant bacterial strains do not have any mechanism for utilization of high-molecular substances. In most cases, the analyzed cultures could grow on these substrates only if glucose was added to them. We established that 5%-solution of $ZnSO_4$ had

more apparent inhibitory effects on microorganisms cultivated on solid and liquid nutrient media. Adding PAM Praestol 857 and PAM Praestol 2530 to solutions of investigated inorganic salts led to an authentic increase in a diameter of a zone with inhibited bacterial growth on an agar medium. This effect might be due to a greater area of a surface being covered with water solutions of PAMs combined with Cu and Zn sulfates; therefore, the analyzed compositions seem quite promising as regards their use for disinfection. In a liquid medium, salts of both metals inhibited growth and viability of all the analyzed microorganisms already in a concentration equal to 0.16 % or lower. Adding PAM Praestol 2530 to a medium to a certain extent weakened antibacterial effects produced by inorganic salts whereas PAM Praestol 857 had practically no influence on their bacteriostatic or bactericidal effects.

Therefore, use of the obtained composite solutions where $CuSO_4$ or $ZnSO_4$, immobilized on a PAM matrix act as an antibacterial component seems a promising way to disinfect objects in the hospital environment. This can significantly reduce risks of hospital-acquired infections. Further research will focus on investigating effectiveness of these created disinfecting compositions as regards clinically significant bacteria types. Various abiotic surfaces will be used in future studies to imitate similar surfaces of medical equipment, furniture etc. in order to identify whether these disinfecting compositions can reduce risks of healthcare-associated infections.

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