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ORIGINAL ARTICLE

GLUCOPROTAMIN ANTIMICROBIAL ACTIVITY AGAINST SELECTED STANDARD ANTIBIOTIC-RESISTANT BACTERIA AND REFERENCE STRAINS USED IN THE ASSESSMENT OF DISINFECTION EFFICACY

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ABSTRACT

Background. The ability of bacteria to develop common mechanisms of resistance to antibiotics and disinfectants raises doubts about the effectiveness of disinfection processes. Glucoprotamin (GP) is an antimicrobial active substance which is widely used to the disinfection in medical area.

Objective. The aim of study was to compare GP's effectiveness with susceptibility of reference strains used for the evaluation of bactericidal efficacy of disinfectants *Staphylococcus aureus (S. aureus)*; *Pseudomonas aeruginosa (P. aeruginosa)* and standard antibiotic-resistant strains: meticillin-resistant *S. aureus* (MRSA) and tetracycline-resistant *P. aeruginosa* (PAO-LAC).

Materials and Methods. Minimum inhibitory concentrations (MICs) of GP and minimum bactericidal concentrations (MBCs) against tested strains were evaluated by serial broth dilution technique. GP's efficiency was examined according to qualitative (phenol coefficient GP-PC) and quantitative (EN 1040: 2006) test methods.

Results. Gram-negative strains were more tolerant to GP than Gram-positive strains among tested strains. MRSA and *S. aureus* exhibited similar susceptibility to GP. PAO-LAC had significantly lower susceptibility to GP than *P. aeruginosa* ($P \leq 0,05$). There were no differences in GP efficiency against these strains based on GP-PC. According to PN-EN 1040: 2006 standard average obligatory reduction $\geq 5 \log_{10,}$ was demonstrated in the active concentration of GP (84 mg/l) at obligatory 5 min contact time for PAO-LAC and *P. aeruginosa*. The differences in basis bactericidal activity between PAO-LAC and *P. aeruginosa* were obtained in the active concentration at 10 and 15 min contact time ($P \leq 0,05$).

Conclusions. Variation in a susceptibility of reference strains and antibiotic-resistant standard strains has no meaning at used clinically GP concentrations, which are higher than concentration causing basis bactericidal activity of GP.

Key words: *glucoprotamin, disinfection efficacy, antibiotic-resistant standard strains*

STRESZCZENIE

Wprowadzenie. Zdolność bakterii do rozwijania wspólnych mechanizmów oporności na antybiotyki i preparaty dezynfekcyjne wywołuje wątpliwości dotyczące skuteczności procesów dezynfekcji. Glukoprotamina (GP) jest substancją aktywną szeroko stosowaną do dezynfekcji w obszarze medycznym.

Cel. Porównanie skuteczności działania glukoprotaminy wobec szczepów referencyjnych stosowanych w ocenie skuteczności bakteriobójczej preparatów dezynfekcyjnych *Staphylococcus aureus (S. aureus)*; *Pseudomonas aeruginosa (P. aeruginosa)* i wobec szczepów antybiotykoopornych: metycylinoopornego szczepu *S. aureus* (MRSA) i tetracyklinoopornego szczepu *P. aeruginosa* (PAO-LAC).

Materiały i metody. Minimalne stężenia hamujące (MICs) i minimalne stężenia bójcze (MBCs) GP były oszacowane wobec badanych szczepów z zastosowaniem metody seryjnych rozcieńczeń w bulionie. Skuteczność GP była badana wg metod jakościowych (współczynnik fenolowy (GP-PC) i ilościowych (EN 1040: 2006).

Wyniki. Badane szczepy Gram-ujemne były bardziej tolerancyjne na GP niż szczepy Gram-dodatnie. MRSA i *S. aureus* wykazywały podobną wrażliwość na GP. PAO-LAC wykazywał znacząco niższą wrażliwość na GP niż *P. aeruginosa* ($P \leq 0,05$). Nie stwierdzono różnic w skuteczności GP wobec badanych szczepów na podstawie GP-PC. Wg normy PN-EN 1040: 2006, średnia wymagana redukcja ($\log_{10}$) ≥ 5 była uzyskana przy aktywnym stężeniu GP (84 mg/l) w obligatoryjnym czasie kontaktu 5 min dla PAO-LAC i *P. aeruginosa*. Różnice w podstawowej bakteriobójczej aktywności PAO-LAC i *P. aeruginosa* stwierdzono w stężeniu aktywnym, w czasach kontaktu 10 i 15 min ($P \leq 0,05$).

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Wnioski. Zmiany wrażliwości szczepów referencyjnych i antybiotykoopornych nie mają znaczenia przy zastosowaniu stężeń użytkowych GP, które są wyższe niż stężenia odpowiedzialne za podstawowe działanie bakteriobójcze GP.

Słowa kluczowe: *glukoprotamina, szczepy antybiotykooporne, dezynfekcja*

INTRODUCTION

The widespread use of antibiotics and antimicrobial compounds causes the occurrence of antibiotic-resistant bacteria which are common source of nosocomial infections. In contrast to the antibiotic-sensitive bacteria, bacteria resistant to antibiotics are associated with infections which are more invasive, with higher mortality rate, increasing length of hospitalization and medical costs. Significantly higher mortality occurred among patients with MRSA infections than those infected by meticillin-susceptible *S. aureus* (MSSA) [19]. The cost of the hospitalization of patients with MRSA bacteraemia was higher because of complications like acute kidney injury occurring during MRSA treatment [6]. *Jefferies* et al. published a systematic review of *P. aeruginosa* infections and colonization outbreaks in neonatal intensive care units in the last decade. In some cases, the source of infections was *P. aeruginosa,* which was resistant to antibiotics or associated with biofilm formation. As in the case of MRSA infections, *P. aeruginosa* infections were also expensive and difficult to treat and eradicate from hospital environment [5].

Disinfection is a basic activity to prevent the spread of pathogenic microorganisms in hospital environment. Disinfection of medical equipment and solid surfaces, which come into direct contact with patients and hospital staff is especially important. Biocidal efficacy of disinfectants is depended on the potency of the active substance, its concentration and contact time. Sustaining these parameters as well as the proper use of disinfectants (as recommended by manufacturer) is extremely important to stop the spread of pathogens. A significant factor in evaluating the effectiveness of disinfectant is intrinsic resistance of bacteria [9]. It is connected with the structure of bacterial cell envelope and its permeability. Changes in composition of proteins, fatty acids and phospholipids in the cell wall, especially in the outer membrane of Gram-negative bacteria, reduce biocidal efficacy. *Walsh* et al. observed that the didecyldimethylammonium chloride-resistant *P. aeruginosa* mutant had a reduced number of one outer membrane protein in comparison with the standard strain [18]. The presence of efflux pumps is another intrinsic resistance mechanism, which decreases intracellular concentrations of toxic compounds including biocides and antibiotics. This mechanism is regarded as giving bacteria common resistance to antibiotics and disinfectants and it also raises doubts whether disinfectants can effectively deactivate these types of bacteria. However, efflux mechanisms were not observed in lethal activity of biocides [9, 15].

Glucoprotamin is one of the most frequently used active substances, that has found its application mainly to the disinfection of surgical instruments, endoscopes and surfaces. This is associated with a broad spectrum of glucoprotamin activity. Glucoprotamin is active against vegetative bacteria including mycobacteria, as well as bacterial spores, fungi and viruses. This active substance is an alternative to aldehydes and phenols because of its greater activity, being non-corrosive and compatible with most materials used in hospitals [17, 20].

Glucoprotamin is a product of a chemical reaction of L-glutamic acid and coco($C_{12/14}$)alkyl-propylene-1,3--diamine. In contrast to aldehydes, chemical structure of glucoprotamin does not impede the removal of protein contamination from surgical instruments surfaces. Clinical trials concerning disinfection of surgical instruments confirmed the effectiveness of glucoprotamin against bacteria before removing protein impurities [20].

Glucoprotamin, as an active substance, found its application in production of biocides such as Sekusept Plus® and Incidin Plus®. These biocides contain 25% and 26% of glucoprotamin, respectively. The biocidal efficiency of these products meets the European requirements for biocidal activity of chemical disinfectant and antiseptic products. Sekusept Plus® demonstrates bacteriocidal, sporocidal, fungicidal and virucidal activity and Incidin Plus® has bactericidal and fungicidal activity [17]. Both products are effective against mycobacteria [11]. These disinfectants are also very effective against bacterial and fungal clinical isolates. Bacterial clinical isolates which had a different susceptibility to antibiotics and chemotherapeutics were sensitive to the low concentration of glucoprotamin-containing disinfectants (0,5%) at short contact time (1 min) [4].

A small amount of published results about the effectiveness of active substance against antibiotic-resistant standard strains raises the question whether the study of disinfectants' efficiency should also be carried out with the use of antibiotic-resistant standard strains. Biocidal efficacy studies performed according to European Standards are made on the reference strains. Although these standards allow use additional test microorganisms, a small amount of disinfectant efficiency research were carried out on antibiotic-resistant standard strains. Test methods of the biocidal efficacy contained in European Standards are meant to determine the "in-use" disinfec-

tant concentration. However, differences in the activity of active substances contained in disinfectants against microorganisms with varied sensitivity to antibiotics can not be assessed by application "in-use" concentrations.

The aim of this study was to present bactericidal properties of glucoprotamin as an active substance against selected Gram-positive and Gram-negative bacterial strains intended for evaluation of the bactericidal efficacy of disinfectant products (*S. aureus*; *P. aeruginosa*) and against antibiotic-resistant strains (MRSA; tetracycline-resistant PAO-LAC). These strains were chosen as standard strains to compare glucoprotamin activity against bacteria with defined antibiotic resistance and strains without evidence of antibiotic resistance.

MATERIALS AND METHODS

Test organisms

All test organisms were obtained from ATCC collection. These strains were maintained on beads in microbank vials and stored at -70ºC. Stock cultures were being recovered from beads every month. The stock cultures of *S. aureus* ATCC 6538; *S. aureus* ATCC 43300 (MRSA); *P. aeruginosa* ATCC 15442 were prepared on Tryptone Soya Agar (TSA; BD Difco) slants and *P. aeruginosa* ATCC 47085 (PAO-LAC) were cultured on LB Agar Miller (A&A Biotechnology) + 10 µg/ml tetracycline slants. Working cultures were prepared from stock cultures by performing two successive subcultures, passaged every 24 h. All strains were incubated at 37°C except PAO-LAC, which was incubated at 30°C. Second subcultures were used in experiments.

Active substance

The 50% concentrate of Glucoprotamin® tested against reference and antibiotic-resistant standard strains was provided by Ecolab GmbH, Düsseldorf, Germany.

Methods

Glucoprotamin minimum inhibitory concentrations (GP-MIC), minimum bactericidal concentration (GP--MBC) were determined against *S. aureus* ATCC 6538; *S. aureus* MRSA ATCC 43300; *P. aeruginosa* ATCC 15442 and *P. aeruginosa* PAO-LAC ATCC 47085.

*Determination of GP-MIC value. A*ssessment was performed using the broth dilution technique as described in method for determination of the bacteriostatic and/or fungistatic activity of chemical disinfectants; National Institute of Public Health - National Institute of Hygiene - PZH DF 07/03: 2003 [7]. Serial dilutions of GP for *S. aureus* and MRSA (2.5-8 mg/l) and for *P. aeruginosa* and PAO-LAC (8-60 mg/l) with final volume of 10 ml were made in growth medium (TSB - BD Difco or LB Broth- Miller + 10 µl/ml tetracycline - A&A Biotechnology, according to the test organisms) in 16×160 mm test tubes. Bacterial suspension was prepared from the second passage and added to each test tube to achieve final inoculum of 10^6 cfu/ml. Tubes with bacterial suspension were agitated and then incubated at 37°C for 48 h. Positive and negative controls were prepared with growth medium with and without bacterial cultures, respectively. Turbidity indicates bacterial growth. The absence of bacterial growth was interpreted as inhibitory activity of GP in given concentration. Each tested concentration was performed in triplicate.

Determination of GP-MBC value. The MBC was determined by sample taken from each test tube, where there was no growth in the MIC assay. The loopful (10 µl) of test sample was transferred to TSB (BD Difco) or LB Brtoth Miller (A&A Biotechnology) with 10 µl/ml tetracycline without GP and incubated for 72 h at 37°C. Concentrations of GP, in which the growth was not observed, act as a bactericide. Tested concentrations ranged from 4.5 mg/l to 25 mg/l for *S. aureus* and MRSA and from 17 mg/l to 75 mg/l for *P. aeruginosa* and PAO-LAC.

Assessment of the GP disinfectant efficacy

Disinfectant efficacy of GP was demonstrated by its phenol coefficient (PC) value determination for all test microorganisms [12]. Additionally, for Gram-negative bacteria (*P. aeruginosa* and PAO-LAC) bactericidal activity of GP was examined according to EN 1040: 2006 method.

PC method. A 2% (w/v) phenol solution was used. Dilutions in sterile distilled water were made from 2% (w/v) phenol and 0.05 % GP (w/v) stock solutions. Subsequently 0.5 ml of test cultures was added to test tubes containing 5 ml of each of the final dilutions of phenol or GP. The test culture was added at 30 s intervals. After 5, 10 and 15 min, one loopful (10 µl) was transferred to subculture medium (TSB or LB Broth Miller + 10 µg/ml tetracycline) without disinfectant. Subcultures tubes were incubated for 3 days at 37°C. Control tubes of cultures were prepared to identify the growth of bacteria. Control tubes with the highest concentrations of phenol and GP were prepared to eliminate results arising from medium turbidity caused by these substances. PC value was the highest dilution of GP that killed test organisms in 10 min, divided by the greatest dilution of phenol showing the same results. Results of PC were expressed as mean value and confidence interval.

EN 1040:2006. 1 ml of water was mixed with 1 ml of bacterial cells suspensions of density 1.5-5×10^8 cfu/ml and with 8 ml of prepared dilutions. The mixture was incubated for 5, 10, 15 min at room temperature. After these periods of time 1 ml of each sample was transfer-

Table 1. Mean number of cells counted per 1 ml of *P. aeruginosa* and PAO-LAC suspensions in different control test mixtures. A, B, C are equal to Nv_0/or greater than 0.5×Nv_0. Glucoprotamin concentration – 0.016%.

Strain	Validation suspension (Nv_0)	Experimental conditions control (A)	Neutralizer control (B)	Method validation (C)
P. aeruginosa	216	116	154	155
PAO-LAC	96	145	158	132

red to neutralizer (3 g/l lecithin, 30 g/l Tween 80.1 g/l L-histidine, 30 g/l saponin in diluent), mixed thoroughly and left at room temperature for 5 min to neutralize the activity of the GP. Then, 1 ml of each sample was inoculated using pour plate technique. Petri dishes were incubated at 37ºC (*P. aeruginosa*) and at 30ºC (PAO-LAC). After 5, 10 and 15 min of contact times surviving cells were enumerated and $\log_{10}$ reduction calculated from the initial populations. Validation of the selected experimental conditions and/ or verification of the absence of any lethal effect in the test condition (A), verification of the absence of toxicity of the neutralizer (B), and dilution-neutralization validation (C) were performed according to validations procedures of EN 1040: 2006. As shown in Table I results of validations meet the assumptions of EN 1040: 2006 standard. Mean number of colonies obtained in A, B and C validations was equal or greater than 0.5× the average number of colonies of validation suspension (Nv_0) for both examined strains (Table I). The presence of lethal effect in the test condition (A), toxicity of the neutralizer (B) and the effect of residual concentration of glucoprotamin (C) on validation suspension (Nv_0) were not noted [2].

Statistical analysis

Average and standard deviation of MIC and MBC values and results of bactericidal activity obtained by PC and EN 1040: 2006 method were analyzed using confidence intervals (CI). CI was determined with two-sided confidence limit and 95% confidence level. 95% confidence level indicates that tested mean value is found within the CI at the significance level of 0.05.

RESULTS

Differences in susceptibility of antibiotic-resistant standard strains and reference strains used in evaluation of bactericidal efficacy of disinfectant to GP

The lowest concentrations that inhibited growth of strains *S. aureus* and MRSA were slightly different. The GP-MIC value was lower for MRSA strain than for *S. aureus* strain. Minimum GP concentrations were 4.0±0.8 mg/l for MRSA and 5.0±0.5 mg/l for *S. aureus.* The concentration of GP lower than 3 mg/l did not inhibit the growth of MRSA and the concentration lower than 4 mg/l did not inhibit *S. aureus*. However, concentrations equal and higher than 6 mg/l inhibited both strains. These results were not significantly different (P>0,05) and indicate that both examined strains had similar susceptibility to GP. Significant differences in GP inhibitory effect were found between a pair of strains *P. aeruginosa* and PAO-LAC (P≤0.05). The lowest concentration of GP, that inhibited growth of *P. aeruginosa*, was 21±3.0 mg/l. The minimum concentration of GP for PAO-LAC, which inhibited growth, was 30±2.6 mg/l. Confidence interval of minimum inhibitory concentrations of GP for PAO-LAC was shifted towards higher values than the confidence inte-

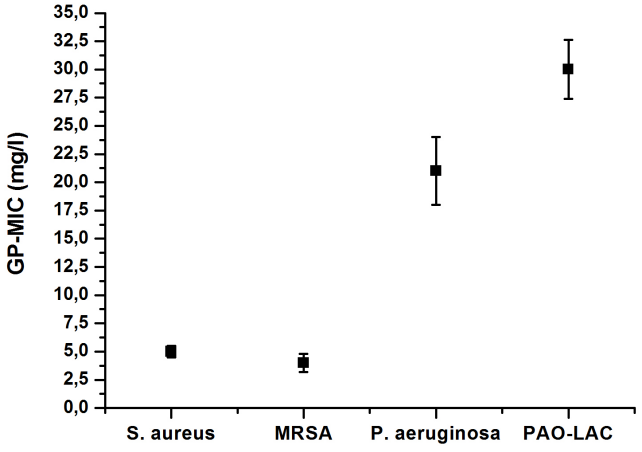

Figure 1. Minimum inhibitory concentrations of antibiotic resistant standard strains (MRSA; PAO-LAC) and strains used in evaluating the effectiveness of disinfectants (*S. aureus*; *P. aeruginosa*). Mean value and confidence interval.

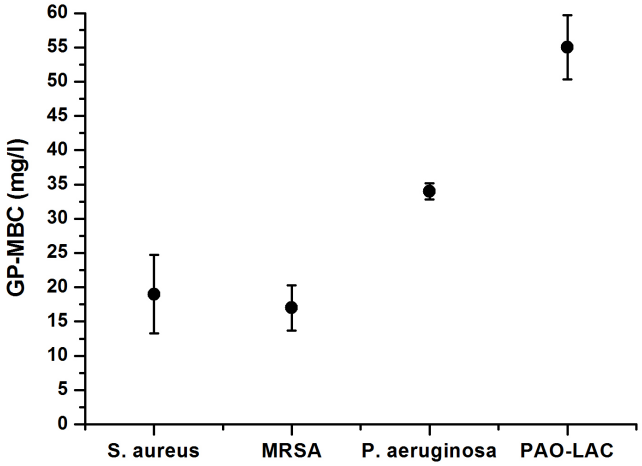

Figure 2. Minimum bactericidal concentrations of antibiotic resistant standard strains (MRSA; PAO-LAC) and strains used in evaluating the effectiveness of disinfectants (*S. aureus*; *P. aeruginosa*). Mean value and confidence interval.

rval obtained for *P. aeruginosa*. The range of minimum values of confidence interval were one and a half times (1.5×) higher for PAO-LAC than for *P. aeruginosa* (Figure 1). These results indicate that GP have a lower inhibitory activity against PAO-LAC than *P. aeruginosa* and PAO-LAC has significant lower susceptibility to GP than *P. aeruginosa*.

The MBCs were higher than the MICs for all tested bacteria. As shown in Figure 2 minimum bactericidal concentration of GP was higher against Gram-negative than Gram-positive bacteria. Mean values of minimum bactericidal concentration of GP were similar for *S. aureus* (19±5.7 mg/l) and MRSA strain (17±3.3 mg/l). A different result of minimum bactericidal concentration of GP was received for *P. aeruginosa* (34±1.2 mg/l) and PAO-LAC (55±4.7 mg/l). A minimum bactericidal concentration of GP was significantly lower for *P. aeruginosa* than for PAO-LAC ($P \leq 0.05$). The strain of PAO-LAC exhibited higher level of GP resistance compared to *P. aeruginosa.*

Glucoprotamin bactericidal efficacy

Glucoprotamin bactericidal efficacy was evaluated based on glucoprotamin phenol coefficient (GP-PC). Average GP-PC values and confidence intervals were determined for *S. aureus* and MRSA strains, which were 4.13±1.46 (n=6) and 4.76±1.22 (n=6), respectively. These results were not significantly different ($P>0.05$). *S. aureus* and MRSA had similar susceptibility to GP. The GP possesses good disinfecting efficiency against Gram-positive bacteria with and without antibiotic resistance.

Average GP-PC value determined for *P. aeruginosa* was 4.29±1.12 (n=7) and 4.16±1.15 (n=7) for PAO-LAC. The efficiency of GP was also similar against these two strains. These results were not significantly different ($P>0.05$).

The efficiency of GP was slightly higher for Gram-positive (4.44±0.81) than Gram-negative bacteria (4.22±0.68). GP possessed similar efficiency against reference *S. aureus* and *P. aeruginosa* strains and was also slightly more efficient to MRSA than to PAO-LAC. Results obtained for these strains were not significantly different ($P>0.05$). GP efficiency was comparable for antibiotic-resistant standard strains and reference strains used in evaluation of bactericidal activity of disinfectants.

In view of the significant differences in the resistance of *P. aeruginosa* and PAO-LAC to GP, bactericidal efficacy of GP against these strains has been defined according to EN 1040: 2006. In contradictions to the results obtained in PC methods antimicrobial efficiency of GP to *P. aeruginosa* and PAO-LAC was variable. Two concentrations of GP were chosen to check bactericidal efficiency of GP, one in the active range – 84 mg/l and one in the non-active range – 42 mg/l. As shown in Figure 3 the required mean reduction ($R_{log10} \geq 5$) for both *P. aeruginosa* and PAO-LAC strains was obtained in active concentration and at obligatory contact time of 5 min. There were no significant differences of GP activity against examined strains ($P>0.05$). The efficiency of GP was significantly higher against *P. aeruginosa* than PAO-LAC in 10 and 15 min contact time. CI of reduction obtained for PAO-LAC range from 4.38 to 5.66 in 10 min contact time and from 4.66 to 5.51 in 15 min contact time. Mean reduction and CI obtained for *P. aeruginosa* were constant with increasing contact time and amounted 5.75±0.02. Efficiency of GP against *P. aeruginosa* fluctuated in a very narrow range.

Efficiency of GP in non active concentration was similar for both tested strains in 5 min contact time and

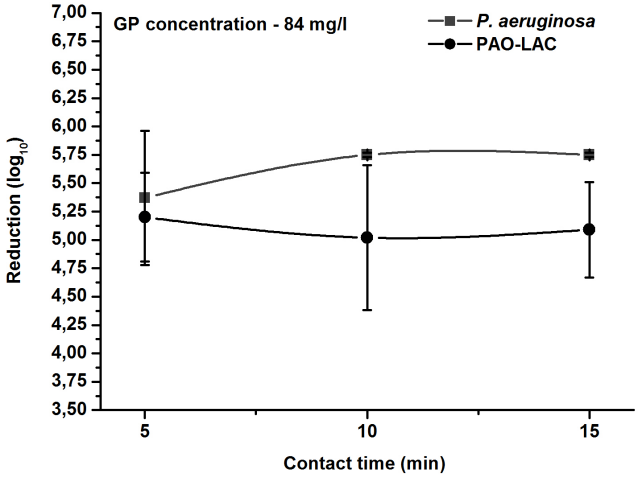

Figure 3. Reduction of *P. aeruginosa* and PAO-LAC in active concentration of glucoprotamin (GP) according to EN 1040: 2006. Mean value and confidence interval.

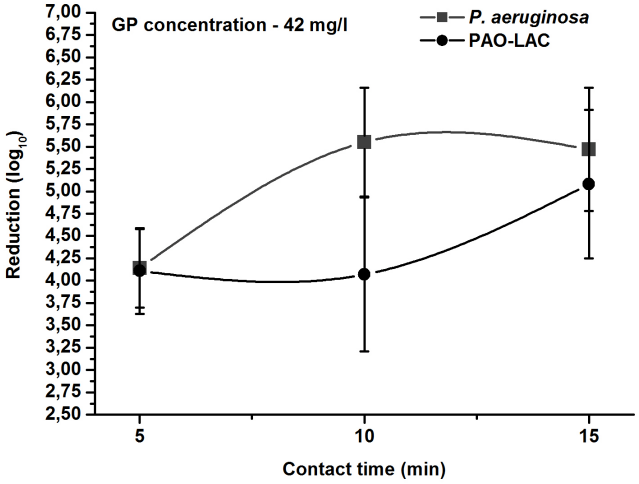

Figure 4. Reduction of *P. aeruginosa* and PAO-LAC in non-active concentration of glucoprotamin (GP) according to EN 1040: 2006. Mean value and confidence interval.

amounted 4.14±0.44 for *P. aeruginosa* and 4.11±0.48 for PAO-LAC. Significant difference was found only at 10 min contact time. Mean reduction obtained for *P. aeruginosa* was higher than 5 $\log_{10}$ (5.55±0.61), while for PAO-LAC still remained in non active range (4.07±0.86). Mean reduction of *P. aeruginosa* (5.47±0.69) and PAO-LAC (5.08±0.83) was not significant different in 15 min contact time (Fig. 4).

These results confirmed that GP efficiency determined by this method did not show significant differences in reduction of *P. aeruginosa* and PAO-LAC in 5 min contact time at active and non active concentrations. Significant differences in efficiency of examined strains were found in active concentration of GP in 10 and 15 min contact time and in these parameters GP possessed lower activity to PAO-LAC than to *P. aeruginosa*.

DISCUSSION

The study concerning differences of glucoprotamin activity against bacterial antibiotic-resistant standard strains and reference strains used in evaluating the effectiveness of disinfectants was conducted by two different groups of methods. Susceptibility of bacterial strains was assessed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration of glucoprotamin (MBC). Bactericidal activity of GP was evaluated by studying the lethal effect of this active substance on bacteria according to qualitative (PC) and quantitative (EN 1040: 2006) test methods of disinfectants' bactericidal efficacy. Susceptibility of MRSA and *S. aureus* to GP were comparable and higher than for PAO-LAC and *P. aeruginosa*. Among tested strains Gram-negative bacteria are more tolerant to glucoprotamin than Gram-positive bacteria. The term "tolerant" has been proposed in the case of the inhibitory effect of disinfectants' active substances due to the fact that strains of bacteria surviving in inhibitory concentrations were still killed at "in use" concentrations [9]. Obtained results indicate differences between the GP bactericidal activity against antibiotic-resistant standard strain PAO-LAC and *P. aeruginosa* reference strain used in determination of the effectiveness of disinfectant preparations. On the other hand, these results are not suitable to demonstrate higher PAO-LAC resistance at "in use" concentrations. However, these results are important, because they may indicate a trend of the same resistance properties of examined antibiotic-resistant strains, especially Gram-negative bacteria on GP. Furthermore, common mechanisms of resistance to disinfectants and antibiotics have been described for Gram-negative bacteria. Resistance of Gram-negative bacteria, especially antibiotic-resistant strains, could be the result of expression of the efflux pump system belonging to Resistance-Nodulation-Cell Division (RND) family**.** *Chuanchuen* et al. (2001) described that the antibiotic-resistant strain PAO 200 with RND efflux system was exposed to antiseptic substance triclosan, and triclosan-resistant mutants were obtained [1]. PAO-LAC is a tetracycline-resistant strain. Resistance of this strain to tetracycline is caused by an efflux pump consisting of Tet-protein [8]. According to own study, tested strains *P. aeruginosa* and PAO-LAC showed varying resistance to tetracycline. The strain PAO-LAC was more resistant to tetracycline (MIC >150 μg/ml) than *P. aeruginosa* (MIC 50 μg/ml). This fact, and higher bactericidal concentration of GP against PAO-LAC than concentration determined against *P. aeruginosa* suggested that an efflux could be a common mechanism, which determined tetracycline and glucoprotamin resistance of PAO-LAC. However, in the context of the methods for testing efficacy of disinfectants, the resistance of PAO-LAC to both tetracycline and to glucoprotamin is irrelevant. Bactericidal concentrations determined by these methods are much higher than those prescribed as the minimum bactericidal concentration. Results of reduction obtained using EN 1040: 2006 standard, confirmed that GP activity is lower for PAO-LAC and *P. aeruginosa*, especially in the active concentration at 10 and 15 contact time (Figures 3 and 4). However, this standard is the first step in determining the effectiveness of disinfectants called as phase 1 and requirements for disinfectant formulations are higher than in this phase. According to them, the concentration of an active substance is determined as effective under a given load of interfering substances and in the case when bacteria are attached to the surface. These standards are included in phase 2, stage 2 [3, 14]. In the light of this knowledge, the MIC/ MBC, PC and EN 1040: 2006 results obtained for GP can not be treated as the "in use" concentration. However, these results provide valuable information about tolerance or resistance of examined strains to GP. GP is an active substance that show bactericidal activity at low concentrations against standard antibiotic-resistant bacteria and reference strains, which are used in evaluating the effectiveness of disinfectant products. The lowest active bactericidal concentration of the glucoprotamin (84 mg/l) was obtained for *P. aeruginosa* and PAO-LAC at 5 min contact time. These test strains showed that in order to ensure effective disinfection, the "in use" concentration of the GP should not be lower than 84 mg/l. Taking into account all of the obtained results, PAO-LAC has the best chance to survive below this concentration. The antimicrobial efficiency of disinfectants containing glucoprotamin was confirmed by *Tyski* et al. who analyzed antibiotic-resistant clinical bacterial strains [17]. Parameters of disinfection determined for this strains, in test method EN 1040: 2006 were lower (0.5%; 1 min) than parameters recommen-

ded by manufacturer (2%; 5 min) [4, 17]. Concentrations of disinfectant formulations containing glucoprotamin recommended by manufacturer designated on the basis of requirements for disinfectant formulations (phase 2, stage 2) are four times higher than obtained by *Tyski* et al. and seems to be sufficient to ensure effective disinfection of antibiotic-resistant standard strains [17].

The efficiency of glucoprotamin as an active substance was also confirmed for bacterial pathogens isolated from access-restricted hematologic transplant unit. Antibiotic-resistant strains were not isolated in this area, but glucoprotamin-containing disinfectant demonstrated similar efficiency against environmental strains like aldehyde-containing product [10]. However, determination of relevant parameters, with using obligatory reference strains should provide effective disinfection of antibiotic-resistant strains in the medical area.

Results of our research on the susceptibility of antibiotic-resistant standard strains and strains used in evaluating the effectiveness of disinfectants to GP seems to be important especially in conditions, in which parameters of disinfections will be reduced. Excessive dilution of the disinfectant, shortened the length of exposure, significant pollution of the organic matter or the presence of biofilms are factors that can reduce the effective concentration of disinfectants [13, 16]. This can lead to selection pressure and to survival of less susceptible strains in the population. Reduced susceptibility of strains to active substances of disinfectants may also have a greater significance in the case when the active substances used in disinfection have low potency of action [15].

In light of this knowledge the introduction of strains with increased resistance to antibiotics, as additional strains to test the effectiveness of disinfectants would constitute good laboratory practice.

CONCLUSIONS

1. There were no differences in susceptibility between strain used in evaluation of antibacterial activity of disinfectants *S. aureus* ATCC 6538 and meticillin-resistant *S. aureus* ATCC 43300 (MRSA) to glucoprotamin.
2. Differences in susceptibility between *P. aeruginosa* ATCC 15442 and tetracycline-resistant *P. aeruginosa* ATCC 47085 (PAO-LAC) to glucoprotamin were not significant for basis bactericidal activity of this active substance.

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Conflict of interest

The authors declare no conflict of interest.

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