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Antimicrobial Activity of Glucoprotamin-Containing Disinfectants

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Abstract

Introduction of a new antimicrobial agent as a drug – for treatment of infections or as a disinfectant and antiseptic, may result in the occurrence of resistance mechanisms against this agent among microorganisms. Two disinfectants of different composition – Incidin Plus for surface disinfection and Sekusept Plus for medical devices disinfection, both containing glucoprotamin as the active substance, were investigated in this study in order to analyze their antimicrobial activity. Standard bacterial and fungal strains recommended by European Standards, established by European Standardization Committee for testing bactericidal and fungicidal activity of chemical disinfectants were used in the study. Furthermore, 60 clinical bacterial strains with different susceptibility to antibiotics and chemotherapeutics, mostly multiresistant, isolated from different specimens from hospitalized patients were analyzed. In addition, 184 fungal clinical strains isolated from hospitalized patients and outpatients were also included in this study. Antimicrobial activity was evaluated according to EN 1040:2005 – using bacterial strains and according to EN 1275:2005 – using fungal strains. Glucoprotamin proved to be a very effective and rapidly acting bactericidal and fungicidal agent. Low concentration of glucoprotamin – 0.5% showed to be very effective (1 min) against clinical bacterial isolates. Incidin Plus was also very effective (5 min) against clinical fungal isolates.

Key words: antimicrobial activity, disinfectant, EN 1040, EN 1275, glucoprotamin

Introduction

Antimicrobial agents for rapid disinfection are very important factors in eradication of hospital infections (Coia *et al.*, 2006; Cookson *et al.*, 2005). Wide antimicrobial spectrum and efficacy complying with European standards are attributes of modern preparation. Alcohols, aldehydes, phenols, biguanides, quaternary ammonium compounds, peroxygens and halogen-releasing agents have been the active substances in disinfectant preparations for years (McDonnell and Russell, 1999). The long time application of known preparations promoted selection of strains with lower sensitivity to active substances and promoted acquiring of resistance mechanisms. Additionally, the transfer of mobile genetic fragments of microorganisms carrying resistance genes resulted in wide distribution of resistance mechanisms among microorganisms. The relevance of these resistance mechanisms for the practical use of disinfectants has, however, been questioned (Meyer, 2006). Glucoprotamin was discovered in the early 1990's (Disch, 1992; Disch, 1994; Von Rheinbaben and Meyer, 2008). It is a multi-component substance, reaction product of L-glutamic acid and N-(alkyl) propylene-1,3-diamines obtained from natural coconut oil. It consists of a mixture of reaction educts and products /N-(C_{12-14}-alkyl)propylenediamines and amides/, showing synergistic activity. This complex substance of wax-like consistency is an active component of some disinfectant agents: Incidin Plus, Incidin Foam, Sekusept Plus, and preparations for mechanical washing and disinfection: Dekaseptol Gel and Sekumatic FDR.

Two disinfectants of different compositions and areas of application – Incidin Plus for surface disinfection

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in health care areas and Sekusept Plus for medical devices disinfection, both containing glucoprotamin, 26% and 25% respectively, as active substance, were analyzed in this study. These preparations possess different content and compositions of supplementary substances – both: nonionic detergents, complexing agents, solvents, dyes, fragrances, besides Sekusept Plus – corrosion inhibitors and Incidin Plus – foam regulator. Because, glucoprotamin preparations have been applied in health care units for almost 15 years, it was interesting to investigate biocidal efficacy toward clinical multiresistant bacterial strains and fungal strains isolated from hospital patients and outpatients. The possibility of the detection of strains with significantly decreased sensitivity to this agent was also the focus of this investigation.

It is worth emphasizing that in this study the antimicrobial activity of glucoprotamin containing agents was tested by standardized assays towards not only collection strains, recommended by ENs, but also against a number of antibiotic-resistant clinical bacterial isolates as well as towards a large group of different clinical fungal isolates. These microorganisms may be transferred from patients to environment and contaminate the medical devices and surfaces. Efficient disinfection in this case is very crucial. It is important to stress that clinical multi drug resistant bacterial strains were isolated from materials from patients staying in the hospital wards, where Incidin Plus has been applied for surface disinfection for several years, so the question might arise, whether this disinfectant agent is effective against the analyzed bacterial strains.

Experimental

Materials and Methods

Bacterial strains. The following 4 standard bacterial strains, recommended by EN 1040:2005, and EN 13727:2003 were used: *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* NCTC 10538 and *Enterococcus hirae* ATCC 10541.

Moreover, sixty clinical strains, each from a separate patient, 10 isolates from each group of: *Enterobacter cloacae*, *Proteus mirabilis* + *Proteus vulgaris*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, methicillin resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* + *Enterococcus faecium*, with different susceptibility to antibiotics and chemotherapeutics, mostly multi drug resistant, isolated during routine clinical microbiological investigations from different specimens of hospitalized patients and identified by biochemical tests – API system (bioMérieux, Lyon, France) were included in the study (Table I).

In order to select multi drug resistant isolates, and include them in the glucoprotamin investigation, the antibiotic susceptibility of bacteria was tested using ATB system (bioMerieux, Lyon, France). Additional standard strains: *E. coli* ATCC 10538, *P. aeruginosa* ATCC 15442, *S. aureus* ATCC 6538, and *E. hirae* ATCC 10541, recommended by CLSI (CLSI, 2006), were used for the determination of MIC values.

Fungal strains. The following 2 standard fungal strains, recommended by EN 1275:2005, were used: *Candida albicans* ATCC 10231 (yeast cells) and *Aspergillus brasiliensis* (former *A. niger*) ATCC 16404 (mould spores). Additionally, 184 clinical strains were included in this study, as follows: *Candida* spp. (n = 133) – including *C. albicans* (65), *C. glabrata* (29), *C. tropicalis* (12), *C. parapsilopsis* (12), *C. krusei* (10), and other *Candida* (5); *Trichophyton* spp. (n = 24) – including *T. mentagrophytes* (18) and *T. rubrum* (6); *Aspergillus* spp. (n=7) – including *A. fumigatus* (4), *A. niger* (2), and *A. flavus* (1); *Microsporum canis* (5), *Geotrichum* spp. (3), *Cryptococcus* spp. (3), *Saccharomyces cerevisiae* (2), *Scopulariopsis brevicaulis* (2), and single strains of *Epidermophyton floccosum*, *Penicillium citrinum*, *Acremonium kiliense*, *Fusarium oxysporum*, and *Zygosaccharomyces* sp. Fungal strains were isolated from different clinical materials of hospitalized patients and outpatients: blood samples, sputum or aspirate samples, stool samples, wound swabs, vaginal swabs, throat or nasal swabs, drain swabs. Strains were identified by biochemical tests – API 20C AUX (bioMérieux, Lyon, France). Additionally, differentiation of *Candida* species was conducted by isolation of colonies on chromogenic medium: CHROM agar *Candida* (bioMérieux, Lyon, France).

Preparations. Commercially available Incidin Plus and Sekusept Plus preparations (both Ecolab GmbH, Düsseldorf, Germany), containing 26 g and 25 g of glucoprotamin in 100 g of preparations respectively, were investigated. According to manufacturer's recommendation they should be diluted with water and used in working concentrations for defined contact times: Incidin Plus – 2–3% for 15–60 min and Sekusept Plus – 1–4% for 15–60 min.

During this study, in order to compare biocidal activity, both preparations were diluted with water to obtain 2% and 0.5% solutions – these values correspond to 0.5% and 0.125% of pure glucoprotamin concentrations, respectively. Incidin Plus was analyzed toward all above indicated microbial strains, while Sekusept Plus evaluated later, when strong biocidal activity of glucoprotamin was already known, was investigated with the application of 4 standard and 30 clinical bacterial strains.

Besides, preparation Glucoprotamin 50 (Cognis Deutschland GmbH & Co. KG, Düsseldorf, Germany), containing 50% w/w solution of the pure active

Table I
Characteristic of bacterial strains analyzed in this study

Strain (number)	Origin of strain	Resistant to* – ATB system applied
S. aureus (1), (2), (4), (10)	nose swabs	OXA
S. aureus (3)	tongue swab	OXA, GEN, ERY, CLI, TET, NOR, LVX, TSU
S. aureus (5)	swab from fistula	OXA, GEN, ERY, CLI, TET, RFA, NOR
S. aureus (6)	wound swab	OXA, ERY, CLI, TET, NOR
S. aureus (7)	wound swab	OXA, GEN, ERY, CLI, TET, MIN, NOR, LVX, FUC, TSU
E. faecium (1)	bile	PEN, AMP, ERY, RFA, CIP, LVX, FUR, GEN
E. faecium (2)	tracheal aspirate	PEN, AMP, ERY, TET, RFA, CIP, LVX, STR, GEN
E. faecalis (3), (4)	urine	TET, CIP, NOR, STR
E. faecalis (5)	urine	TET, STR, GEN
E. faecalis (6)	tracheal aspirate	PEN, AMP, ERY, TET, CMP, RFA, CIP, LVX, QDA, STR, GEN
E. faecium (7)	bile	PEN, AMP, ERY, TET, RFA, CIP, LVX, FUR, STR
E. faecium (8)	abdominal cavity contents	PEN, AMP, ERY, RFA, CIP, LVX, FUR, STR, GEN
E. faecalis (9)	wound swab	ERY, TET, CMP, RFA, CIP, LVX, QDA, STR, GEN
E. faecalis (10)	urine	PEN, AMP, PIC, CIP, STR, GEN
A. baumanii (1)	tracheal aspirate	AMO, AMC, PIC, TIC, TZP, TCC, CTX, CAZ, CXT, CXM, GEN, AKN, CIP, TSU
A. baumanii (2)	intubation tube	AMO, AMC, PIC, TIC, TZP, TCC, CTX, CXT, FEP, CAZ, CXM, TOB, GEN, AKN, NET, CIP, TSU
A. baumanii (3)	external ear swab	AMO, AMC, PIC, TIC, TZP, TCC, CTX, FEP, CXT, CAZ, CXM, GEN, AKN, NET, CIP, TSU
A. baumanii (4) *A. baumanii* (5) *A. baumanii* (7)	wound swab sinus fluid tracheal aspirate	AMO, AMC, PIC, TIC, TZP, TCC, CTX, FEP, CXT, CAZ, CXM, GEN, AKN, CIP, TSU
A. baumanii (6)	tracheal aspirate	AMO, AMC, PIC, TIC, TZP, TCC, CTX, FEP, CXT, CAZ, CXM, TOB, GEN, AKN, NET, CIP, TSU
A. baumanii (8)	drain	AMO, AMC, PIC, TIC, TZP, TCC, CTX, FEP, CXT, CAZ, CXM, GEN, AKN, CIP, TSU
A. baumanii (9)	bile	AMO, AMC, PIC, TIC, TCC, MERO, CTX, FEP, CAZ, CXT, CXM, TOB, GEN, AKN, NET, CIP, NOR, TSU, FOS
A. baumanii (10)	urine	AMO, AMC, PIC, CTX, CXM, CXT, GEN, NET, CIP, NOR, NAL, FUR
E. cloacae (1)	urine	PIC, CTX, CAZ, CXM, TOB, GEN, AKN, NET, CIP, NOR, NAL, FUR, TSU
E. cloacae (2)	urine	PIC, TZP, TCC, CTX, CAZ, CXM, TOB, GEN, CIP, NAL, FUR
E. cloacae (3)	urine	PIC, CTX, CAZ, CXM, TOB, GEN, TSU
E. cloacae (4)	urine	PIC, CTX, CAZ, CXM, TOB, GEN, AKN, CIP, NET, NOR, NAL, TSU, FOS, FUR
E. cloacae (5)	sputum	PIC, CTX, CAZ, CXM, TSU, NOR, NAL, FUR, FOS
E. cloacae (6)	urine	PIC, CTX, CAZ, CXM, TOB, GEN, AKN, CIP, NET, NOR, NAL, TSU, FUR
E. cloacae (7), (8), (9)	urine	PIC, CTX, CAZ, FEP, CXM, TOB, GEN, AKN, NET, NOR, CIP, NAL, TSU, FOS, FUR
E. cloacae (10)	urine	PIC, CTX, CAZ, FEP, TOB, GEN, AKN, NET, CIP, NOR, NAL, TSU, FUR, FOS
P. aeruginosa (1)	urine	PIC, TIC, TZP, TCC, FEP, TOB, GEN, AKN, CIP, NET
P. aeruginosa (2)	ulceration	TOB, CIP, NET
P. aeruginosa (3)	wound swab	TOB, GEN, AKN
P. aeruginosa (4)	sputum	NET
P. aeruginosa (5)	urine	PIC, TIC, MERO, TZP, IMI, CAZ, TOB, GEN, AKN, NET, CIP
P. aeruginosa (6) *P. aeruginosa* (10)	Foley'a catheter wound swab	PIC, TIC, TZP, TCC, MERO, IMI, FEP, TOB, GEN, AKN, NET, CIP
P. aeruginosa (7)	urine	PIC, TIC, TZP, TCC, FEP, TOB, GEN, AKN, CIP
P. aeruginosa (8)	urine	PIC, TIC, CAZ, FEP, TOB, GEN, AKN, CIP
P. aeruginosa (9)	urine	PIC, TIC, MERO, TZP, CAZ, FEP, TOB, GEN, CIP
P. mirabilis (1)	urine	AMC, PIC, CFT, CXT, TOB, GEN, NET, CIP, NOR, NAL, TSU
P. vulgaris (2)	urine	AMC, PIC, CFT, CXT, CTX, CAZ, GEN, NET, CIP, NOR, NAL, TSU

Table I continued

Strain (number)	Origin of strain	Resistant to* – ATB system applied
P. mirabilis (3)	urine	PIC, CFT, CTX, NAL
P. vulgaris (4)	blood	TSU
P. mirabilis (6)	abscess	AMC, PIC, TIC, TCC, CXT, CFT, CTX, CAZ, TOB, GEN, AKN, NET, CIP, TSU
P. vulgaris (7)	preservative liquid	PIC, TIC, CFT, CTX, CXT, CAZ, FEP, TOB, GEN, AKN, NET, CIP, NOR, NAL, TSU, FOS
P. vulgaris (8)	urine	AMC, PIC, TIC, CFT, CTX, CXT, CAZ, GEN, NET, CIP, NOR, NAL, TSU, FOS
P. mirabilis (9)	urine	PIC, CFT, CTX, TOB, GEN, AKN, NET, CIP, NOR, NAL, TSU
P. mirabilis (10)	urine	AMC, PIC, CFT, CTX, CXT, CAZ, CIP, NAL , NOR

* AMO – amoxicillin, AMC – amoxicillin + clavulanate acid, AMP – ampicillin, OXA – oxacillin, PEN – penicillin, PIC – piperacillin, TZP – piperacillin + tazobactam, TIC – ticarcillin, TCC – ticarcillin + clavulanate acid, IMI – imipenem, MERO – meropenem, FEP – cefepime, CXT – cefoxitin, CAZ – ceftazidime, CXM – cefuroxime, CMP – chloramfenicol, ERY – erythromycin, AKN – amikacin; GEN – gentamicin, NET – netilmicin, TOB – tobramycin, STR – streptomycin, CIP – ciprofloxacin, NOR – norfloxacin, NAL – nalidixic acid, LVX – levofloxacin, FUR – nitrofurantoin, QDA – quinupristin-dalfopristin, RFA – rifamycin, TEC – teicoplanin, VAN – vancomycin, MIN – minocycline, TET – tetracycline, CLI – clindamycin TSU – co-trimoxazole, FOS – fosfomycin, FUC – fusidic acid.

glucoprotamin in diethylenglycol-monobutylether : water (20 : 30, w/w), was also applied in MIC value determination assay, in comparison with both commercial preparations – in order to detect the influence of additional substances, which might affect antimicrobial activity.

Bactericidal activity testing. Antibacterial activity of Incidin Plus and Sekusept Plus were evaluated using clinical isolates, according to EN 1040 : 2005. One ml of water was mixed with 1.0 ml of standardized bacterial cells suspensions of density 1.5––5.0×10^8 cfu/ml of all tested strains and 8.0 ml of preparations dilutions. Mixture was incubated for 1 and 5 min in 37°C. Then 1.0 ml of sample was placed in a tube containing 8.0 ml of neutralizer + 1.0 ml of water and placed in the water bath at 37°C. The following solution was effective in neutralization of glucoprotamin antimicrobial activity: lecithin 3 g/l, polysorbate 80 30 g/l, sodium thiosulphate 5 g/l, L-histidine 1 g/l, saponin 30 g/l in diluent (tryptone, pancreatic digest of casein 1.0 g/l, sodium chloride 8.5 g/l).

After neutralization time – 5 min, 1.0 ml sample was inoculated using pour plate technique. The test method was validated. Numbers of cells per ml in the test mixture at the beginning of the test and number of surviving cell after 1 and 5 min contact time with disinfecting agent were counted. Reduction of cells count in log scale was calculated. The antibacterial preparation fulfills EN criterion when reduction of 5 log bacteria cells count (cfu/ml) of analyzed strain, takes place.

Minimal inhibitory concentrations (MIC) of both preparations as well as of pure glucoprotamin, were estimated by agar dilution method towards 4 ATCC strains recommended by European Standards for antiseptics and disinfectants testing as well as selected 18 multiresistant clinical strains – 3 from each group of: *E. cloacae*, *P. mirabilis* + *P. vulgaris*, *A. baumanii*, *P. aeruginosa*, *E. faecium* + *E. faecalis* and MRSA.

Fungicidal activity testing. Antifungal activity of Incidin Plus after dilution was analyzed, according to EN 1275:2005. Incidin PLUS showed a very strong fungicidal efficacy at the use concentration recommended by the manufacturer. In order to differentiate the susceptibility of analyzed strains to the preparation, the lowest concentration of the product, which causes at least 4 log reduction of fungal cells counts during test conditions, was established toward 2 standard and 184 clinical fungal strains.

One ml of water was mixed with 1.0 ml of fungal cells suspensions of density 1.5–5.0×10^7 cfu/ml of tested strains and 8.0 ml of appropriate dilution of Incidin Plus. Mixture was incubated for 5 min in 20°C. Then 1.0 ml of sample was placed in a tube containing 8.0 ml of neutralizer (described above) +1.0 ml of water and placed in the water bath at 20°C. After 5 min of neutralization, 1.0 ml sample was inoculated using pour plate technique. The numbers of cells per ml in the test mixture at the beginning of the test and number of surviving fungal cells after 5 min contact time with disinfecting agent were counted. Reduction of cells count in log scale was calculated. The antifungal preparation fulfills EN criterion when reduction of 4 log fungal cells count (cfu/ml) of analyzed strain, takes place.

Results

When bactericidal activity of Incidin Plus and Sekusept Plus in both concentrations 0.5% and 2%, was evaluated, according to EN 1040:2005, it was shown, that bacterial cells of all isolates, regardless their antibiotic resistance pattern, were reduced over 5 log during only 1 min of contact time by 0.5% disinfectant. This means that the applied concentration 2% and contact time 5 min were higher than needed in this case.

In order to detect any interaction of additional substances present in commercial disinfectant, which may affect antimicrobial effect, MIC values of glucoprotamin contain in 3 preparations were determined. These values, vary from below 0.0039% to 0.0625% and depend on strains tested (4 standard and 18 different clinical isolates) but were exactly the same when 3 preparations were compared. It means that no interaction was observed and glucoprotamin activity in disinfectant preparations was the same as in compared reference solution.

When the fungicidal activity of Incidin Plus was tested, in order to perform comparative evaluation, analyzed strains were gathered into 3 groups, *Candida* spp. (n-133), *Trichophyton* spp. (n-24) and other fungal strains (n = 27), which included also 7 *Aspergillus* spp. isolates. Susceptibility of isolates was determined according to EN 1275:2005. The lowest glucoprotamin concentrations causing at least 4 log reduction of viable cells of analyzed *Candida* strains, ranges: 0.02% – 0.12% (Fig. 1). The values of lowest glucoprotamin concentration causing at least 4 log reduction of viable cells of 50% and 90% analyzed *Candida* strains, were 0.03% and 0.06%, respectively.

The lowest glucoprotamin concentrations causing at least 4 log reduction of viable cells of analyzed *Trichophyton* strains, ranges: 0.02–0.24% (Fig. 1) and the values of lowest glucoprotamin concentration causing at least 4 log reduction of viable cells of 50% and 90% analyzed *Trichophyton* strains was 0.06% in both cases.

The lowest glucoprotamin concentrations causing at least 4 log reduction of viable cells of analyzed other fungal strains, ranges: 0.005–0.24% (Fig. 1). The values of lowest glucoprotamin concentration causing at least 4 log reduction of viable fungal cells of 50% and 90% analyzed other fungal strains, were 0.02% and 0.12%, respectively.

Calculating values for the total group of 184 fungal strains, the lowest glucoprotamin concentrations causing required reduction of viable fungal cells of, varied from 0.005% to 0.24% and the values of lowest glucoprotamin concentration, which caused required reduction of fungal cells of 50% and 90% analyzed all fungal strains, were 0.03% and 0.12%, respectively.

Discussion

The wide-spread occurrence of different microorganisms, very often with high antibiotic and chemiotherapeutic resistance, especially among hospitalized patients as well as on the surfaces of medical devices and in health care facilities, requires specific antisepsis and disinfection procedures performed with the use of efficient antimicrobial preparations. This antimicrobial efficacy of chemical antiseptic and disinfection agents, toward different microorganisms: bacteria and fungi, including their spores and viruses is currently evaluated by appropriate normalized standards. European Committee of Standardization during the recent years, established standards (EN) dedicated to antimicrobial activity testing of antiseptics and disinfectants. Especially test methods of phase 1 for evaluation of basic biocidal activity should be applied for antimicrobial efficacy testing of active substances.

In this study we were interested in evaluating the basic antibacterial and antifungal activity of glucoprotamin containing preparations, not only to test strains

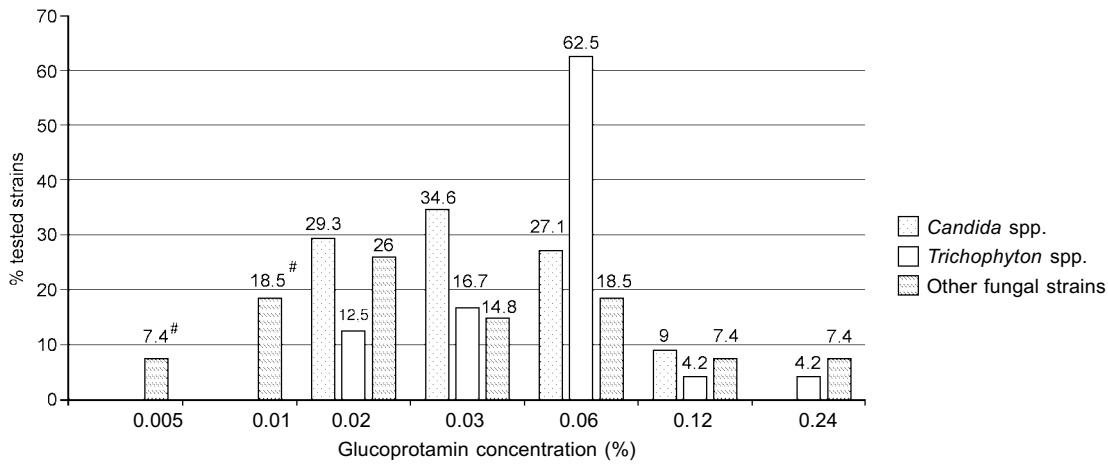

Fig. 1. Susceptibility of analyzed clinical fungal strains* to Incidin Plus (glucoprotamin 26%), evaluated according to EN 1275:2005 /5 min contact time/.

*Analyzed strains: *Candida* spp. (n = 133 = 100%): *C. albicans* (65), *C. glabrata* (29), *C. tropicalis* (12), *C. parapsilopsis* (12), *C. krusei* (10), and other *Candida* (5); *Trichophyton* spp. (n = 24 = 100%): *T. mentagrophytes* (18) and *T. rubrum* (6); other fungal strains (n = 27 = 100%): *Aspergillus* spp.*: A. fumigatus* (4), *A. niger* (2), and *A. flavus* (1); *Microsporum canis* (5), *Geotrichum* spp. (3), *Cryptococcus* spp (3), *Saccharomyces cerevisiae* (2), *Scopulariopsis brevicaulis* (2), and single strains of *Epidermophyton floccosum*, *Penicillium citrinum*, *Acremonium kiliense, Fusarium oxysporum*, and *Zygosaccharomyces* sp.
susceptibility of *Aspergillus* spp. isolates to glucoprotamin.

from culture collections, recommended by EN 1040 and EN 1275, but first of all to different clinical bacterial and fungal isolates, which might be less susceptible to glucoprotamin than standard strains. To keep the unified conditions of investigation the test methods described in these ENs were performed here.

Although glucoprotamin was discovered more than 15 years ago, there are only a few publications describing the activity of glucoprotamin-containing disinfectants against different microorganisms (Disch, 1992; Disch, 1994; Von Rheinbaben and Meyer, 2008; Widmer and Frei, 2003; Meyer and Kluin, 1999; Allen, 2004). In the first, published by Disch (1992, 1994), besides chemical-physical data of glucoprotamin, condensed microbiological information is presented, including values of minimal inhibitory concentrations of glucoprotamin and its activity after 60 min contact time, toward different genera of bacteria and fungi. A shorter time was not investigated. Differentiated activity, depending on pH value was noticed. Glucoprotamin at pH 9 showed 5–20 times higher antimicrobial activity than at pH 6.

A clinical study performed by Widmer and Frei (2003) proved excellent efficacy (cfu/instrument bacteria reduction over 5 log) of diluted Secusept Plus (1.5% glucoprotamin) after 1h contact, toward aerobic and anaerobic microorganisms contaminated instruments (specula and forceps). The instruments were not washed before the disinfection process and were soiled with secretions and blood. The average bioburden per instrument used in the hospital was between 10^5 and 10^7 cfu. The authors did not check the antimicrobial efficacy of more diluted Secusept Plus nor used shorter contact time. Mycobacteria are generally recognized as the bacterial forms most resistant to disinfectants except for bacterial spores. Meyer and Kluin (1999) using suspension method without organic load, documented significant antimycobacterial activity of 1% Sekusept Plus after 15 min and total mycobacteria destruction after 60 min.

In our study, bacterial and fungal isolates commonly existing in many hospitals were under investigation. Basic suspension methods were applied to investigate the susceptibility of different bacteria and fungi to glucoprotamin. Incidin Plus and Sekusept Plus proved to be very efficient antimicrobial agent for surface and medical devices disinfection, even in very low – 0.5% concentration. However, such low concentration is not recommended by manufacturer, because isolates with lower susceptibility to glucoprotamin may appear on disinfected objects. Also contact times: 1 min for bacteria and 5 min for fungi which were quite sufficient in this study, may be not long enough, in case of more resistant microorganisms, high load of organic contamination or presence of biofilm structure, so contact time supposed to be extended, even to 1 h – maximal time provided by disinfection standards.

Bacterial strains analyzed in this study, belonging to several species, significant from clinical points of view and showed different patterns of resistance to antibiotics and chemotherapeutics. However bactericidal effect of glucoprotamin was so strong and rapid, that all analyzed microorganisms regardless to their antimicrobial drug sensitivity patterns, were reduced over 5 log just after 1 min of contact with 0.5% disinfectant. Taking into consideration the so great power of cells destruction during recommended routine application, there was no reason to investigate the influence of multi-time exposure of microorganisms to extremely low glucoprotamin concentration, on bacteria susceptibility – the procedure frequently applied in case of antimicrobial drugs investigations. As can be observed in this study, the mode of glucoprotamin action, dealing with rapid and massive cell wall and membrane destruction, is not modified by different mechanisms of bacterial resistance. So, there was no interest to analyze the relationship between susceptibility of clinical isolates to antimicrobial drugs and susceptibility to glucoprotamin. Applying the glucoprotamin disinfectants according to manufacturers recommendation (longer contact time and higher concentrations) analyzed microorganisms had no chance to survive in as environment in which the cleaning and disinfection procedures were carried out properly. The investigated clinical strains were isolated from different clinical materials obtained from hospitalized patients, where Incidin Plus is being routinely used for hospital surfaces disinfection for a long time. It can be expected that some of these strains may contaminate the hospital environmental, so it was especially interesting, whether analyzed strains were susceptible to disinfectant preparation applied in the hospital. We have concentrated on survival of typical resistant clinical isolates, and we have not included in this study, the environmental strains form hospital surfaces and air, the population which is not stable and characteristic, but undergo fluctuations after performing of routine cleaning and disinfection procedures.

Analyzing the susceptibility of different fungal isolates to Incidin Plus, some variations were observed, however concentrations applied were much lower than recommended for routine application. Only single isolates of *Trichophyton mentagrophytes*, *Penicillium citrinum* and *Scopulariopsis brevicaulis* showed slightly lower susceptibility to glucoprotamin comparing to *Candida* spp. strains. On the contrary, clinical *Aspergillus* spp. isolates proved to be very susceptible to glucoprotamin.

To conclude, glucoprotamin is a very effective and rapidly acting antibacterial and antifungal agent. Low concentration of this agent – 0.50% in Incidin Plus

and Sekusept Plus proved to be very effective even after 1 min, against all analyzed clinical bacterial isolates. Also this concentration of glucoprotamin in Incidin Plus acted very effectively after 5 min of contact time against clinical fungal isolates. It can be expected, that proper application of glucoprotamin containing disinfectants sufficiently eliminate contaminating microorganisms from disinfected objects.

Some preliminary results were presented as posters at two conferences: 6th International Conference of the Hospital Infection Society, Amsterdam, 2006 (Tyski *et al.*, 2006) and 8th Congress of the International Federation of Infection Control, Budapest, 2007 (Tyski *et al.*, 2007).

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