Cetylpyridinium-chloride (CPC) and Miramistin (MST) compared to established antiseptics under protein challenge in-vitro - evaluating alternative agents for wound cleansing

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Research article

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Abstract

Background: Due to rising numbers microbial resistance to established antibiotics and first described tolerance developments for local wound antimicrobials a continuous need for alternative antimicrobial agents exists. Due to complex conditions in the microenvironment of especially chronic wounds, such as high protein levels, novel antimicrobials need to meet advanced requirements. Aim: Compare the antimicrobial efficacy of Cetylpiridinium-chloride (CPC) and miramistin (MST) to established antimicrobials under protein-challenge in-vitro. Methods: Antimicrobial activity of octenidin-dihydrochloride, povidon-iodine, polyhexamethylene-biguanide hydrochloride, chlorhexidine, cetylpiridinium-chloride and Miramistin after 0.5, 1, 3, 5 and 10 min of exposure against S. aureus, P. aeruginosa, E. coli, E. faecium and C. albicans was tested, using a quantitative suspension method with either 0.3% or 3% bovine albumin challenge, based on DIN EN 13727 ('Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)'). Results: CPC and MST demonstrated no inferiority to the established agents in-vitro. Especially CPC showed equal reduction rates as octenidin and povidon-iodine and achieved significantly higher reduction rates within shorter exposure times than polyhexanide and chlorhexidine ($p \leq 0.01$) for S. aureus, P. aeruginosa, E. faecium and C. albicans. Both agents demonstrated no significant loss of efficacy under high protein-challenge (3% albumin). Conclusion: In terms of antimicrobial activity cetylpyridinium-chloride and miramistin proved to be at least equally effective as established agents. No protein error was detected in the tested concentrations. More complex in-vitro assays and comprehensive in-vivo and clinical studies will be needed to determine their clinical value.

Background

Wound infection remains a major issue and challenge for health-care providers and patients. An increasingly elderly patient population is at higher risk of developing surgical site infections (SSI) as well as chronic wounds due to predisposing factors such as diabetes, peripheral arterial disease or chronic venous insufficiency. [1] Chronic wounds are generally considered to be at least colonized by microorganisms, whereby recent studies stated up to 78% to be challenged by biofilm formation [2]. Surgical site infections (SSI) account for on average 20% of hospital-acquired infections in Europe. [3-6] Antimicrobial wound cleansing and local antisepsis are key factors in the treatment of acute and chronic wound infections. Octenidin-dihydrochloride (OCT), povidon-iodine (PVP-I), polyhexamethylene-biguanide hydrochloride (PHMB; polyhexanide) and chlorhexidine (CHX) represent the main antimicrobial agents used in wound management. So far no single antimicrobial has proven to be generally superior regarding treatment of local wound infection or promotion of wound healing. [7, 8] [9] With adverse effects on essential cells for skin regeneration, such as fibroblasts and keratinocytes, reported for each agent [10, 11], antibiotic resistance on the rise [12] and first reports of developing tolerance to local antiseptics [13, 14], investigations into alternative antimicrobial agents with comparable efficacy and low cytotoxic impact to human skin cells are necessary.

Two potential alternatives are the quaternary ammonium compounds Cetylpyridinium-chloride (CPC) and Miramistin (MST), belonging to the family of cationic surface-active agents. Based on first promising results regarding antimicrobial efficacy and cytotoxicity to human cells *in-vitro* [15], this study aimed to compare MST and CPC to OCT, PVP-I, PHMB and CHX in challenging conditions simulating a high-protein wound environment, with short exposure times (as in clinical practice) against common wound pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecium* and *Candida albicans*).

Methods

Preparation of antiseptic solutions

Octenidin-dihydrochloride (Octenisept®; OCT, 0.1%; Schülke & Mayr GmbH, Norderstedt, Germany) and povidone-iodine (Betaisodona®; PVP-I, 10%; Mundipharma GmbH, Limburg, Germany) were used as readily available customary products of everyday clinical use (concentrations as indicated by manufacturer).

Polyhexamethylene-biguanide (PHMB, 20%; Bonding, Shanghai, China) and chlorhexidine (CHX, 2%; Carl Roth, Karlsruhe, Germany) were prepared in distilled water to reach desired final test concentrations of 0.02% (v/v; PHMB) and 0.2% (v/v; CHX) in compliance with previously published studies and available wound cleansing products.

Cetylpyridinium-chloride (CPC; Sigma-Aldrich, Schnelldorf, Germany) and Miramistin (MST; Farmhim, Shostka, Ukraine) were purchased as powders and also prepared in distilled water to reach best suited final test concentrations of 0.5% (w/v; CPC) and 0.05% (w/v; MST) according to previous experiments [15]. An overview as well as specifications for the tested products/substances is provided in table 1.

Test organisms and nutrient solutions

In this work, *Pseudomonas aeruginosa* (DSM-939), *Staphylococcus aureus* (DSM-799), *Escherichia coli* (DSM-11250), *Enterococcus faecium* (DSM-2146) and *Candida albicans* (DSM-1386; all DSMZ, Braunschweig, Germany) were used as bacterial test strains. As nutrient solution sterile casein/soy peptone broth (CSB) was prepared consisting of 15 mg ml-1 casein peptone, 5 mg ml-1 soy peptone and 5 mg ml-1 sodium chloride diluted in distilled water. The pH value was adjusted to 7.2 using 5N sodium hydroxide (all AppliChem, Darmstadt, Germany). One fresh colony of each bacterial strain was added to 50 ml CSB and incubated over night at 37°C under aerobic conditions and agitation. Bacterial test solutions were adjusted using a spectrophotometer to result in initial counts of ~ 1.5 – 3.0 x 108 CFU ml-1. Fungal test solutions were prepared in the same manner using malt/soy peptone broth (MEB) and malt agar (MEA). Initial microbial CFU ml-1 counts were controlled by spreading serial dilutions of untreated microbial test solutions of each experiment onto agar plates allowing exact calculations of reduction rates.

Challenge and neutralisation solutions

To simulate a challenging wound environment and determine possible decrease in efficacy of the tested antiseptics especially under high-protein conditions, bovine albumin (Carl Roth, Karlsruhe, Germany) was added to the experimental setup (as recommended in the standard DIN EN 13727 by the german institute for standardization (DIN)). [16] Challenge solutions contained either 3 mg ml-1 (0.3%) or 30 mg ml-1 (3%) albumin and were prepared using an autoclaved casein/natriumchloride solution dissolved in distilled water. The challenge solution was subsequently aliquoted and stored at -18°C until usage.

To prevent further antimicrobial activity beyond sampling time points, a neutralisation solution was used in accordance with DIN EN 13727, comprising 3 g l-1 sodium thiosulfate, 30 g l-1 saponine, 30 g l-1 polysorbate 80 (Tween 80), 3 g l-1 lecithin, 1 g l-1 L-histidine and 1 g l-1 L-cysteine (all Carl Roth, Karlsruhe, Germany) diluted in distilled water and autoclaved for sterility. Both, challenge and neutralization solution, were individually validated regarding their purpose and showed no antimicrobial effects of its own.

Quantitative suspension method

The test method for evaluation of antimicrobial efficacy was based on DIN EN 13727 and slightly adjusted to fit the purpose. [16] Briefly, 1 ml microbial and 1 ml challenge solution (0.3% or 3% bovine albumin) were carefully mixed for two minutes and 8 ml of antimicrobial test solution was added. After 0.5 , 1, 3, 5 and 10 min of exposure 1 ml of the resulting solution was transferred into the prepared neutralisation solution (8 ml neutralizer and 1 ml distilled water) and continuously agitated. After 10 sec, 0.5 ml was again transferred into 4.5 ml neutralisation solution to thoroughly block further antimicrobial activity. Subsequently, this sample was serially tenfold diluted in either CSB (for bacteria) or MEB (for yeast), 25 l of every dilution step were seeded on either CSA or MEA plates and incubated at 37°C under aerobic conditions overnight. Surviving microorganisms (in CFU ml-1) were counted. Experiments were performed threefold at different times and in duplicates for each tested antiseptic and microorganism challenged with either 3 mg ml-1 or 30 mg ml-1 albumin solution.

Statistical analysis

Reduction rates were calculated for all tested antimicrobials (in Δlog10 CFU ml-1). For bacteria, a high antimicrobial efficacy (reducing at least 99.999% of initial bacterial counts) is indicated by a reduction of at least 5 log10 reduction steps within 1 min as specified in DIN EN 13727. [16] For yeast the cut-off for a high efficacy is considered at least 4 log10 reduction steps as specified in DIN EN 13624. Mean values and SEM were calculated and differences considered statistically significant at $p < 0.05$.

Statistical evaluations of the antimicrobial efficacy were performed using two-way repeated measures ANOVA with Tukey's HSD test as post-hoc analysis for multiple comparisons. The statistic package GraphPad PRISM (GraphPad Software, Inc., La Jolla, United States of America) was used for statistical analysis.

Results

Antimicrobial efficacy on tested pathogens

An overview on the reduction rates (in Δlog10 CFU ml-1 ± SEM reduction steps compared to initial CFU ml-1) of the tested antimicrobial agents is given in table 2.

Octenidin-dihydrochloride/phenoxyethanol (OCT) and povidone-iodine (PVP-I)

OCT and PVP-I achieved significant and complete eradication against all tested microorganism within 0.5 min of exposure ($p < 0.0001$). Increased protein challenge of 0.3% or 3% albumin did not exert a negative effect on their antimicrobial activity (fig. 1 -5).

Polyhexamethylene-biguanide hydrochloride (PHMB)

PHMB showed to be less effective in the conducted experiments. After 0.5 min of exposure with 0.3% protein challenge, PHMB achieved significantly lower reduction rates compared to other tested antimicrobials, such as OCT or PVP-I ($p \leq 0.01$). A significant and strong antimicrobial efficacy (> 5 log10 steps within 1 min; $p < 0.0001$) was achieved against all investigated microorganism, but for complete eradication extended exposure times were necessary: 3 min for *S. aureus* (fig. 1) and *P. aeruginosa* (fig. 3) and 5 min for *E. faecium* (fig. 4) and *C. albicans* (fig. 5). Only against *E. coli* (fig. 2) PHMB did not achieve complete eradication within the investigated exposure time (maximum reduction: 7.38±0.81).

Under higher protein-challenge (3% albumin), PHMB showed comparable results, achieving complete eradication for all tested microorganism except for *E. coli* (fig. 2). Longer exposure times were necessary under increased protein-challenge, especially to eradicate *S. aureus* and *C. albicans* (10 min for both) and reduction rates were lower at tested time-points compared to 0.3% protein challenge, yet not statistically significant (fig. 1 and 5). Against P. aeruginosa and E. faecium, PHMB achieved complete eradication within 3 min (fig. 3 and 4).

Chlorhexidine (CHX)

CHX challenged with 0.3% albumin achieved complete eradication of *E.coli* (within 0.5 min; $p < 0.0001$, fig. 2), *P.aeruginosa* (within 1 min; $p < 0.0001$, fig. 3) and *E. faecium* (within 5 min; $p < 0.0001$, fig. 4) after varying exposure times. Against *S. aureus* and *C. albicans* CHX did not manage complete eradication under 0.3% challenge within the tested exposure time, but achieved significant and required reduction rates (> 5 log10 steps within 1 min; $p < 0.0001$, fig. 1 and 5). This standard criterion was not met for *E. faecium*, despite a complete eradication (table 1 and fig. 4).

Under 3% protein challenge CHX achieved comparable results, demonstrating no significant difference in efficacy between a 0.3% and 3% challenge. Only for *S. aureus* an extended exposure time was needed to achieve the required cut off (3 min; 5.991.99, fig. 1). Also, compared to the lower challenge, CHX achieved a complete eradication of *C. albicans* within 3 min of exposure (fig. 5).

Cetylpyridinium-chloride (CPC)

Regardless of the protein challenge, CPC achieved a strong antimicrobial efficacy, as indicated by the standard, within 0.5 min of exposure against each tested microorganism. Complete eradication was achieved within 0.5 min against *S. aureus*, *E. coli* and *E. faecium* ($p < 0.0001$; fig. 1, 2 and 4). For *C. albicans* under 0.3% challenge (fig. 5) and *P. aeruginosa* under 3% challenge (fig. 3), 1 min of exposure was needed for complete eradication.

Miramistin

Against *E. coli* and *E. faecium* MST managed complete reduction within 0.5 min under lower (0.3%) as well as higher (3 %) protein challenge (fig. 2 and 4). Generally, MST met the criteria set by the standards (> 5 log10 steps within 1 min) for all tested microorganism.

Challenged with 0.3% albumin, MST managed complete reduction of *S. aureus* and *C. albicans* within 3 min (fig. 1 and 5; $p < 0.0001$), while for *P. aeruginosa* 5 min of exposure were needed (fig. 3; $p < 0.0001$).

Under higher protein challenge (3%), results were similar compared to 0.3% challenge with MST achieving complete eradication of *S. aureus* and *C. albicans* within 3 min (fig. 1 and 5; $p < 0.0001$). For *P. aeruginosa* only 1 min of exposure was needed under 3% albumin challenge (fig 3; $p < 0.0001$).

Discussion

Due to the globally rising challenge of antibiotic resistance and tolerance development in microorganim [12], advancements to counteract such challenges and investigations into new local antimicrobial agents are indispensable. Based on previously published data on the basic efficacy of CPC and MST as alternative antimicrobials and their cytotoxicity to human skin cells (keratinocytes and fibroblasts) [15], as relevant side-effects in wound management, this study extended the previous work to evaluate CPC and MST compared to established antiseptics. To especially account for the high-protein environment of wounds, bovine albumin was used in the experimental setup as a challenge substance to evaluate the protein-error of the tested agents, capable of reducing an antimicrobials efficacy. [17, 18]

As expected, the established agents OCT, PVP-I, CHX and PHMB managed the broad range of tested microorganism well and achieved a high antimicrobial efficacy, as reported in several previous studies [11, 19, 20]. All investigated agents, except PHMB and CHX, achieved the required reduction of > 5 log10 phases within 1 min of exposure demanded by the international standard and managed complete eradication within the investigated time-course. Even though PHMB and CHX failed complete eradication in individual cases, they demonstrated an overall strong antimicrobial efficacy. PHMB only failed to completely eradicate *E. coli* in this study (fig. 2), while CHX demonstrated weaknesses against *S. aureus* and *C. albicans* (fig. 1 and 5). Of the established antimicrobials, OCT and PVP-I proved to be most effective, followed by PHMB and CHX.

The alternative agents investigated, especially CPC, demonstrated an antimicrobial efficacy comparable to the most effective established antiseptics OCT and PVP-I within the tested exposure times (fig. 1-5). Only in one case (against *P. aeruginosa* under 3% challenge), CPC needed insignificantly longer to achieve complete eradication (1 min instead of 0.5 min). In all other experiments, CPC performed as well as OCT or PVP-I regardless of the administered protein challenge. Especially under high protein challenge (3%) within the first 1-3 min of exposure, CPC demonstrated significantly higher and faster reduction rates than PHMB and CHX (fig. 1-5; $p < 0.0001$). MST overall showed a slightly lower efficacy and partly longer exposure times than OCT, PVP-I and CPC, yet only statistically significant against *S. aureus* ($p < 0.0001$) and *P. aeruginosa* ($p < 0.01$) within the first 0.5 min. Compared to PHMB and CHX, MST proved significantly more effective against most microorganisms ($p < 0.01$).

As described in various studies, several antiseptics show a significant loss of efficacy under high-protein challenge. The local wound microenvironment contains about 3 to 5 % total protein in acute and chronic wounds. [21, 22] When assessing antimicrobial substances this influencial factor needs to be taken into account to approximate their efficacy in the *in-vivo* setting. Kapalschinski *et al.* demonstrated, that the addition of 0.3% albumin in *in-vitro* settings already significantly reduces the antiseptic potency of PHMB, OCT and PVP-I depending on the antiseptics concentration and that with rising protein concentration the antimicrobial efficacy is further diminished. [17, 18] These results are in line with our previous investigations of antiseptic wound dressings, demonstrating a significant dose-dependent loss of efficacy for some silver- and PHMB-containing foam dressings challenged with human acute wound fluid (median protein content of 3.9%) [23], as well as the results in this study: especially the biguanides PHMB and CHX, to some extend, demonstrate differences in performance under 0.3% and 3% albumin challenge (e.g. against *S. aureus* (fig. 1)). The fact, that this solely occurs for PHMB and CHX without statistical significance, presumably arises from the differences in evaluated concentrations. The reported significant results of Kapalschinski *et al.* became especially apparent in lower concentrations of the tested antiseptics (e.g. 0.005% PHMB), while the concentrations of other publications and commercially available products is usually higher (0.02%, 0.04% or higher) and less affected by the investigated amounts of protein. Nevertheless, 0.02% PHMB yielded comparable decreases in reduction rates (~ 1 log10 difference between 0.3% and 3% albumin) against *S. aureus* in both studies. The tested concentrations of OCT and PVP-I showed no reduction in efficacy under high protein challenge, which is also in line with previously reported results. [17] The alternatives of interest, CPC and MST, demonstrated varying results: while the efficacy of CPC remained unaltered under higher protein challenge, MST showed a slight decrease in efficacy, yet only against *S. aureus* within the first 0.5 min of exposure (~ 3 log10 reductions; fig. 1). Therefore, in this study CPC, OCT and PVP-I showed no, MST a slight and CHX as well as PHMB a comparable higher protein-error in the tested concentrations. Generally, the reported results indicate a certain extend of protein error in a complex high-protein microenvironment of the wound, depending on the concentration of the used antimicrobial, the amount of challenging total protein as well as the class of antimicrobials. For biguanides such as CHX and PHMB high protein challenge seems to be more relevant than for other antimicrobials. Especially in the case of PHMB the results have

to be interpreted with regard to the used lower antimicrobial concentration available as commercial product (0.02%), so that higher concentrations of PHMB are unlikely to yield a relevant protein error.

In light of the presented results, compared to established antimicrobials, the general antimicrobial efficacy of CPC and MST, as well as challenged with a higher protein concentration (3%), proved to be at least equal, even superior in certain cases, against a broad range of microorganisms encountered in wound management. Especially CPC demonstrated the same efficacy as the highly potential antiseptics OCT and PVP-I with no protein error. MST proved to be only slightly less effective, yet still significantly more effective than the biguanides PHMB and CHX. Additionally, in the context of biocompatibility to human wound cells, which should be given equal consideration as previously described by Mueller & Kramer [20], the alternative agents demonstrated promising toxicity profiles in earlier published *in-vitro* evaluations. [15] For OCT and PVP-I severe cytotoxic effects on human keratinocytes and fibroblasts *in-vitro* have been reported [20], even stating dilutions of commercially available products as low as 12.5% (OCT) and 7.5% (PVP-I) to reduce cell viability and proliferation of fibroblasts and keratinocytes to 0% [24]. MST (0.05%) in comparison only demonstrated a reduction of cell vitality to about 30% for keratinocytes and fibroblasts in a previous study [15], being still highly toxic but more biocompatible than OCT or PVP-I. CPC on the contrary proved even less toxic, showing only a 50% reduced cell vitality within 60 min of exposure [15], ranging its toxicity towards human skin cells considerably lower than OCT or PVP-I while achieving the same antimicrobial efficacy.

Conclusion

Overall, CPC and MST feature a high potential as alternative antimicrobials based on their *in-vitro* efficacy profiles compared to established antiseptics. Especially CPC proved to be at least equally, in case of PHMB and CHX even significantly ($p < 0.0001$) more effective against tested microorganisms than established antiseptics. Neither CPC nor MST suffered a significant loss in efficacy under challenging high-protein (3% albumin) conditions. Considering that the here tested concentration of CPC (0.5%) also demonstrated lower toxic side-effects in earlier studies than those reported for OCT anf PVP-I, especially CPC should be further persecuted in more complex *in-vitro* and comprehensive *in-vivo* studies, as a potential new antimicrobial agent in wound management.

List Of Abbreviations

CFU – Colony forming unit

CHX – Chlorhexidine

CPC – Cetylpyridinium chloride

CSA – casein/soy peptone agar

CSB – casein/soy peptone broth

DIN – Deutsches Institut für Normung (German institute for standardization)

DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Culture)

HAI – Hospital-acquired Infection

MEA – malt/soy peptone agar

MEB – malt/soy peptone broth

MST – Miramistin

OCT – Octenidin

PHMB – Polyhexamethylene-biguanide hydrochloride

PVP-I – Povidone-Iodine

RKI – Robert Koch-Institute

SSI – surgical-site infection

v/v – volume per volume percentage

w/v – weight per volume percentage

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare, that they have no competing interests.

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Authors contributions

JDR and EKS designed the study. VT and NH performed the experiments. JDR and VT performed statistical analysis and designed the figures. JDR, VT and EKS drafted the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 - Specifications on test substances. Information have been obtained from the manufacturer. The investigated antimicrobial agent/product is stated in the first row, followed by corresponding chemical group, manufacturer, composition as well as final test concentration of the antimicrobial agent tested in this study (commercially available products were used in undiluted formulation as provided by manufacturer).

Table 1

Antimicrobial agent (commercial product)	Chemical group	Manufacturer	Composition	Final test concentration
Octenidin-dihydrochloride (Octenisept®)	Bis-pyridinamine	Schülke & Mayr GmbH, Norderstedt, Germany	0.1% octenidin-dihydrochloride with 2% phenoxyethanol in 100ml aqueous solution	0.1 %
Polyhexamethylene-biguanide hydrochloride	Polymeric biguanide	Bonding, Shanghai, China	20% polyhexamethylene-biguanide hydrochloride stock solution in distilled water	0.02 %
Chlorhexidindigluconat -solution 2%	Bis-biguanide	Carl Roth, Karlsruhe, Germany	2% chlorhexidindigluconat stock solution in distilled water	0.2 %
Povidone-iodine (Betaisodona®)	Iodine	Mundipharma GmbH, Limburg, Germany	10% povidone-iodine, glycerol, nonoxinol 9, disodium hydrogen phosphate, citric acid, sodium hydroxide, potassium iodate in aqueous solution	10 %
Miramistin (Miramistin®)	Quaternary ammonium compound (QAC)	Farmhim, Shostka, Ukraine	0.5 mg ml-1 miramistin powder in distilled water	0.05 %
Cetylpyridinium-chloride	Quaternary ammonium compound (QAC)	Sigma-Aldrich, Schnelldorf, Germany	5 mg ml-1 cetylpyridinium chloride in distilled water	0.5 %

Due to technical limitations, Table 2 is only available as a download in the supplemental files section.

Figures

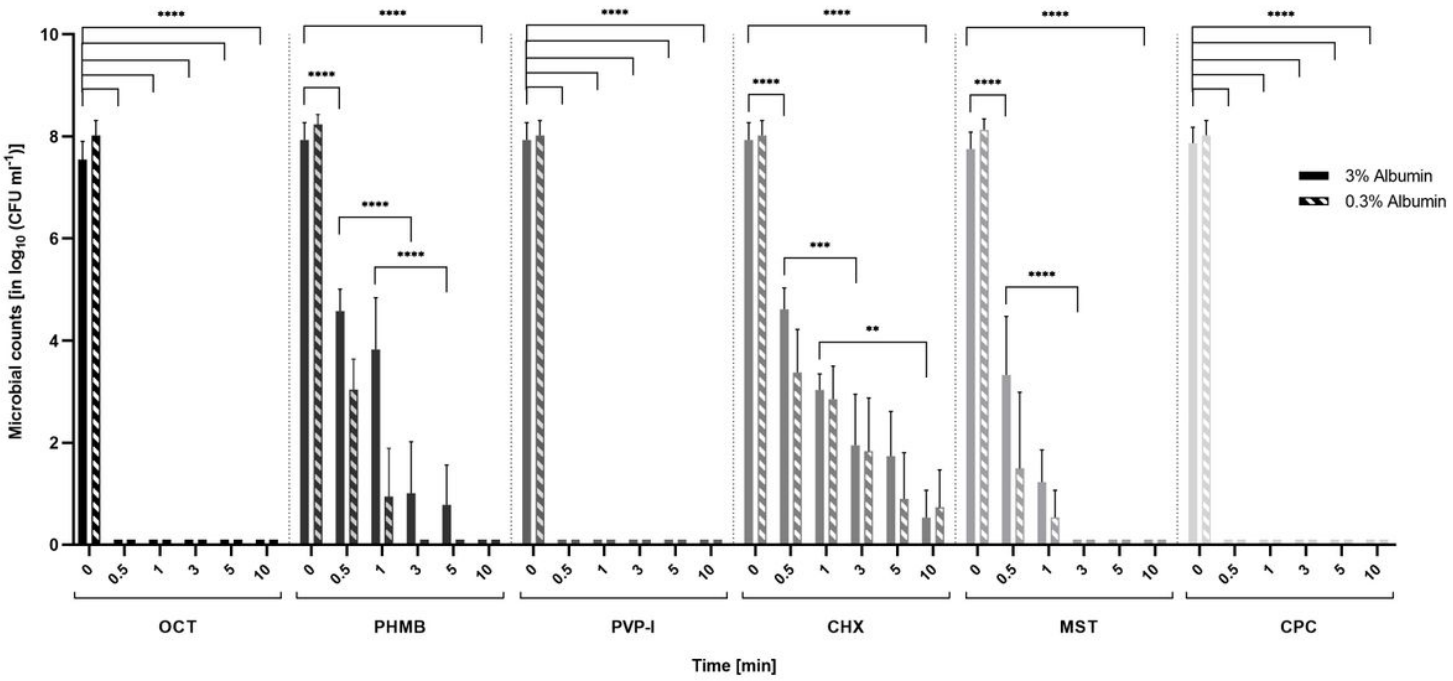

Figure 1

Reduction rates of tested antimicrobials against S. aureus under 0.3% or 3% protein-challenge. Microbial counts are expressed as log10 CFU ml-1 over time. Antimicrobials are displayed individually with a side by side comparison of the reduction rates under 3% and 0.3% protein-challenge. OCT, PVP-I and CPC achieved complete reduction of S. aureus after 0.5 min regardless of the challenge. PHMB and MST required longer exposure times to achieve complete reduction and yielded lower reduction rates under high-protein challenge (3%). CHX achieved the required ≥ 5 log reductions, but did not completely eradicate S. aureus (significant reductions over time are indicated as *p < 0.05, **p < 0.01, ***p <0.001, ****p < 0.0001).

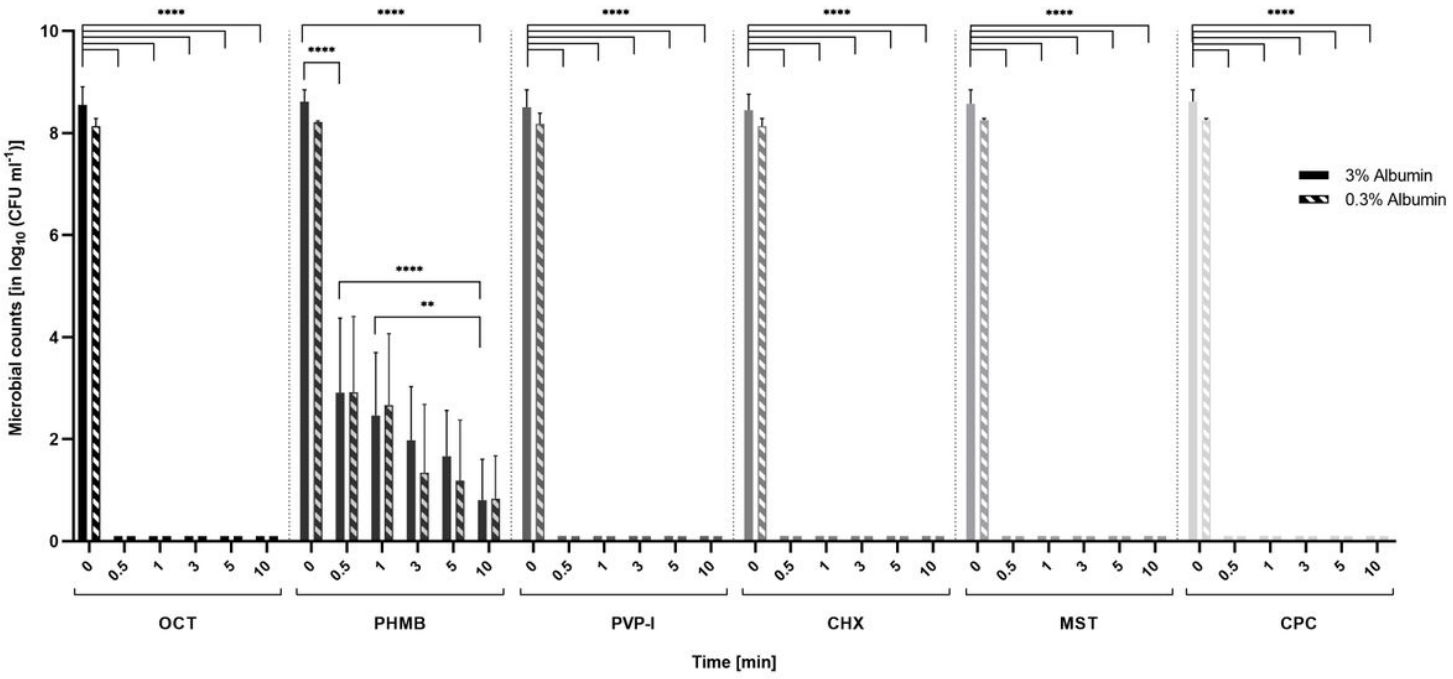

Figure 2

Reduction rates of tested antimicrobials against E. coli under 0.3% or 3% protein-challenge. Microbial counts are expressed as log10 CFU ml-1 over time. Antimicrobials are displayed individually with a side by side comparison of the reduction rates under 3% and 0.3% protein-challenge. Except for PHMB, all tested antimicrobials achieved complete reduction of E. coli within 0.5 min of exposure regardless of the challenge. PHMB on the contrary achieved significant and required reductions, but failed to completely eradicate E. coli in these experiments (significant reductions over time are indicated as *p < 0.05, **p < 0.01, ***p <0.001, ****p < 0.0001).

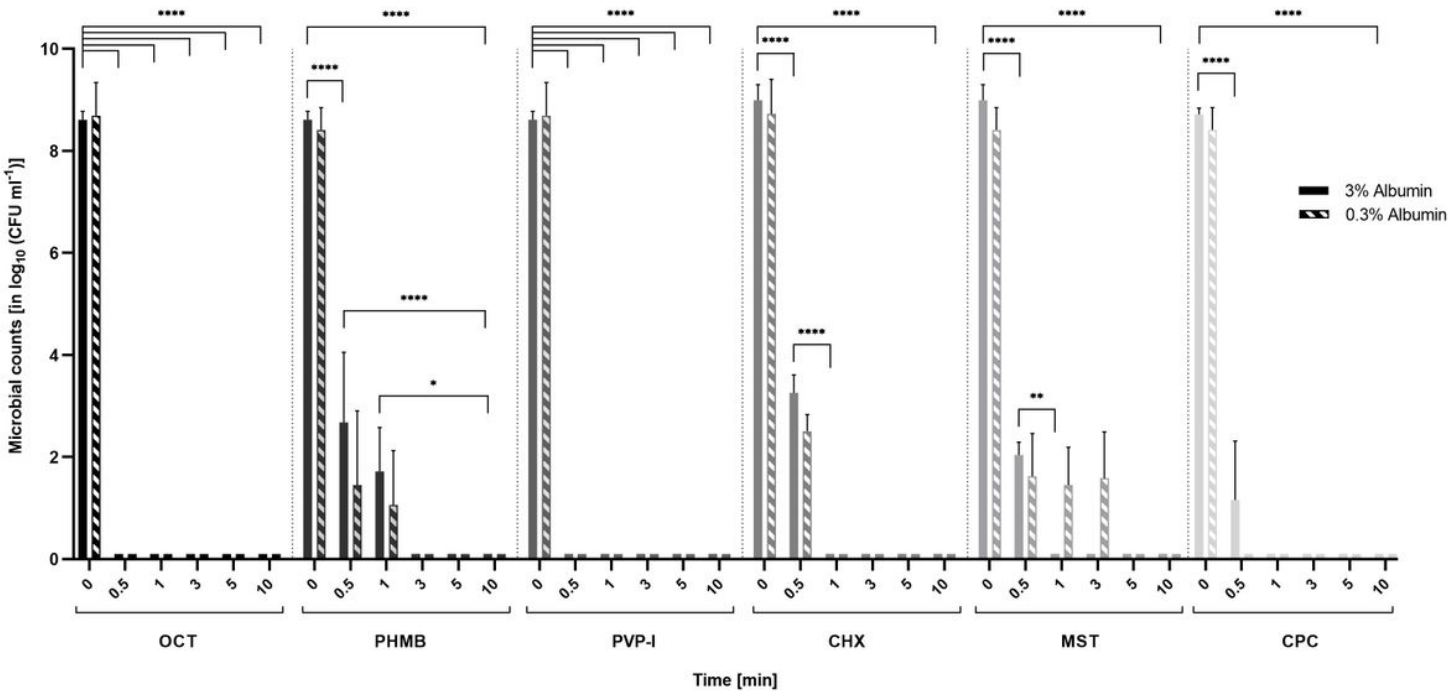

Figure 3

Reduction rates of tested antimicrobials against P. aeruginosa under 0.3% or 3% protein-challenge. Microbial counts are expressed as log10 CFU ml-1 over time. Antimicrobials are displayed individually with a side by side comparison of the reduction rates under 3% and 0.3% protein-challenge. OCT and PVP-I achieved complete eradication of P. aeruginosa within 0.5 min. CHX, MST and CPC needed a prolonged exposure time of 1 min under 3% protein-challenge to achieve full reduction. After 3 min, PHMB also managed a complete eradication of P. aeruginosa (significant reductions over time are indicated as *p < 0.05, **p < 0.01, ***p <0.001, ****p < 0.0001).

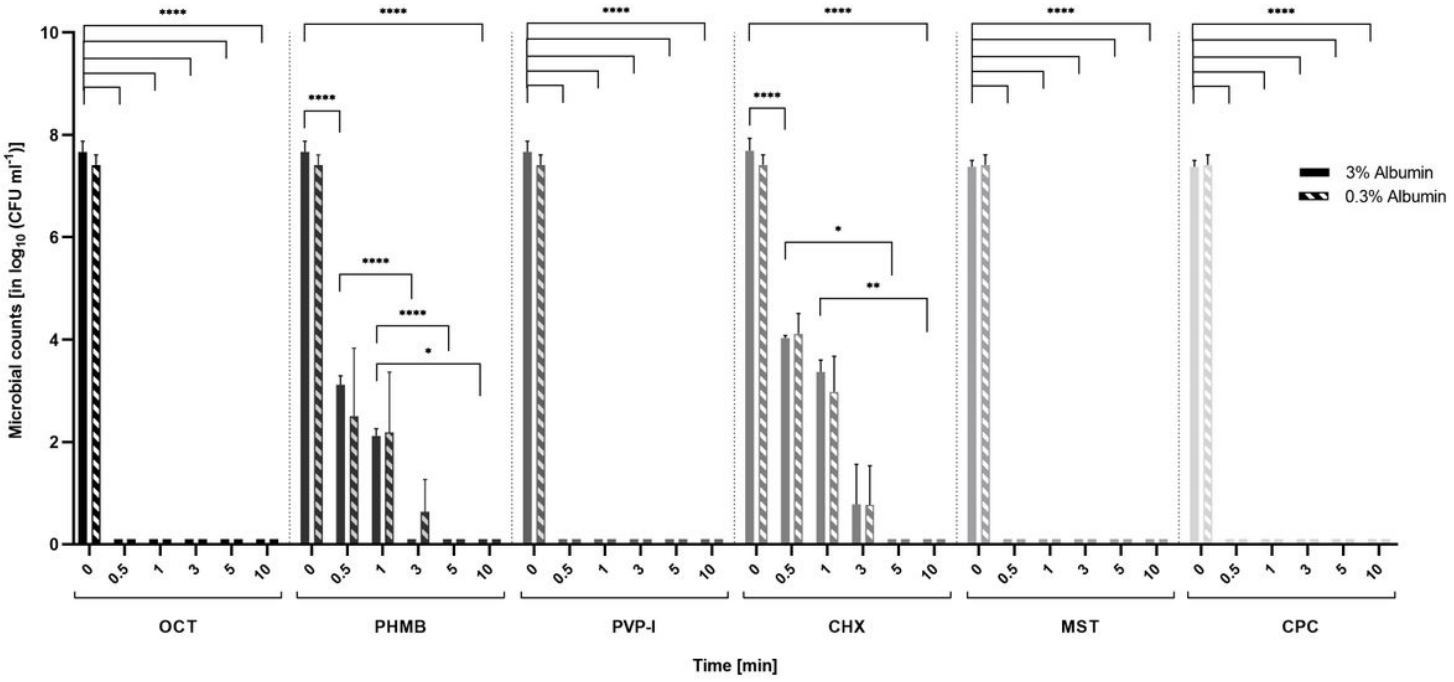

Figure 4

Reduction rates of tested antimicrobials against E. faecium under 0.3% or 3% protein-challenge. Microbial counts are expressed as log10 CFU ml-1 over time. Antimicrobials are displayed individually with a side by side comparison of the reduction rates under 3% and 0.3% protein-challenge. OCT, PVP-I, MST and CPC completely reduced E. faecium within 0.5 min of exposure. PHMB needed a prolonged exposure time of 3 min to achieve complete eradication, while CHX required 5 min. (significant reductions over time are indicated as *p < 0.05, **p < 0.01, ***p <0.001, ****p < 0.0001).

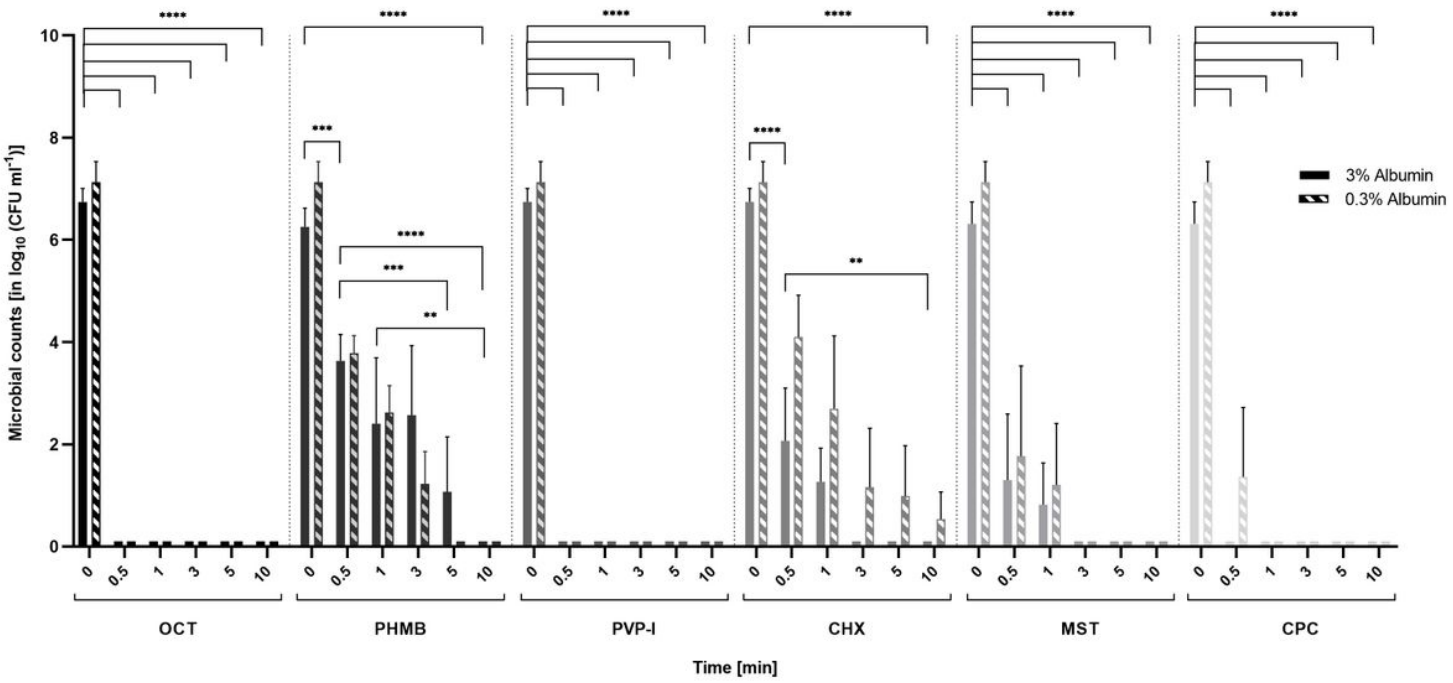

Figure 5

Reduction rates of tested antimicrobials against C. albicans under 0.3% or 3% protein-challenge. Microbial counts are expressed as log10 CFU ml-1 over time. Antimicrobials are displayed individually with a side by side comparison of the reduction rates under 3% and 0.3% protein-challenge. OCT and PVP-I achieved complete reduction of C. albicans within 0.5 min of exposure. CPC also achieved complete reduction within 0.5 min under high-protein challenge (3%), but needed 1 min under lower challenge. MST and PHMB needed prolonged exposure times with MST achieving complete reduction within 3 min and PHMB within 10 min. CHX did not achieve full reduction of C. albicans within 10 min (significant reductions over time are indicated as *p < 0.05, **p < 0.01, ***p <0.001, ****p < 0.0001).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf