Anti-Biofilm Effect of Octenidine and Polyhexanide on Uropathogenic Biofilm-Producing Bacteria

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**Abstract**

**Background:** A catheter allowing a release of antibacterial substances such as antiseptics into the bladder could be a new way of preventing biofilm formation and subsequent catheter-associated urinary tract infections. **Methods:** Minimal inhibitory and bactericidal concentration (MIC/MBC) determinations in cation-adjusted Mueller-Hinton broth and artificial urine were performed for 4 antiseptics against 3 uropathogenic biofilm producers, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. Furthermore, effects of octenidine and polyhexanide against catheter biofilm formation were determined by quantification of biofilm-producing bacteria. **Results:** Sodium hypochlorite showed MIC/MBC values between 200 and 800 mg/L for all strains tested. Triclosan was efficient against *E. coli* and *P. mirabilis* (MIC ≤2.98 mg/L) but ineffective against *P. aeruginosa*. Octenidine and polyhexanide showed antibacterial activity against all 3 species tested (MIC 1.95–7.8 and 3.9–31.25 mg/L). Both octenidine and polyhexanide were able to prevent biofilm formation on catheter segments in a concentration dependent manner. Furthermore, adding 250 mg/L of each biocide disrupted biofilms formed by *E. coli* and *P. mirabilis*, whereas even 500 mg/L was not sufficient to completely destroy *P. aeruginosa* biofilms. **Conclusion:** Octenidine- and polyhexanide-containing antiseptics showed a broad effect against typical uropathogenic biofilm producers even in high dilutions. This study provides a basis for further investigation of the potential of octenidine and polyhexanide as prophylaxis or treatment of catheter biofilms.

**Keywords**

Urinary tract infection · Catheter biofilm · Antiseptics

**Introduction**

Urinary tract infections (UTIs) are among the most common nosocomial infections and up to 90% of these are catheter associated. Indwelling catheters are a major risk factor for ascending UTIs and catheter-associated UTIs (CAUTIs) as well as other complications and are especially important in urology [1, 2]. The most significant catheter complications are severe mechanical traumas such as perforation, partial urethral damage and uri-
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Materials and Methods

Bacteria

From each species tested a reference strain (E. coli ATCC25922, P. aeruginosa ATCC27853, and P. mirabilis ATCC35659) as well as a clinical isolate from patients with UTI (E. coli UTI89, P. aeruginosa 568, and P. mirabilis CHD71) was used.

Determination of Minimal Inhibitory and Bactericidal Concentrations (MIC/MBC)

The following disinfectants were tested: FARCO-fill® Protect (triclosan 3,000 mg/L; Farco-Pharma, Köln, Germany), LAVAN- OX® (<0.08% sodium hypochlorite <800 mg/L; Serag Wiessner, Naila, Germany), Octenisept® (octenidine 1,000 mg/L; Schülke & Mayr, Norderstedt, Germany), and Prontosan® (polyhexanide 1,000 mg/L; B. Braun Melsungen AG, Melsungen, Germany). MIC and MBC determinations were described elsewhere [20]. All determinations were repeated thrice.

Determination of Catheter Biofilm Preventing and Removing Activities

Sterile latex catheters (Rüsch Gold; Teleflex Medical, Perak, Malaysia) were cut in 1 cm segments. These segments were placed in artificial urine with 1–10 × 10^8 CFU/mL of the tested bacteria. For biofilm-preventing assays, disinfectants were added in parallel to the bacteria and catheter segments were subsequently incubated for 1 day (P. aeruginosa and P. mirabilis) or 3 days (E. coli) at 37°C without shaking for biofilm formation. In case of the biofilm-re-

nary leakage, symptomatic bacterial infection, anaphylaxis, catheter toxicity, and hypersensitivity [3]. Especially CAUTIs are related to increased morbidity and mortality and costs such as increased length of stay and hospital costs [4]. Catheter-associated bacteriuria is associated with biofilm formation along the catheter surface [5]. Biofilms are surface-associated bacterial conglomerates that enable bacteria to survive and persist on abiotic and biotic surfaces. Biofilms are encased within a self-produced extracellular polymeric matrix which enables pathogens to escape host defences but also enhances antimicrobial resistances. The organisms that commonly contaminate indwelling urinary catheters and develop biofilms are Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Staphylococcus epidermidis, and other gram-negative bacteria [6, 7]. A recent systematic review on CAUTIs revealed that there is no sufficient evidence to show a benefit of alternative type of catheters compared to standard catheters, or alternative catheter materials, or alternative antiseptic-impregnated catheters [8]. In addition there is no consensus on the best scientific approach, how to study CAUTIs, and preventive strategies. Comparative studies however have investigated how to best quantify catheter biofilm formation [9] or biofilm location on catheters [10]. Several evidence-based guidelines provide recommendations for the prevention of CAUTI including avoidance of catheter use, improving catheter design, or fabricating catheter coatings [6, 11]. Nitrofurazone-coated catheters have been shown to decrease the risk for CAUTI development [12]. However, these catheters are accompanied with a more frequent removal and increased patient discomfort, as well as a possible emergence of antimicrobial resistance [12].

There is therefore a strong need for novel urinary catheter designs, aiming at decreasing complications, such as CAUTIs [3]. A catheter allowing a continuous release of antibacterial substances as antiseptics into the bladder could be a new way of preventing biofilm formation and subsequent catheter-associated UTIs [13]. Such a catheter could be used for biofilm prophylaxis by rinsing the bladder and the space between the catheter and the urethra, either intermittently or constantly with the antibacterial solution under development. Since antimicrobial resistance is a worldwide major public health problem, the usage of antibiotics should be prudent, thoughtful, and rational [14]. Hence, the undirected use of antibiotics as prophylactic therapy using the improved catheter should be avoided. As a replacement, antiseptics could be used as reservoir filling. A wide variety of active biocides contained in antiseptics have been used for hundreds of years for disinfection and preservation. Biofilm preventing or disrupting activity of some antiseptics could be shown before. Thus, cationic octenidine dihydrochloride (referred further as octenidine) inactivates Acinetobacter baumannii and Staphylococcus aureus biofilms formed on different surfaces including catheters [15, 16]. Octenidine and polyhexanide reduced P. aeruginosa biofilms grown on polycarbonate slides [17]. In an in vitro urinary tract model, triclosan-impregnated urinary catheters were more effective in preventing the formation of S. aureus and S. epidermidis biofilms compared to nitrofural-treated catheters [18]. In addition, filling the retention balloon of urinary catheters with 10 g/L triclosan in a catheterized bladder model prevented the biofilm formation of several uropathogens [19].

The objective of this study was to investigate the efficacy of the biocides octenidine, polyhexanide, triclosan, and sodium hypochlorite against the biofilm-producing uropathogenic species E. coli, P. aeruginosa, and P. mirabilis in artificial urine and the relevant concentrations needed. This should serve to assess the possible use of these agents as an antimicrobial filling for continuous release catheters as CAUTI prophylaxis.

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moving assays, biofilms were allowed to form before disinfectants were added and biofilms were quantified after further overnight incubation. For quantification of the planktonic bacteria, supernatants of the catheter segments were serial diluted in PBS and plated on cation-adjusted Mueller-Hinton broth (CAMHB) agar plates. For biofilm quantification, catheter segments were washed thrice with PBS. After placing them in 1 mL of PBS in 1.5-mL reaction tubes, they were vortexed 1 min at full speed. Afterwards, sonification of the catheter segments was performed using ultrasonic bath Sonoex TK30. For *E. coli* biofilms, catheter segments were sonicated for 10 min [21]. *Pseudomonas*- and *Proteus*-treated catheter segments were sonicated twice for 5 min with a 2-min vortexing in between [22]. Determination of biofilm-forming CFU was carried out after another 1 min of vortexing step by serial dilution and plating on CAMHB agar plates. Data were plotted using GraphPad Prism 8.4.3. software (San Diego, CA, USA). Differences were identified following ANOVA and, when appropriate, Dunnett’s test analysis of significance for each of the variables.

**Results**

*Determination of Inhibitory and Bactericidal Titres*

Four commercial disinfectants were used to test the inhibitory and bactericidal activity of the disinfecting agents triclosan (FARCO-fill® Protect), sodium hypochlorite (LAVANOX®), octenidine (Octenisept®), and polyhexanide (Prontosan®) against 3 uropathogenic species. FARCO-fill Protect containing a concentration of 3,000 mg/L triclosan showed almost no activity against *P. aeruginosa*, while it was highly bactericidal with MBC values of at least 5.86 mg/L against *P. mirabilis*. For *E. coli*, MIC values were below the tested minimum of 2.93 mg/L but MBC values ranged between 46.88 and 187.5 mg/L suggesting more bacteriostatic activity against these bacteria. Furthermore, it seemed to be less active in artificial urine compared to CAMHB (Table 1). MIC and MBC values for sodium hypochlorite ranged from 800 to 400 mg/L and 400–200 mg/L for all tested strains in CAMHB and artificial urine, respectively. The treatment of *P. mi-

| Table 1. Median minimal inhibitory and bactericidal concentrations (mg/L) in CAMHB and AU of triclosan, sodium hypochlorite, octenidine, and polyhexanide. |
|----------------|----------------|----------------|----------------|----------------|
|                | CAMHB          | AU             | CAMHB          | AU             |
|                | MIC            | MBC            | MIC            | MBC            |
| Triclosan (FARCO-fill Protect®) | 3,000          | >3,000         | 3,000          | >3,000         |
| Sodium hypochlorite (LAVANOX®)   | 400–200        | 400–200        | 400–200        | 400–200        |
| Octenidine (Octenisept®)         | 400–200        | 400–200        | 400–200        | 400–200        |
| Polyhexanide (Prontosan®)        | 15.6–31.25     | 15.6–31.25     | 15.6–31.25     | 15.6–31.25     |

CAMHB, cation-adjusted Mueller-Hinton broth; AU, artificial urine; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; Ec, *E. coli*; Pa, *P. aeruginosa*; Pm, *P. mirabilis*. *Octenisept caused turbidity in AU with *P. mirabilis* leading to false positive values.

**Fig. 1.** Activity of polyhexanide and octenidine against bacterial catheter biofilms. Polyhexanide and octenidine in different concentrations were added once at the beginning of the biofilm formation process to artificial urine containing bacteria and catheter segments (a, c) or biofilms formed on catheter segments in artificial urine were treated with 250 mg/L (*E. coli* and *P. mirabilis*) or 500 mg/L (*P. aeruginosa*) polyhexanide (P) and octenidine (O) for 24 h. Planktonic (c, d) and biofilm-forming bacteria (a, b) were quantified. Open symbols show the values of the individual measurements. Horizontal lines show median values. ctrl, control without adding disinfectants; LOD, limit of detection; nd, not determined. *p < 0.05 over control, **p < 0.01 over control, ***p < 0.001 over control, ****p < 0.0001 over control. (For figure see next page.)
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**E. coli UTI89**

**P. aeruginosa 568**

**P. mirabilis CHD71**

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**Legend:**
- E. coli UTI89
- P. aeruginosa 568
- P. mirabilis CHD71

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**Log10 CFU/cm catheter**

**Log10 CFU/mL**

**LOD**

**Polyhexanide (mg/L)**

**Octenidine (mg/L)**

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**Ctrl**

**P**

**O**

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**Table:**

|          | Ctrl | 15.6 | 62.5 | 250 | 15.6 | 62.5 | 250 |
|----------|------|------|------|-----|------|------|-----|
|          | Polyhexanide | mg/L | Octenidine | mg/L |
| E. coli  | UTI89 |       |       |     |      |      |     |
| P. aeruginosa | 568 |       |       |     |      |      |     |
| P. mirabilis | CHD71 |       |       |     |      |      |     |
rabilis with Octenisept® in artificial urine caused turbidity also in wells where no bacteria were found after plating. Due to these false positives, the MIC values appear to be higher than they really are. Based on the MBC values, it can be assumed that the MIC values for P. mirabilis in artificial urine were at least 3.9 mg/L (Table 1). MIC and MBC values of octenidine ranged between 1.95 and 3.9 mg/L for E. coli and between 7.8 and 15.6 mg/L for P. aeruginosa, with a slight reduction of bactericidal activity in artificial urine for Pseudomonas. MIC values of polyhexanide ranged from 3.9 to 15.6, 7.8–31.25, and 3.9–31.25 mg/L for E. coli, P. aeruginosa, and P. mirabilis, respectively. MBC values were equal or 1–2 dilution steps lower (Table 1).

**Determination of Anti-Biofilm Activities**

In the next step, we were interested in the effectivity of octenidine and polyhexanide against bacterial catheter biofilms. Therefore, on the 1 hand the disinfectants were added during the process of biofilm formation on catheter segments to test their preventing activity. On the other hand, both were added after the biofilm has formed to determine the biofilm destructive potential. While 62.5 mg/L of polyhexanide killed all planktonic E. coli, biofilms on the catheter segments were still detectable but reduced. Using 62.5 mg/L of octenidine, reduced numbers of planktonic as well as biofilm bacteria were detected, compared to the untreated control. In contrast, using 250 mg/L of both biocides completely prevented biofilm formation (Fig. 1). In addition, 250 mg/L of octenidine as well as polyhexanide were able to almost completely disrupt the formed E. coli biofilm on the catheter segments (Fig. 1). For P. mirabilis 15.6 mg/L of both disinfecting agents were sufficient to kill all planktonic bacteria and to significantly reduce biofilm formation on catheter segments; 62.5 mg/L almost completely abolished biofilm formation, whereas no biofilms were detected at all with 250 mg/L of polyhexanide as well as octenidine. In addition, formed P. mirabilis biofilms could be completely disrupted using 250 mg/L of both biocides (Fig. 1). Biofilms formed by P. aeruginosa were more robust against the disinfectants. Concentrations of 62.5 mg/L had almost no effect on the planktonic as well as biofilm bacteria for both biocides, whereas 250 and 500 mg/L killed the planktonic bacteria. In contrast to the Enterobacteriaceae, using 250 mg/L of polyhexanide or octenidine only reduced the numbers of biofilm P. aeruginosa. Here 500 mg/L were needed to completely prevent a biofilm formation. In addition, even incubation with 500 mg/L of both biocides was not able to completely destroy the formed biofilms (Fig. 1).

**Discussion**

Catheter-associated complications such as infections are very frequent [3]. Apart from avoiding urinary catheters or reducing catheter lengths, there is very little evidence showing a benefit for other preventive strategies, such as alternative type of catheters, different catheter materials, antiseptic impregnated catheters, or other measures [8]. There is therefore a strong need to reduce catheter-associated complications by evaluating novel catheter designs and preventive measures [3]. Despite the heterogeneous methodology used to assess and evaluate catheters and the involved biofilm formation, studies have shown how to best quantify biofilm formation and standardize the assessment of biofilm in and around a catheter [9], which was used in this study. MIC/MBC determination of the 4 antiseptics tested revealed sodium hypochlorite (LAVANOX®) as the least active substance against the 3 uropathogen species. Triclosan was bactericidal against E. coli and P. mirabilis but not against P. aeruginosa. Triclosan inhibits the fatty acid synthesis by binding to bacterial enoyl-acyl carrier protein reductase enzyme FabI. P. aeruginosa in contrast to the tested Enterobacteriaceae, encodes a second enoyl-acyl carrier protein reductase which is resistant to triclosan [23]. Antiseptics containing the biocides octenidine and polyhexanide showed the best activity against all 3 bacterial species tested. Furthermore, determination of the anti-biofilm activity showed that Octenisept® containing octenidine as well as Prontosan® containing polyhexanide were able to prevent biofilm formation of E. coli, P. aeruginosa, and P. mirabilis in a concentration-dependent manner. Using the new developed catheter with slow release of these biocides into the bladder could possibly be used as biofilm prophylaxis. However, it is important to note that for a complete biofilm prevention, concentrations of at least 250 mg/L of both disinfecting agents were required. This corresponds to a maximum 1:4 dilution of the disinfectant, assuming an average urine production of 1.5 L per day will lead to a 1:25 dilution for a disinfectant released with 60 mL/24 h from the catheter reservoir. Thus, only a reduction but no complete prevention of biofilm formation would be expected. However, our experimental design tested only a single-dosage treatment. A treatment approach using multiple doses while additional imitation of the bladder voiding would reflect a more accurate picture of the in vivo situation. Here, an increase of the anti-biofilm effectiveness and a reduction in the required concentration of the bio-
cides would be expected. However, this still has to be proven in further experiments. In addition, safety issues have not been evaluated in this in vitro study. However previous studies with keratinocytes indicated that Prontosan is better tolerated than Octenisept for concentrations up to 175 mg/L [24]. Therefore, adding cell culture experiments to the experimental set-up could evaluate some of the safety issues. Therefore, the sensitivity of the bladder cells to the antiseptics and various concentrations has yet to be tested.

**Statement of Ethics**

Ethics approval was not required.

**Conflict of Interest Statement**

M.L., L.P., and M.W. declare no conflict of interest. K.G.N. reports personal fees from Adamed, Allecra, Apogepha, Bionorica, Enteris Biopharma, Galenus, GlaxoSmithKline, Hermes, Leo, Medice, MerLion, MSD Sharp & Dohme, Paratek, Roche, Rosen, Saxonia, and Vifor outside the submitted work. F.M.E.W. reports personal fees and others from Achaogen, personal fees from AstaZeneca, personal fees from Bionorica, others from Enteris Biopharma, others from Helperby Therapeutics, personal fees from Janssen, personal fees from LeoPharma, personal fees from MerLion, personal fees from MSD, personal fees from OM Pharma/Vifor Pharma, personal fees from Pfizer, personal fees from RosenPharma, personal fees and others from Shionogi, personal fees from VenatoRx, and personal fees from GSK outside the submitted work.

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**Author Contributions**

M.L. designed and carried out all experiments, performed the analysis, and wrote the manuscript in close cooperation with all coauthors considering their critical comments. K.G.N., L.P., M.W., and F.M.E.W. developed and initiated the project.

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