In vitro effectiveness of colistin, tigecycline and levofloxacin alone and combined with clarithromycin and/or heparin as lock solutions against embedded Acinetobacter baumannii strains

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Objectives: To determine the in vitro stability and efficacy of colistin, tigecycline and levofloxacin alone or in combination with clarithromycin and/or heparin as lock solutions against biofilm-embedded Acinetobacter baumannii strains.

Methods: Candidate antibiotics (colistin, tigecycline and levofloxacin) were investigated in vitro, either alone or in combination with clarithromycin and/or heparin in solution. The efficacy of antibiotic lock solutions was tested in an in vitro catheter biofilm model against A. baumannii isolated from catheter-related bacteremia.

Results: Candidate antibiotics at 400× MICs combined with clarithromycin (200 mg/mL) and/or heparin (1000 U/mL) were compatible. Colistin, tigecycline and levofloxacin and their combinations with clarithromycin demonstrated bactericidal activity against the biofilm-embedded A. baumannii strains. Compared with other antibiotics alone, the lock solution including only colistin was the best agent to eradicate A. baumannii embedded in the catheter model. When tested antibiotics were used in combination with clarithromycin, the combinations were significantly more effective and more rapid in reducing the live cell number or eliminating A. baumannii colonization in biofilms than each of the antibiotics alone.

Conclusions: Catheter lock solutions containing colistin may have the most promise for treating or preventing biofilm-producing catheter infections caused by A. baumannii. Clarithromycin was ultimately effective with the studied antibiotics to reduce live cell number or eradicate A. baumannii colonization in biofilms and could serve as an antibiotic enhancer. Our in vitro model findings now warrant clinical trials to investigate their real role in the management of catheter-related bacteremia.

Keywords: Gram-negatives, bacterial biofilms, catheter-related infections, antibiotic therapy, polymyxin B

Introduction

The use of central venous catheters (CVCs) has dramatically increased during the last decade worldwide. Catheter-related bacteremia (CRB) infections associated with the insertion and maintenance of CVCs are among the most common and dangerous complications that can occur. When CRB is diagnosed, the guidelines recommended that the patients for whom CVCs cannot be removed should be treated for 2 weeks with systemic therapy and antibiotic lock therapy (ALT), as they often suffer from Gram-negative bacteremia.1 ALT is based on the instillation of high concentrations of an antimicrobial agent into the lumens of infected CVCs for extended periods to overcome the relative antimicrobial resistance of biofilm bacteria.2-5 Although most investigations have focused on CVC infections involving Gram-positive bacteria, Gram-negatives are also important pathogens in catheter-related infections.5-7 Gram-negative bacteria accounted for 19% and 21% of CRBs reported to the CDC8 and the Surveillance and Control of Pathogens of Epidemiological Importance (‘SCOPE’) database,9 respectively. Acinetobacter baumannii is an important organism that causes catheter-related infections and often difficult-to-eradicate bloodstream infections.6,10 Seifert et al.11 showed that of patients with A. baumannii bacteremia, 91% were hospitalized in an intensive care unit (ICU) and 99% had indwelling vascular catheters.

In the present study, we examined the in vitro activity of ALT using colistin, tigecycline and levofloxacin alone or in combination with clarithromycin and/or heparin, an anticoagulant agent, against biofilm-embedded A. baumannii.
Materials and methods

Bacterial strains used

A. baumannii AB-1 was isolated from patients with CRB. A. baumannii ATCC 19606 (AB-19606) is known to form biofilms. These two strains were confirmed for biofilm-forming ability.

Determination of MICs

MICs were determined by the broth microdilution method according to the CLSI guidelines.12 AB-19606 was used as a control strain.

In vitro antibiotic lock model

Segments (1 cm) of 7-French, triple-lumen CVCs (Cook, Inc., Bloomington, IN, USA) were incubated in bacterial suspensions that contained bacteria at 10⁶ cfu/mL in Trypticase soy broth to allow biofilm formation. After incubation at 37°C for 24 h, segments were removed and excess broth was shaken off. Three catheter segments were rinsed and cultured to obtain a baseline value, and the remaining segments (three replicates per condition) were suspended for 24, 48, 72 and 96 h at 37°C in one of the following treatment solutions: colistin (sulphate) alone, tigecycline alone, levofloxacin alone, clarithromycin alone, colistin/clarithromycin, colistin/heparin, colistin/clarithromycin/heparin, tigecycline/clarithromycin, tigecycline/heparin, tigecycline/clarithromycin/heparin, levofloxacin/clarithromycin, levofloxacin/heparin and levofloxacin/clarithromycin/heparin. PBS was used as a control. Colistin, tigecycline and levofloxacin were used at concentrations of 400× MIC alone or in combination for the organisms tested in the planktonic phase. Clarithromycin was used at 200 mg/mL (~100× serum concentration) and heparin was used at 1000 U/mL. Then, catheter segments were removed and washed 10 times with PBS to remove planktonic bacteria. These sections were individually sonicated for 10 min and vortexed for 30 s in 1 mL of PBS. After successive dilutions, if needed, all original sonication fluids were inoculated onto Trypticase soy agar plates (limit of detection, <10 cfu) and the plates were incubated at 37°C overnight. The median colony count of the three 1 cm sections was considered the representative value for that segment.13 The experiments were repeated three times and the mean values for the biofilm bacteria were compared between groups for each antibiotic alone or in combination. The cfu/cm² values of catheters for different groups were compared by one-way ANOVA followed by Bonferroni’s multiple comparison test (GraphPad Software Inc., San Diego, CA, USA) and a P value of <0.05 indicated significance.

Drug stability and compatibility

Each solution was incubated for 96 h and then evaluated for physical compatibility by particulate formation, colour change or gas evolution.

Results

The MICs of the antimicrobial agents for each of the planktonic forms of A. baumannii are shown in Table 2. AB-19606 was susceptible to all antibiotics tested in this study, whereas AB-1 was resistant to levofloxacin. Clarithromycin, which is already not a therapeutic choice for A. baumannii infections, showed high MIC values.

Colistin at 400× MIC completely eradicated the AB-19606 and AB-1 biofilm bacteria within 3 days (Figure 1a and b). In particular, AB-19606 and AB-1 biofilms were sterilized after 2 days of exposure to colistin/clarithromycin combinations. Although the colistin/heparin and colistin/clarithromycin/heparin combinations did not eradicate AB-1 or AB-19606 biofilm bacteria within 3 days, the same combinations eradicated AB-19606 within 4 days.

Four antibiotic lock model

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Discussion

Although CVCs are increasingly used to save lives in ICUs and to administer medicine and fluids,1 the use of CVCs may result in serious bloodstream infections. According to a clinical study by Krishnasami et al.,7 ALT was successful in achieving a clinical and bacteriological cure of dialysis CRB in 64.5% of patients, without requiring catheter replacement. Similarly, Fundleras et al.14 suggested that ALT combined with a systemic antibiotic provided a 95% cure rate for 37 patients with Gram-negative bacteraemia.

According to our results, colistin alone was significantly more effective than tigecycline or levofloxacin for inhibiting A. baumannii organisms embedded in a biofilm (P<0.05). Colistin...
at 400× MIC for a sufficient dwell time may be an option in catheters to prevent colonization and biofilm formation or to eliminate biofilm-embedded microorganisms. However, the use of tigecycline or levofloxacin alone might be associated with the regrowth of *A. baumannii*, so these drugs alone are not fully effective for preventing or eliminating the microbial burden of *A. baumannii* colonization in biofilms.

On the other hand, clarithromycin was found to be highly active in reducing *A. baumannii* colonization in biofilms on catheter segments compared with the control (P<0.05). Notably, clarithromycin was highly efficacious in enhancing the activities of all the antibiotics tested. These combinations involving clarithromycin acted more rapidly in reducing or eradicating both of the tested *A. baumannii* strains compared with the antibiotics tested alone. Figure 1 shows that colistin/clarithromycin killed biofilms of both *A. baumannii* strains within 2 days (3 days alone) and that tigecycline/clarithromycin and levofloxacin/clarithromycin could eradicate AB-1, which is an antimicrobial-resistant pathogen, within 2 and 3 days, respectively (no eradication when alone). Similarly, enhancement of the antibacterial activities of

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**Figure 1.** *In vitro* activity of ALT including the tested agents—colistin (CST), tigecycline (TGC), levofloxacin (LVX), clarithromycin (CLR) and heparin (HEP)—against biofilms formed by a representative strain of *A. baumannii*, AB-19606 (a, c and e), and a clinical strain of *A. baumannii*, AB-1, (b, d and f).
other antibiotics when combined with clarithromycin has previously been demonstrated against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria embedded in biofilms.\(^5\)\(^-\)\(^7\)\(^,\)\(^1\)\(^9\)\(^,\)\(^2\)\(^0\) Additionally, Kandemir et al.\(^1\)\(^8\) have shown that clarithromycin was the most common constituent of antibiotic combinations active against *Pseudomonas aeruginosa* in biofilms. Because this is the first investigation in which clarithromycin combinations were more effective in a lock solution to eradicate or reduce live cell numbers in biofilm formation in *A. baumannii* compared with the antibiotics used alone, additional studies are needed to investigate the mechanism of action of clarithromycin against *A. baumannii* biofilms.

Generally, ALT is used in conjunction with the administration of systemic antibacterial and/or anticoagulant agents to prevent thrombosis or obstruction of the catheter.\(^7\)\(^,\)\(^1\)\(^9\)\(^,\)\(^2\)\(^0\) However, bacteria such as staphylococci may survive and grow in heparin-locked catheters.\(^1\)\(^9\) We found that when heparin is included, combinations of colistin, tigecycline or levoflaxacin were less effective than antibiotics used alone in ALT for *A. baumannii* biofilms (\(P>0.05\)). On the other hand, because ALT use also increases the frequency of catheter thrombosis, we evaluated our three antibiotics in combination with a clarithromycin/heparin solution to assess the change in antimicrobial activity. Clarithromycin/heparin rarely reduced the antimicrobial effect of the antibiotics compared with when they were used alone. The interaction between the used antibiotics and heparin in catheter lock solutions needs to be evaluated in future studies.

In conclusion, colistin was significantly more active than tigecycline and levoflaxacin against *A. baumannii* embedded in biofilms. Colistin, alone or in combination, eliminated and prevented the regrowth of organisms embedded in biofilms for 96 h and could be considered a most effective therapeutic option for the treatment or prevention of catheter-related infections due to *A. baumannii*.

Clarithromycin was highly effective when combined with the studied antibiotics to reduce the number of live cells of *A. baumannii* in biofilms. Notably, clarithromycin was efficacious in enhancing the activities of all the antibiotics tested. Our in vitro model findings about these solutions warrant clinical trials to investigate their real role in the management of CRB.

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**Transparency declarations**

None to declare.

**References**