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Short communication

Selenium nanoparticles render pandrug-resistant *Acinetobacter baumannii* susceptible to colistin

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Introduction

Colistin is used as a "last resort" drug in the treatment of carbapenem-resistant *Acinetobacter baumannii* infections. Though not the most convenient option due to the serious side effects, this antibiotic can be very efficient if administered properly (Garnacho-Montero and Timsit, 2019). However, for several years now, we are witnessing a steady emergence of colistin-resistant (Col^R) strains, with the constant threat of a sudden burst worldwide (Nowak et al., 2017). Having in mind that these strains are usually non-susceptible to any of the existing treatments, i.e. pandrug-resistant (PDR), the development of new therapeutic strategies is desperately needed.

In the present study, we showed that colistin susceptibility of Col^R *A. baumannii* strains can be fully restored in the presence of very low amounts of selenium nanoparticles (SeNPs). To demonstrate potent synergistic interactions between colistin and SeNPs, we performed checkerboard and time-kill analyses.

Materials and methods

Bacterial strains

A total of eight Col^R *A. baumannii* isolates identified by 16S rRNA gene sequencing, originating from Clinical Hospital Centre Dr Dragiša Mišović Dedinje, Clinic for Gynecology and Obstetrics of University Clinical Centre of Serbia, and Beo-lab outpatient laboratory, were used in this study. The isolates were further genotyped by Pulsed-field gel electrophoresis (PFGE) (Kojic et al., 2005). Colistin resistance in the isolates was confirmed by performing broth-microdilution method (CLSI, 2015). *A. baumannii* ATCC 19606 was used as a reference strain.

Selenium nanoparticles (SeNPs)

Sodium selenite was reduced by ascorbic acid (VWR BDH Prolabo) in the presence of 0.87% BSA to obtain BSA-stabilized SeNPs (Filipović et al., 2021). Antimicrobial activity of SeNPs against *A. baumannii* isolates was tested by broth-microdilution method.

Checkerboard test

Antimicrobial activity of colistin in the presence of SeNPs against Col^R *A. baumannii* was assessed by checkerboard test (Odds, 2003). Colistin and SeNPs were used over the concentration ranges of 0.004–4 and 0.125–8 μg/mL, respectively. The interaction types were interpreted according to fractional inhibitory concentration indices (FICIs), as follows: ≤0.5, synergy; >4.0, antagonism; >0.5–4.0, no interaction.

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Time-kill assay

Time-kill test was employed to compare the growth kinetics of Col^R *A. baumannii* strains treated with colistin/SeNPs combination to the same strains treated with colistin only (CLSI, 1999). Based on the clinical susceptibility breakpoint values (EUCAST), colistin was applied at 0.5, 1, and 2 µg/mL, with or without SeNPs at 0.5 µg/mL. Viable cell counts were made at 0, 4, 8, and 24 h, by drop plate method.

Results and discussion

Col^R *A. baumannii* isolates (n = 8) were collected from three different health facilities in Belgrade over the relatively short period of time (six months). Seven of them displayed highly similar, although unique *Apa*I restriction fragments, indicating a high level of genetic relatedness among them. Notably different was, especially in the area of larger fragments, the PFGE profile of one strain (namely D1010), meaning that two distantly related clones of Col^R strains were used in this study.

The isolates displayed variable levels of colistin resistance, based on the obtained minimum inhibitory concentrations (MICs), which ranged between 16-256 µg/mL. MICs of SeNPs were either 128, or 256 µg/mL. When the two agents were used in combination, dramatically lower concentrations of both were sufficient to induce the growth inhibition of the tested isolates. It was only 0.25-0.5 µg/mL of colistin that, when used with 0.5 µg/mL of SeNPs, resulted in no visible bacterial growth after 24 h of incubation. The existence of an exceptionally potent synergistic interaction between the two agents was further confirmed by calculating the FICI values (0.004–0.035). What is most important, the isolates, which were resistant to colistin and thus regarded as PDR, were made susceptible to colistin, since the MIC of colistin was reduced below the clinical breakpoint value (2 µg/mL).

Further, based on the growth kinetics, it was shown that when colistin was used in combination with SeNPs, the level of viable bacterial cell counts decreased by 0.4 $\log_{10}$ CFU in average during the first 4 h of incubation. Such phenomenon, which resulted in a growth delay, was absent in groups treated with colistin only, and thus may explain why bacteria did not reach the visibility threshold after 24 h. Considering that reducing the rate of reproduction can significantly decrease the level of bacterial burden in living tissues, which largely affects the clinical outcome of an *A. baumannii* infection (*Wong et al.*, 2017), this result further supports the beneficial effect of the combined colistin/SeNPs administration.

Conclusion

In this study we showed that colistin susceptibility of PDR *A. baumannii* isolates can be restored if colistin is used in combination with SeNPs. Furthermore, synergistic concentration of SeNPs was low enough to presume that it should be readily achieved *in vivo* following the systemic administration.

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