Silver Resistance identified in clinically isolated Enterobacteriaceae: Major Implications for Burn and Wound Care

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Abstract

During the last 40 years, inorganic silver has become a popular additive for many medical devices including burn and wound dressings. As a result, concerns of widespread silver-resistance emerging in clinical bacteria have been raised. Previously, we identified the first clinical bacteria (Klebsiella pneumoniae and Enterobacter cloacae) capable of luxuriant growth at exceedingly high concentrations of silver. Additional DNA sequence analysis revealed PCR products from these isolates had a high degree of similarity to other plasmids containing genes which encode for heavy metal resistance. These plasmids included pKPN1, pMG101, and many megaplasmids in the Klebsiella taxid. This finding suggests the existence of many genetically similar plasmids all with potential capabilities of expressing high levels of silver-resistance. Further antimicrobial testing against commercially-available silver dressings showed after 24 hours of dynamic contact, the silver-resistant isolates were largely resistant to many of the dressings. Data from a corrected zone of inhibition (CZOI) assay supported these findings. Neither of the silver-resistant isolates produced significant zones of inhibition after contact with the dressings, while the non-resistant organisms yielded a measurable zone of inhibition. Swabs from underneath the dressing post-incubation revealed the silver-resistant bacteria remained viable. Research revealed non-silver based wound dressings maintained high antimicrobial efficacy against these clinical bacteria without the risk of resistance. The development of acute silver-resistance would have significant consequences on wound care and patient outcomes. There is a significant need for non-silver based dressings that can effectively manage bioburden while minimizing resistant risks. Due to a current lack of antimicrobial stewardship practices for silver-based treatments, continued monitoring for silver-resistance is warranted.

Methods

Bacterial isolates
After IRB approval, 859 bacterial strains were isolated from patients at a tertiary care hospital in Springfield, Missouri. Species identification was conducted using the VITEK 2® System and initially screened by ability to grow on Ag+ agar.

Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy
SRKP, SREC, and controls were prepared and examined by SEM (JEOL-7600F). Using the modified ASTM designation E2149-01. Non-Silver based Dressing
A non-silver-based dressing utilizing DACC technology was examined as an alternative to silver dressings. DACC dressing function via natural hydrophobic interactions between bacteria and DACC. After exposing the DACC dressing to highly silver-resistant bacteria, SEM images were taken to determine binding.

Results

Figure 1. Preliminary screening of 859 isolates identified 67 samples capable of luxuriant growth on LB agar supplemented with Ag+. These samples were further screened using PCR. Representative MIC images.

Table 1. Out of 859 bacteria isolates, 31 carried at least 1 silver resistant gene. MIC testing revealed most strains displayed little or no increase in silver resistance (200 - 300µM Ag+). However, two isolates (K. pneumoniae and E. cloacae) had significantly higher MIC values (5,500µM Ag+). Silver-resistant Enterobacter cloacae and Silver-resistant Klebsiella pneumoniae bond to DACC dressing.

Figure 2. Representative plate images of the silver-resistant isolate grown on LB agar supplemented with increasing silver concentration over time. (A) 0.0 mM Ag+ at 24 hrs. (B) 3.0 mM Ag+ at 24 hrs. (C) 3.0 mM Ag+ at 48 hrs. The bacteria’s pigment darkened over time when exposed to high concentrations of silver.

Figure 3. SEM of SRKP grown on LB agar supplemented with 3.0 mM Ag+ indicating the presence of silver nanoparticles. Subsequent EDS analysis confirmed the presence of silver localized on the cell surface. Control SEM images of SRKP and SSKP grown in the presence and absence of sub-MIC concentrations of Ag+.

Figure 4. Immobilized antimicrobial testing under dynamic conditions showed the Sil + resistant bacteria were at least 1,000 times more resistant to commercially available Silver-based wound dressings compared to their Sil-negative counterparts. Values are means of 2 independent experiments (n=3).

Summary

1 First clinical bacteria identified to display clinically significant silver resistance expression.
2 A need for increased antimicrobial stewardship and alternatives to silver dressings.
3 DACC’s ability to bind highly silver resistant bacteria addresses resistant and stewardship.
4 Future studies will investigate whether bacteria can share the smaller cryptic plasmids.
5 There is a significant need for non-silver based dressings that can effectively manage bioburden while minimizing resistant risks.