In vitro assessment of the synergistic effects of antibiotics and wound dressings on biofilms from diabetic foot pathogens

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Background

The impact of biofilm in the effective control of wound microbiome is an ongoing dilemma which has seen the use of different treatment strategies. The effects of wound dressings and antibiotics on both planktonic bacteria and biofilms have been separately evaluated in previous studies.[1,2,3,4] Some of the methods used include 96-well microtitre plate assay, 6-well plate assay, isothermal calorimetry, microscopy and the constant depth film fermentation.[1,2,5,7,9]

However, the quest for a more sensitive and reproducible method to mimic the biofilm phenotype is ongoing. One such method uses poloxamer gel to grow biofilm phenotype due to its proven ability to promote sessile growth and maintain the biofilm architecture to mimic clinically conditions.[8]

Aim of Study

The aim of this study was to assess the combined effects of some selected wound dressings (silver-impregnated: Acticoat (ACT) and Silvercel (SIL); and honey-impregnated: Medihoney™ Apinate (MA)) and antibiotics (ceftazdime and levofloxacin) on *Klebsiella pneumoniae* and *Proteus mirabilis* biofilms using a standard agar assay. The ability of poloxamer gel cultures to support sessile growth and maintain biofilm architecture was also assessed in comparison with the standard agar method.

Methods

The two multidrug resistant diabetic foot isolates were initially grown overnight and diluted to final broth suspensions of 10^8 colony forming unit (CFU)/mL.[5] 1 mL volumes of *K. pneumoniae* and *P. mirabilis* suspensions containing ceftazidime (CAZ) and levofloxacin (LEV) at final concentrations of 256, 512, 1024 and 5120 µg/mL were inoculated on Mueller Hinton agar (MHA) and 30% (w/v) Kolliphor® P 407 (poloxamer) gel plates and allowed to dry.

Wound dressings cut into circular shapes (2cm-diameter) were aseptically placed on the agar and gel plates and incubated at 35 – 37° C for 24 hours. ZOIs produced by the 3 antibacterial dressings after 24 hours were measured and compared with a control dressing (Atrauman (ATR) – with no antibacterial activity).

The ZOIs measurements are presented as means (±SEM) and statistically analyzed using GraphPad Prism software.

Results

ZOIs associated with ACT, SIL and MA dressings augmented with CAZ and LEV were compared with no antibiotic that of ATR controls. All three dressings showed significant ($p < 0.05$) biofilm-inhibiting activity against both bacteria at antibiotic concentrations of 1024 and 5120µg/mL with ZOI between 17.5 and 35mm on MHA (Figure 2. (**A**) and **(B**)). Similarly, significant ZOIs on Kolliphor® P 407 gels were between 22 and 30mm at the same concentrations (Figure 2. (**C**) and (**D**)).

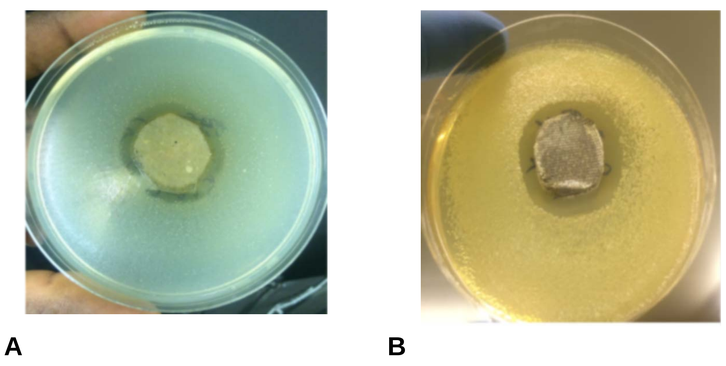

Figure. 1. *P. mirabilis* biofilm phenotypes with ZOI on (**A**). Kolliphor gel and (**B**). MHA

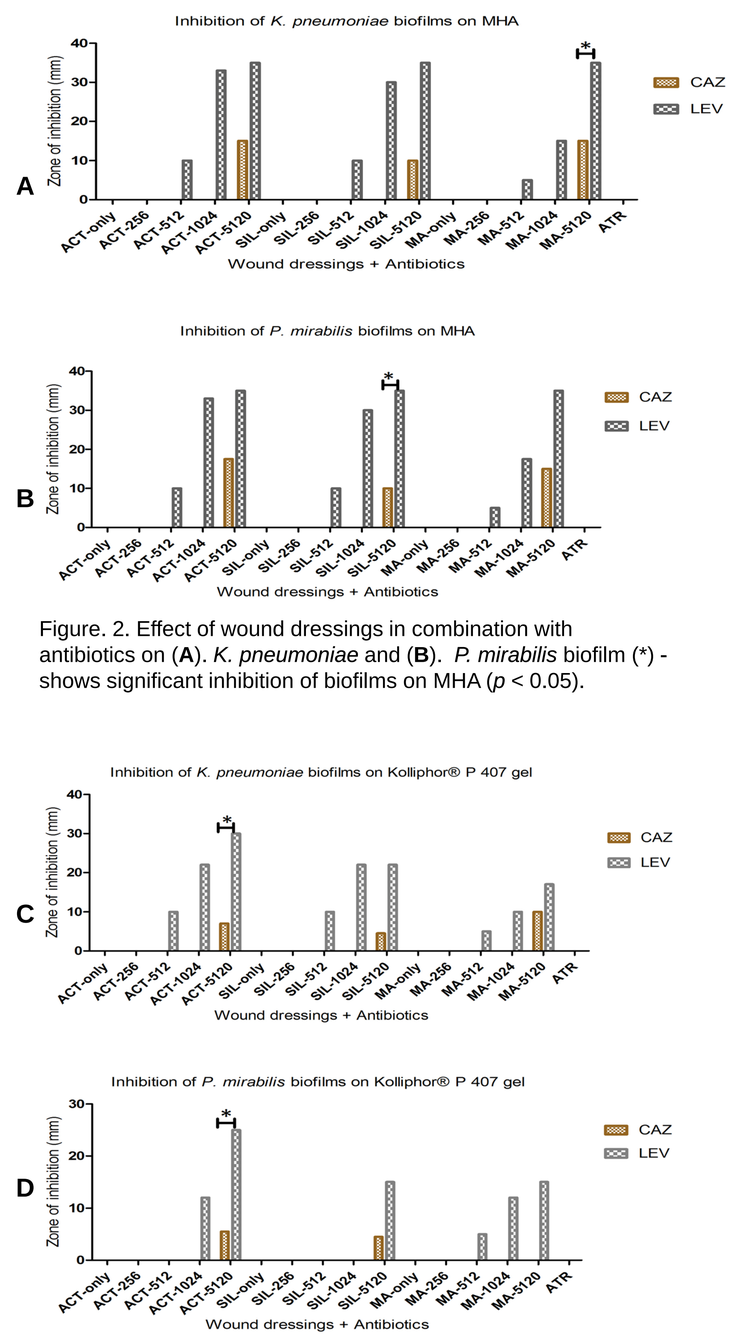

Figure. 2. Effect of wound dressings in combination with antibiotics on (**A**). *K. pneumoniae* and (**B**). *P. mirabilis* biofilm (*) - shows significant inhibition of biofilms on MHA ($p < 0.05$).

Figure. 2. Effect of wound dressings in combination with antibiotics on (**C**). *K. pneumoniae* and (**D**). *P. mirabilis* biofilms. (*) - shows significant inhibition of biofilms on Kolliphor gel ($p < 0.05$)

Discussion

This study has demonstrated that clinical strains of *K. pneumoniae* and *P. mirabilis* biofilms are highly resistant to current antimicrobial agents on the market. They are however sensitive to higher concentrations of antibiotics which are not clinically applicable.

In this study, ACT produced greater ZOIs against both *K. pneumoniae* and *P. mirabilis* biofilms than SIL and MA. This suggests that the continuous treatment of infected diabetic foot with ACT can improve healing.

It was also observed that, all 3 dressings were less effective on Kolliphor-grown biofilms than on MHA. This is because standard agar only promotes the growth of colony forming units in a semi-sessile state as previously described.[10]

As already established and confirmed in this study, biofilms are difficult to treat.[5,6] Their presence in wounds prolong healing, increase cost of treatment and affect the quality of life of affected individuals. Therefore, the effective management of chronic wounds must encompass a holistic approach that includes antimicrobial chemotherapy, surgical debridement, physiotherapy and periodic reviews.

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