Cinnamaldehyde increases the susceptibility of quorum-sensing-mediated biofilms to conventional antibiotics

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Background

Bacterial communication, also known as quorum sensing (QS) has been linked to regulate cellular processes such as bioluminescence, antibiotic production, antibiotic resistance, biofilm formation and virulence expression.[1,2,3] Previous studies have demonstrated that cell-to-cell signalling is essential in the differentiation of bacterial cells into complex multicellular structures called biofilms.[1] Biofilms are enclosed in a self-produced extracellular matrix called extracellular polymeric substance (EPS).[5, 6]

The EPS mostly made up of a polysaccharide biopolymers, proteins or DNA provides stability to the biofilms. The EPS also acts as a diffusion barrier that inhibits and prevents the entry of large antimicrobial peptides and antibiotics thereby conferring resistance to the biofilm.[6]

QS has also been found to regulate bacterial cell surface interactions during biofilm formation.[7] The inhibition of quorum sensing has been considered as an alternative strategy to antibiotic treatment that can be useful in the treatment of chronic infections through the prevention and disruption of biofilm formation. Quorum sensing inhibitors (QSI) such as cinnamaldehyde (Cinn), baicalin hydrate and hamamelitannin have been found to influence EPS production and biofilm;[4] a fundamental step in the development of anti-biofilm strategies.

Aim of Study

In this current study, the synergy between a quorum sensing inhibitor (cinnamaldehyde) and two antibiotics (ceftazidime and levofloxacin) was evaluated in an attempt to develop a strategy for biofilm disruption using the high-throughput Physiology and Genetics (P & G) minimum biofilm eliminating concentration (MBEC™) assay.

Methods

Two multidrug resistant diabetic foot isolates, *Klebsiella pneumoniae* and *Proteus mirabilis* biofilms of initial broth suspensions of 10^8 colony forming units (CFU)/mL, cultivated on the pegs of the MBEC device were challenged with 5120 µg/ml of ceftazidime (CAZ) and levofloxacin (LEV) in a double dilution assay in the presence of 500 µM cinnamaldehyde (Cinn).

The rest of the test was carried out following the manufacturer's (Innovotech, Alberta, Canada) instructions.

Values obtained from the assays are presented as means (±SEM) at a confidence interval of 95% and statistically analyzed using GraphPad Prism software.

Results

The minimum inhibitory concentrations (MIC) in the presence of sub-inhibitory concentration of Cinn for CAZ and LEV were 0.125% (640 µg/mL) and 0.0625% (320 µg/mL) respectively with no significant bacterial growth on LB agar. The MICs for CAZ and LEV were previously determined as > 1280 µg/mL for *P. mirabilis* and > 2560 µg/mL for *K. pneumoniae* respectively.

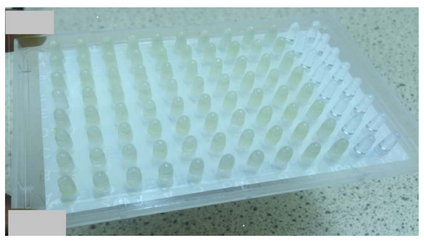

Figure 1. MBEC peg lid showing biofilm growth after 24 hours of incubation (This study).

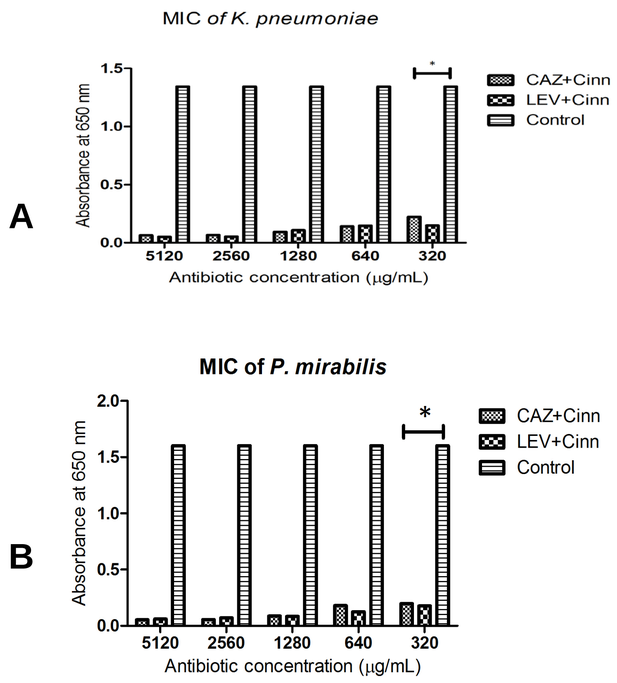

Figure 2. MIC values for ceftazidime and levofloxacin determined in the presence of cinnamaldehyde for **A**. *K pneumoniae* and **B**. *P. mirabilis*. * The combined concentrations of CAZ+Cinn and LEV+Cinn were found to significantly inhibit biofilm formation ($p < 0.01$)

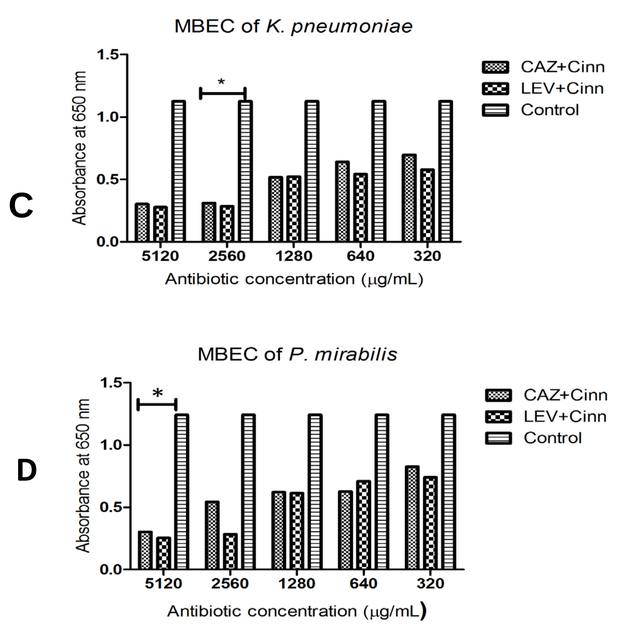

Figure 2. MBEC values for ceftazidime and levofloxacin determined in the presence of cinnamaldehyde for **C**. *K pneumoniae* and **D**. *P. mirabilis*. * The combined concentrations of CAZ+Cinn and LEV+Cinn were found to significantly reduce biofilm ($p < 0.01$)

The MBECs for CAZ and LEV were above 5120 and 2560 µg/mL respectively which yielded over 70% reduction in both *K. pneumoniae* and *P. mirabilis.* The MIC for Cinn against *P. mirabilis* and *K. pneumoniae* were also determined to be > 1000 µM.

Discussion

Quorum sensing systems have been found to control a variety of microbial phenotypic expressions through cell-density dependent regulation of gene expression. The ability to modulate QS systems in bacteria is essential to either maximize benefits or minimise their adverse effects.[9]

Cinnamaldehyde, a phenylpropanoid compound which acts as an antioxidant and antibacterial agent has widely been used for medical and biotechnological purposes.[8] It is a natural compound produced from cinnamon bark and cassia oils.

In this study, the combined effects of Cinn and conventional antibiotics (CAZ and LEV) on multidrug resistant diabetic foot biofilms were evaluated. On their own, CAZ and LEV are only effective at high concentrations (up to 100-fold their MICs). Though sub-inhibitory concentrations of Cinn significantly increased the susceptibility of quorum sensing-mediated biofilms to CAZ and LEV, their MBEC values still remain outside the therapeutic range.

The above results indicate the possibility that the synergy between antimicrobial agents may lead to biofilm eradication.

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