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Activity of combinations of an enzymatic cocktail (CDD) with Tobramycin, Amikacin , Ciprofloxacin, Moxifloxacin, Vancomycin or Linezolid against biofilms of clinical isolates from different ESKAPE pathogens

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DETECT & DISSOLVE BIOFILM MATRIX

Introduction

The capacity of bacteria to form biofilms is a major cause for nosocomial infections. *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are ESKAPE pathogens that are frequently associated with biofilm infections. Antibiotics and antiseptics are usually considered as weakly active against bacteria growing in biofilms as they have only a poor access to bacteria. We developed an enzymatic cocktail containing DNase and polysaccharidases (CDD) which allows the dispersion of bacterial biofilms of species mentioned above. We examined whether CDD could restore activity of antibiotics against these biofilm, taking TOB, AMK, CIP, MXF, VAN and LDZ as examples.

Materials and Methods

We used 6 clinical isolates of *ESKAPE pathogens* (coming from infections on medical devices). Biofilms were grown for 24 h in 96-wells plates and then exposed for 24 h to Cmax of CIP, TOB, AMK, MXF, VAN and LDZ, alone or in combination with CDD. Bacterial viability in biofilms was quantified using the redox indicator resazurin as previously described (1). Toxicity induced by CDD (5X concentrated) was tested using a predictive test methods (detailed in the guidelines TG 439 (2) to identify a potential irritant effect of CDD using *in vitro 3D* Reconstructed Human Epidermis (RHE/S/17, SkinEthicTM, Lyon, France). Cell viability was determined by the MTT colorimetric and quantitative assay and an analysis of the tissues morphologies by histology (haematoxylin/eosin staining) (H&E) was performed in triplicates.

Results

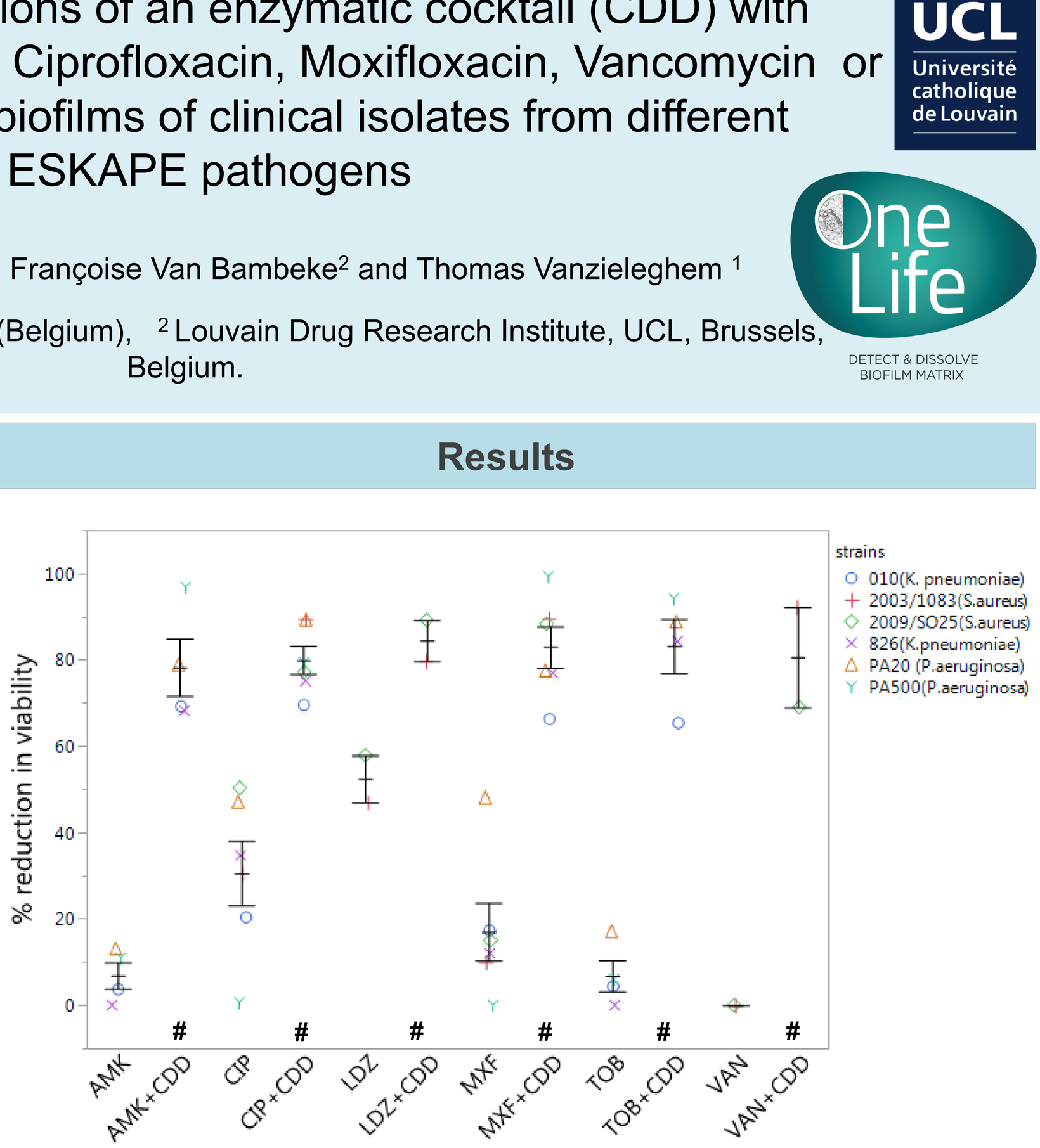

Figure1 : Percentage of bacterial viability reduction in the biofilm after antibiotics treatment alone or combined with CDD enzyme cocktail in a static biofilm model # highlights combinations for which the mean reduction was higher than that observed for drugs alone (Statistical analysis: one-way ANOVA with Tukey's post-hoc test) reduction in viability compared to untreated control

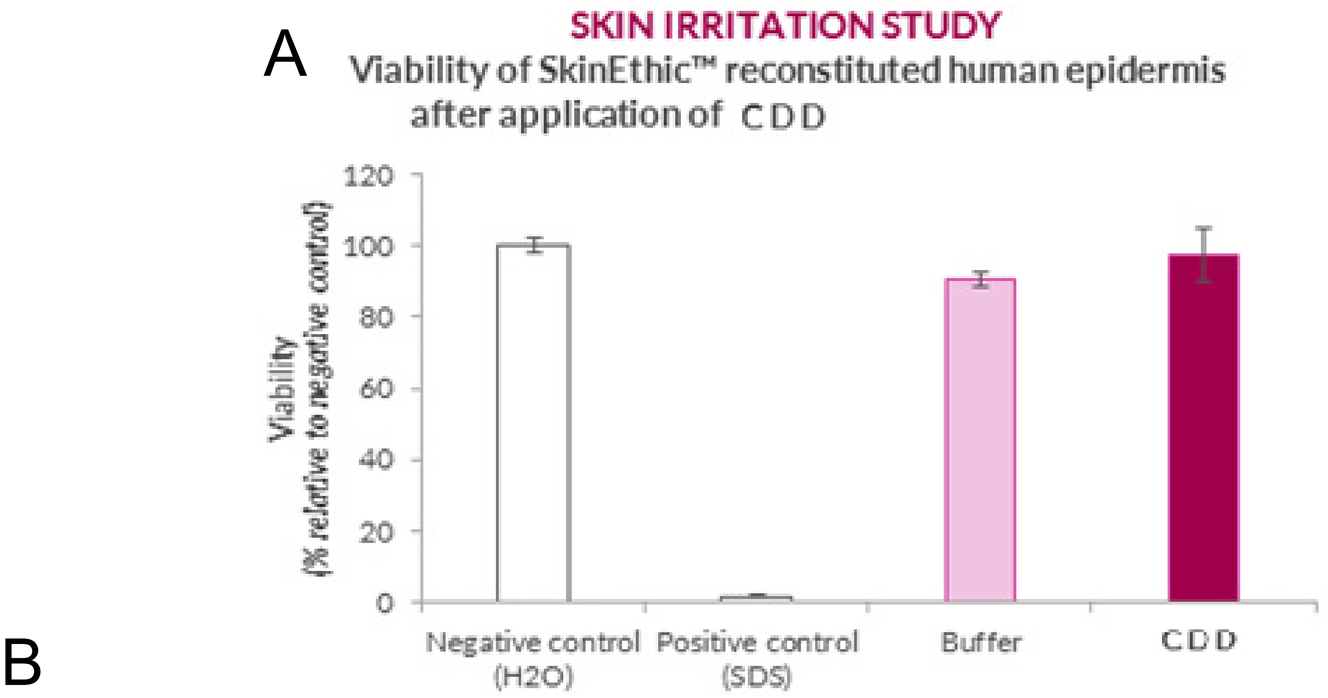

Figure 2: Assessment of CDD toxicity against reconstituted human epidermis. skin Irritation test (A), and Histological analysis (B) performed on reconstituted human epidermis (RHE) treated with CDD. These tests are in accordance with the guidelines of the OECD irritation test (OECD 439)

Conclusions

Combining CDD with 6 antibiotics belonging to 4 classes proves highly synergistic against biofilms of 6 clinical isolates. This opens perspectives for testing these enzymes as adjuvant for the treatment of biofilm infections

References

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2. OECD GUIDELINES FOR THE TESTING OF CHEMICALS: IN VITRO SKIN CORROSION: RECONSTRUCTED HUMAN EPIDERMIS (RHE) TEST METHOD. 1-33. 2014.

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