Activity of combinations of an enzymatic cocktail (CDD) with antibiotics against biofilms of clinical isolates of ESKAPE pathogens

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“ESKAPE” Pathogens

- Enterococcus faecium
- Staphylococcus aureus
- Klebsiella pneumoniae
- Acinetobacter baumannii
- Pseudomonas aeruginosa
- Enterobacter species

There is no Escape from the ESKAPE Pathogens

DEVICE-RELATED INFECTIONS
- Ventricular derivations
- Oro-tracheal tubing
- Vascular central
- Prosthetic cardiac valves and pacemakers
- Peripheral vascular
- Urinary catheters
- Orthopedic prosthesis

CHRONIC INFECTIONS
- Oral infections
- Endocarditis
- Cystic fibrosis
- Urinary tract infections
- Chronic wounds
Biofilms facts

- 99% of bacteria grow as aggregated, sessile communities (biofilm)
- Bacteria within biofilm are highly protected and highly resistant to antibacterial treatments (up to 1000 times more resistant to antibiotics than planktonic bacteria)
- Bacteria within biofilm are genetically different than bacteria in the planktonic state
- NIH estimates more than 80% of infections in humans are caused by microbial biofilm.
Current therapy and prophylaxis of Biofilm Infections

**Physical and surgical methods**: in cases of infected medical devices, removal of the device is often necessary to treat the infection.

**Antimicrobial therapy**: poor access
β-lactams, fluoroquinolones, aminoglycoside,

**Preventing microbial attachment**

**Goal of the study**
Develop a new enzymatic combination to specifically restore activity of antibiotics and eradicate ESKAPE biofilm.
Methods

**In vitro** static biofilm model

- Assessment of enzymatic activity against Biofilm matrix
  - Crystal violet assay

**Ex vivo** biofilm model:
- Human urinary catheter

- Assessment of enzymes-antibiotics activity against bacterial viability
  - Resazurin assay
Design of a broad spectrum enzymatic cocktail

![Graph showing biofilm removal percentages for E.coli and P.aeruginosa with different enzymes.](image-url)
Design of a broad spectrum enzymatic cocktail

**E. faecalis**

- Amylase
- Cellulase
- Dispersin B
- **Dnase I**
- Lipase
- Mannanase
- Protease
- Viscozyme

**S. aureus**

- Amylase
- Cellulase
- Dispersin B
- **Dnase I**
- Lipase
- Mannanase
- Protease
- Viscozyme
Design of a broad spectrum enzymatic cocktail

**K.pneumoniae**

**S.epidermidis**

![Graphs showing biofilm removal for K. pneumoniae and S. epidermidis using different enzymes.](image-url)
Percentage of biofilm removal after exposure to combinations used for enzymatic cocktail design

![Graph showing biofilm removal percentages for different strains after exposure to enzymatic cocktail CDD.](chart.png)

**Legend:**
- E.coli
- E.faecalis
- E.faecalis
- K.pneumoniae
- P.aeruginosa
- S.aureus
- S.epidermidis

**Enzymatic cocktail CDD**
Percentage of biofilm removal after exposure to enzymatic cocktail CDD in *In vitro* biofilm models

*In vitro static biofilm model*
Percentage of biofilm removal after exposure to enzymatic cocktail CDD in *Ex vivo* biofilm models

*Ex vivo* biofilm model: Human urinary catheter

Crystal violet assay

Biofilm removal %

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# 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
CDD showed highest biofilm removal against ESKAPE biofilms of *S.aureus* (89%), *S.epidermidis* (94%), *P.aeruginosa* (83%), *E.faecalis* (81%), *E.coli* (74%) and *K.pneumoniae* (55%)

At human Cmax TOB, AMK, MXF, CIP, VAN, LDZ were weakly active against bacteria growing in biofilms

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.
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Thank you for your attention!

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