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The antifungal caspofungin (CAS) increases moxifloxacin (MXF) activity against *Staphylococcus aureus* biofilms *in vitro* or in a mice subcutaneous model

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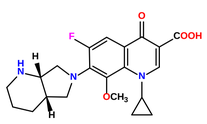
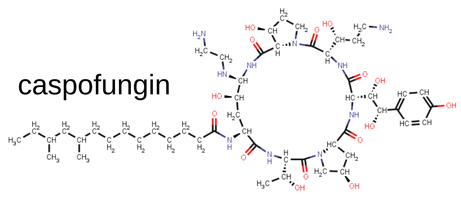
Introduction

Staphylococcus aureus is an important human pathogen causing chronic infections that are difficult to treat. Biofilm contributes to the persistence of infections, by protecting bacteria from immune system and antimicrobial agents. We showed that many antibiotics are poorly active on biofilms [1], especially against clinical isolates from persistent infections [2]. In a preliminary screening of combinations of moxifloxacin (MXF) with drugs selected based on their amphiphilic character, we observed that the antifungal caspofungin (CAS) was synergistic. Our aim was now to test this combination on biofilms preformed on catheters *in vitro* and *in vivo*. [2]



moxifloxacin

caspofungin



Materials and Methods

*B*iofilms were grown inside 1cm polyurethane catheters at 37°C for 24h (initial inoculum: 5.10^6 cells/ml).

In vitro, 7 clinical isolates strain were used. Biofilms grown on catheters were placed in 24-well plates, incubated with MXF (10mg/L); CAS (80mg/L) or MXF/CAS for 48 h. Catheters were washed, sonicated, and CFUs/catheter were counted.

In vivo, 5 catheters with pre-grown biofilms of the 2011S027 clinical isolate were implanted subcutaneously in the back of mice. Animals were treated intravenously with MXF (40 mg/kg twice daily), CAS (4 mg/kg/day) or with the MXF/CAS combination during 7 days. CFUs/catheter were counted.

Scanning electron microscopy (SEM) of *in vivo* biofilms: catheters were retrieved from the back of the mice after 7 days of treatment, fixed in 2% paraformaldehyde–2.5% glutaraldehyde and, after washing steps with PBS, postfixed with 1% osmium tetroxide, rinsed with PBS, and dehydrated using a series of washes with ethyl alcohol (30 to 100%). Representative SEM images were obtained as described previously [3].

References

1-Bauer, J., Siala, W., Tulkens, P. M. & Van Bambeke, F. (2013) *Antimicrob. Agents Chemother.* **57**, 2726-2737.

2-Siala, W., Mingeot-Leclercq, M. P., Tulkens, P. M., Hallin, M., Denis, O. & Van Bambeke, F. (2014) *Antimicrob. Agents Chemother.* **58**, 6385-6398.

3- Kong, F., Kucharíková, S., Van Dijck, P., Peters, B.M., Shirtliff M.E. & Jabra-Rizka, M.A. (2015) *Infection and immunity*. **83**, 604-613.

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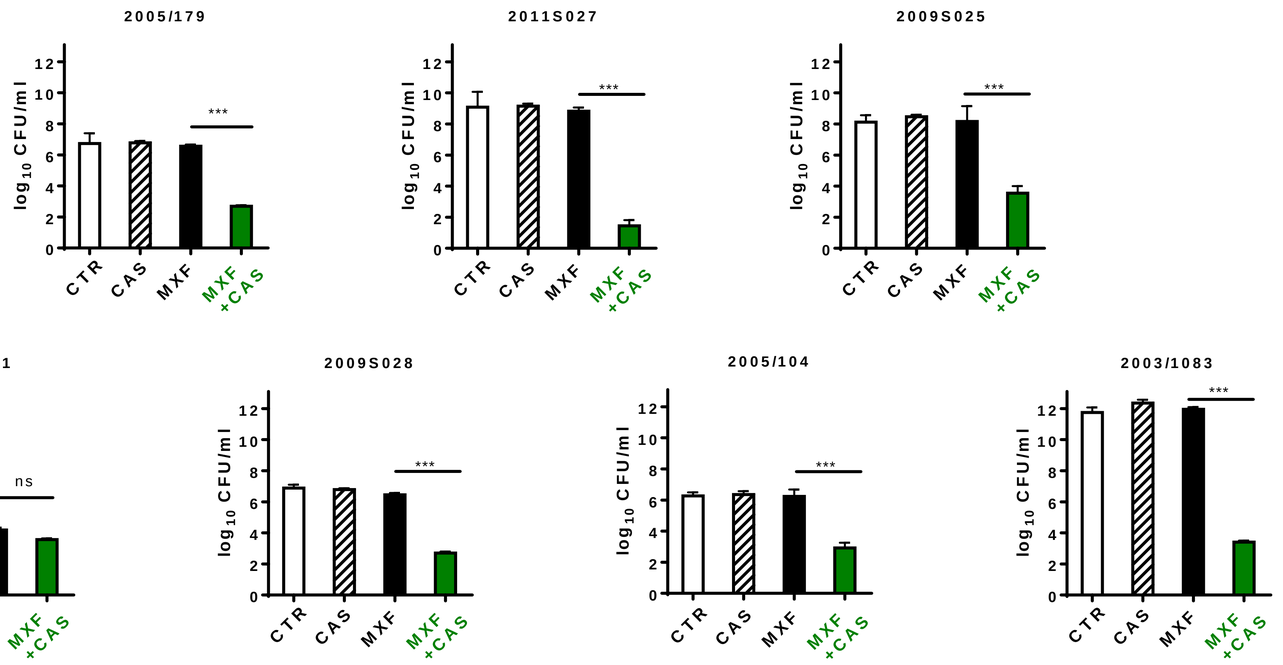
This poster will be available after the meeting at http://www.facm.ucl.ac.be/posters

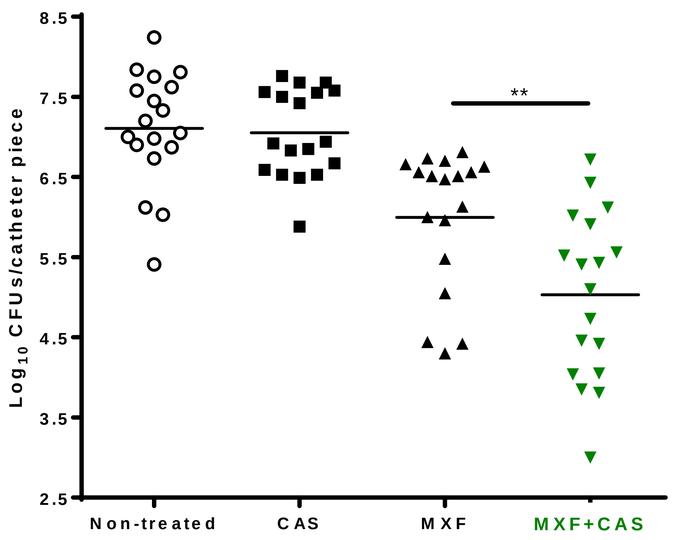
Results

In vitro



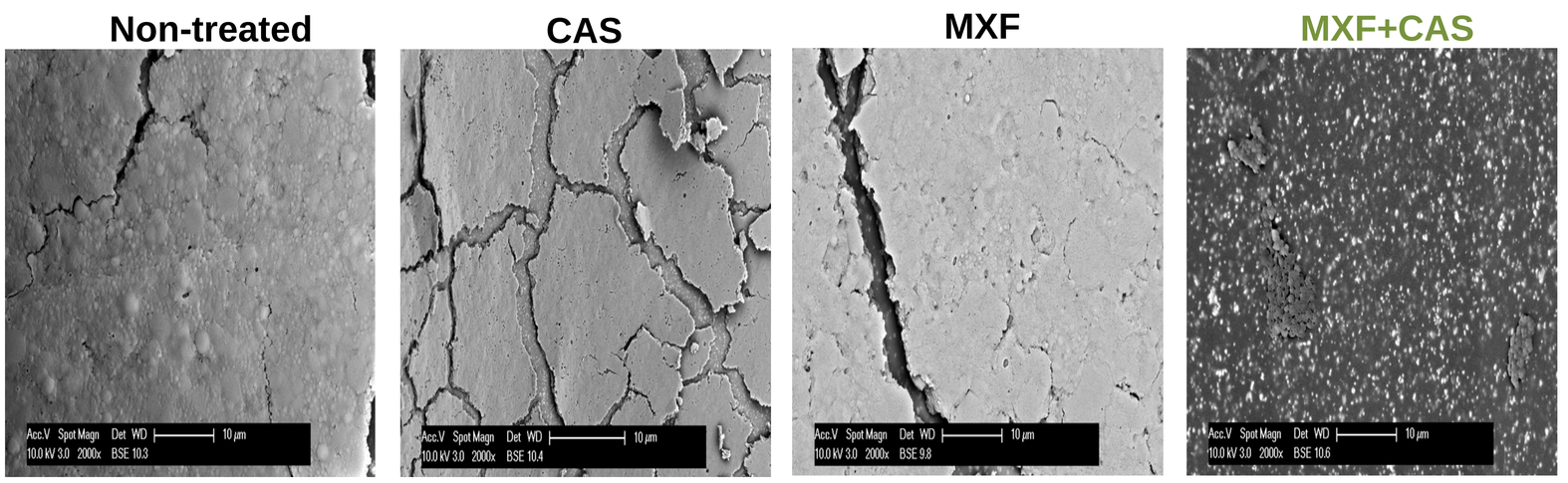
Effect of MXF, CAS or MXF-CAS combination on biofilms of clinical strains in catheters in vitro model
Results represent $\log_{10}$ CFU per catheter (3 catheters per treatment).
Statistical analysis (ANOVA): Differences between the MXF-treated group and the MXF+CAS-treated group were statistically significant in 6 out of 7 strains (P value≤ 0.0002).

In vivo



Effects of the administration of MXF, CAS, and MXF+CAS on clinical isolate (2011S027) biofilms developed in subcutaneous mice model.
The horizontal lines indicate the median values for $\log_{10}$ CFU (7.12; 6.93; 6.49; 5.25 for the untreated, CAS, MXF and MXF-CAS respectively).
Statistical analysis (ANOVA): p <0.01 when comparing MXF-treated group and MXF-CAS group

Scanning electron microscopy



Representative SEM images of catheters implanted in mice infected with clinical isolate 2011S027 biofilm
Images demonstrating the massive biofilm matrix formed on the surface of the catheters after 7 days treatment. The untreated, CAS and MXF treated group showed a thick biofilm structure and few single cells visible. The MXF+CAS treated group showed a destroyed biofilm structure, less biofilm was visible and cells in patches can be seen spread on the surface.

Conclusions

Combining MXF with CAS proves highly synergistic *in vitro* and *in vivo* against staphylococcal biofilms of clinical strains.

This opens promising perspectives for new therapeutic strategies directed towards *S. aureus* biofilm-related infections.