

Influence of biofilm age, media and exposure time on biofilm removal by vancomycin and tobramycin

Xiaofeng Chen¹, Yijuan Xu^{1,2}, Heinz Winkler³, Trine Rolighed Thomsen^{1,2}

Xiaofeng Chen
xch@bio.aau.dk

1. Center for Microbial Communities, Aalborg University, Aalborg, Denmark
2. Life Science Division, The Danish Technological Institute, Aarhus, Denmark
3. Osteitis Centre, Privatklinik Döbling, Vienna, Austria

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Introduction

Osteomyelitis is an inflammatory process with bone destruction caused by infecting microorganisms. *Staphylococcus aureus* is the most common organism in osteomyelitis.

Chronic osteomyelitis is especially hard to treat due to biofilm formation. Comparing to planktonic bacteria, biofilm is known to be difficult to remove.

Aim

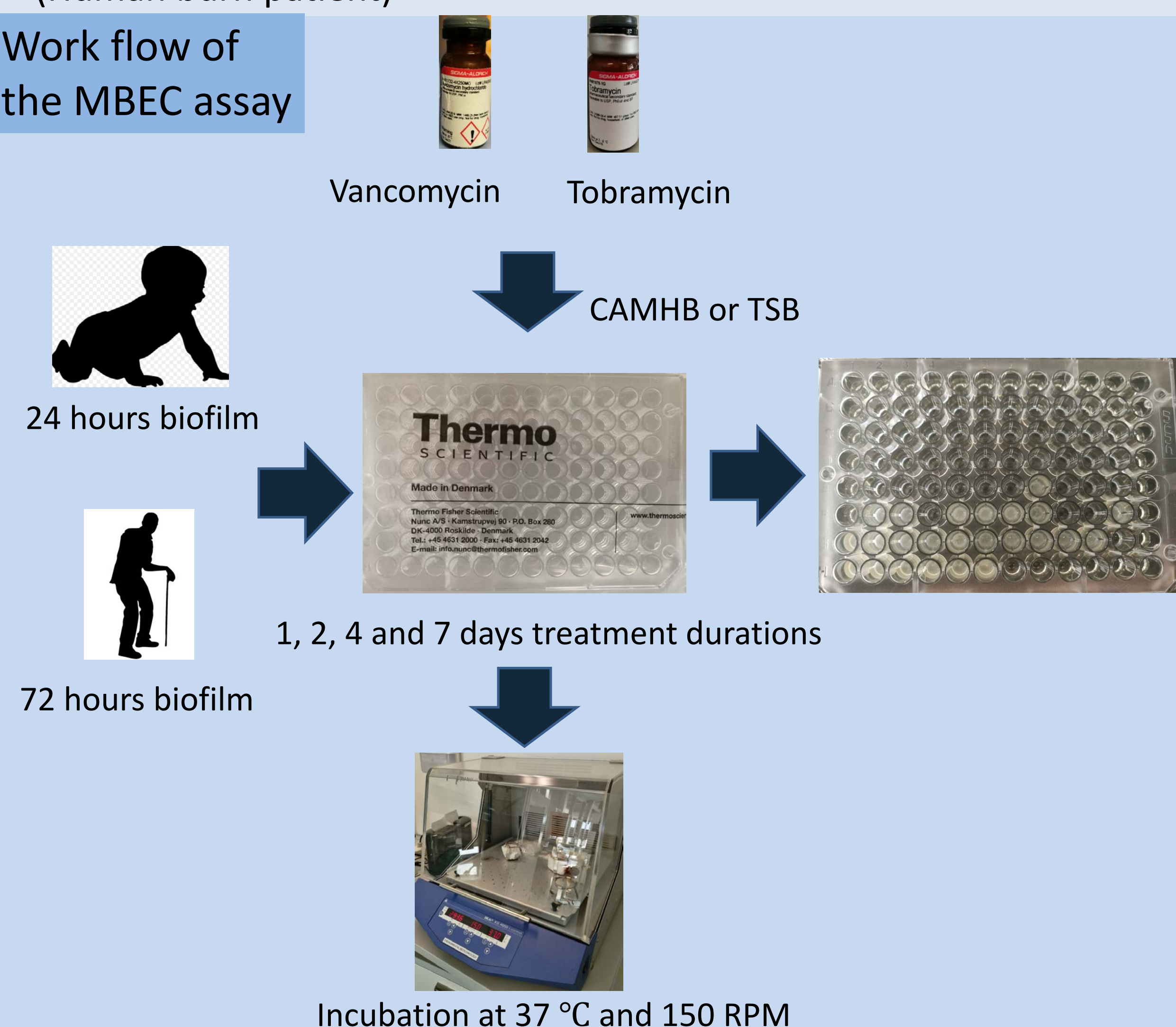
In this study, we investigated the possibility of eradicating biofilm *in vitro* by using vancomycin and tobramycin in clinically relevant concentrations. Secondly, we studied influence of biofilm age, growth media, and antibiotics exposure time on the biofilm removal.

Methods

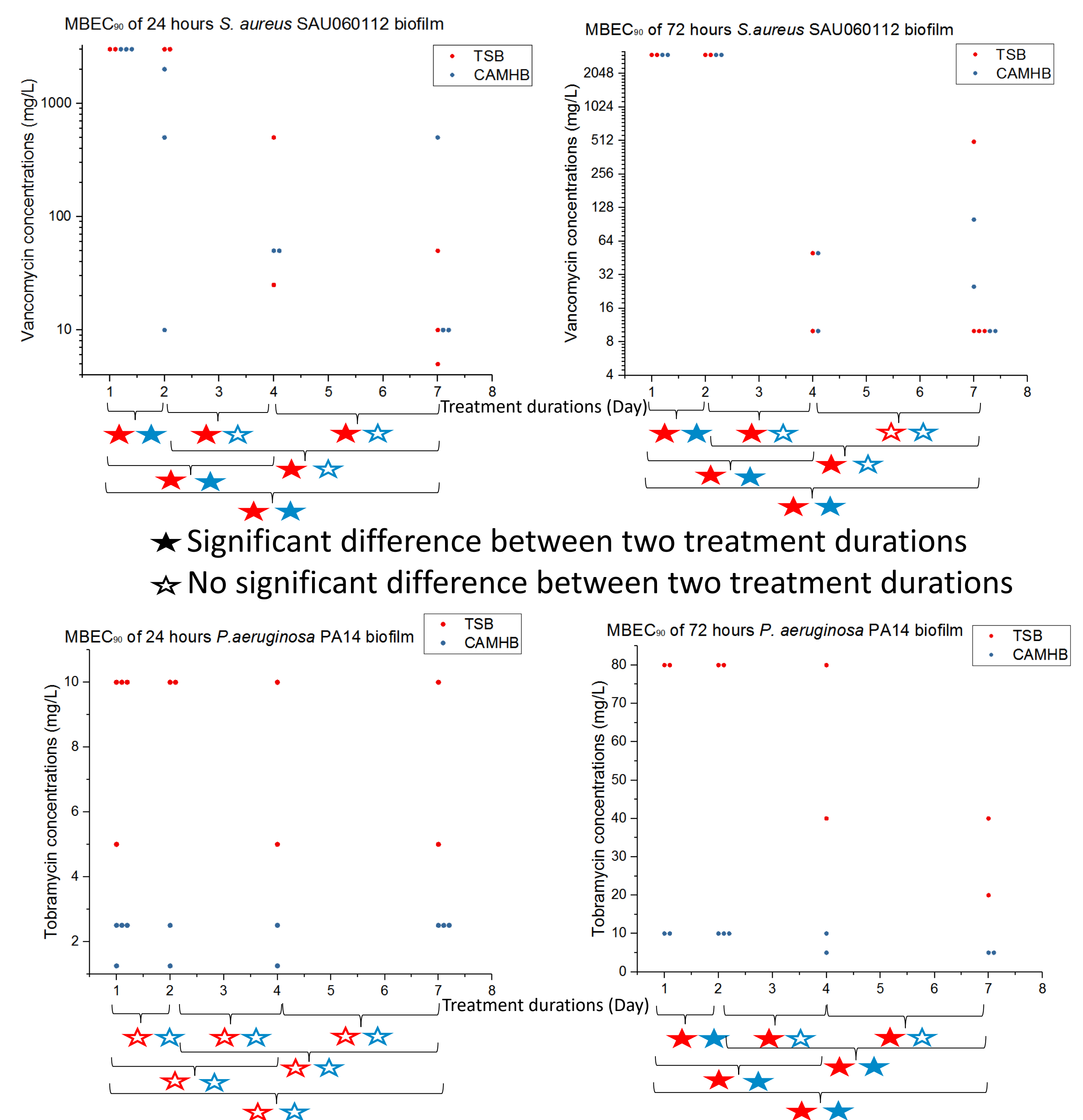
Two strains were tested by the MBEC™ assay (a high throughput screening assay to determine efficacy of antimicrobials against biofilm) with following conditions. Each test was performed at least twice. Minimum inhibitory concentration (MIC), minimum biocidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) were determined in each test.

Strains	Antibiotics	Conditions		
		Media	Biofilm growth (hour)	Treatment durations (day)
<i>S. aureus</i> SAU060112 (Prosthetic joint infection)	Vancomycin	TSB, CAMHB	24, 72	1, 2, 4, 7
<i>P. aeruginosa</i> PA14 (Human burn patient)	Tobramycin	TSB, CAMHB	24, 72	1, 2, 4, 7

Work flow of the MBEC assay



Results



MBEC₉₀ is the lowest concentration of antibiotics which removes all biofilms in 90% or more replicates.

Factors	Biofilm removal efficacy analysis by binary logistic regression
Media	In both <i>S. aureus</i> and <i>P. aeruginosa</i> strains, biofilm challenged in TSB was more difficult to remove than in CAMHB media.
Biofilm age	In both <i>S. aureus</i> and <i>P. aeruginosa</i> strains, 72 hours biofilm was more difficult to remove than 24 hours.
Treatment duration	<i>S. aureus</i> biofilm removal efficacy was enhanced generally when treatment duration was extended except 72 hours biofilm from 4 days to 7 days treatment durations when tested in TSB. In CAMHB, the efficacy was enhanced from 1 day to 2 days treatment durations but not further enhanced thereafter. <i>P. aeruginosa</i> 24 hours biofilm removal efficacy was not enhanced with longer treatment durations while the efficacy of 72 hours biofilm was always enhanced in TSB and enhanced from 1 day to 2 days treatment durations in CAMHB media.

Conclusions

- It is possible to remove biofilm by antibiotics using clinically relevant concentrations *in vitro*.
- Choice of test conditions (biofilm age, media and treatment durations) can influence MIC, MBC and MBEC values. Therefore, for future clinical use, a standardized MBEC method for a specific strain with defined conditions is needed.