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Sub-inhibitory concentrations of antibiotics increase tolerance of *Staphylococcus aureus* to different classes of antibiotics

Poster A-532

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Abstract

Background: During antibiotic treatment, bacteria are likely exposed to varying concentrations of the drug. Exposure to sub-inhibitory concentrations of antibiotics (SICAB) has revealed mechanisms in bacteria that induce tolerance against the same drug. The information on response to SICAB is generally very limited and the **aim** of the present study was to investigate whether exposure to SICABs belonging to different classes will increase tolerance to the antibiotic itself and/or antibiotics belonging to other functional classes. We used the pathogen *Staphylococcus aureus* as our model organism.

Methods: We used a microtiter-plate approach and measured OD_{600} every 30 min for a 16h period. Cultures were grown for 5 generations with and without SICAB before transfer to microtiter wells containing either no antibiotic or rifampicin (RIF), erythromycin (ERM), vancomycin (VAN) at 2×MIC, 1×MIC, 0.5×MIC and 0.1×MIC.

Data: We exposed *S. aureus* to sub-inhibitory concentrations (SIC) of antibiotics belonging to 4 different classes: inhibitors of translation, transcription, DNA replication and cell wall synthesis and investigated the tolerance to subsequent exposure to antibiotics from 3 different functional classes: inhibitors of transcription, translation and cell wall synthesis.

When pre-exposed to SIC of translation and transcription inhibitors and subsequently exposed to inhibitory concentrations of RIF, ERM and VAN the cells had in all cases both higher growth rate and final yield compared to cells that were not pre-exposed. This data suggests that small doses of certain antibiotics induce a cellular response which promotes a general tolerance to antibiotics.

In contrast, pre-exposure to SIC of cell wall inhibitors only increased tolerance against other cell wall inhibitors and only when certain representatives were used for the pre-exposure. This might indicate a possible cell wall specific response.

Summary and conclusions: We have demonstrated that pre-exposure to SICABs belonging to translation and transcription inhibitors induce a cellular response that renders the cells more tolerant to a range of different antibiotics with diverse mode of actions.

Introduction

While extensive research has focused on how bacteria develop resistance to antibiotics, there has been little focus on the bacterial response to sub-lethal concentrations of antibiotics. Such low concentrations are likely to occur in the external environment, where fungi and bacteria produce antibiotics, but will also occur during treatment of infections, where the actual concentrations will depend on numerous factors, including dosage and distribution in tissue. Some studies have shown that bacteria do respond specifically to non-lethal antibiotic exposure[1,2]. Therefore, we would like to understand how bacteria sense the presence of antibiotics and what the biological consequences of low dose antibiotic exposure are.

We have chosen to investigate this issue using the model organism *Staphylococcus aureus*, which is renowned for its remarkable ability to develop resistance to antibiotics. This bacterium causes a multitude of clinical conditions and is a major problem in relation to hospital-acquired infections that may be life threatening. We have exposed *S. aureus* to low concentrations of antibiotics that target important steps in the lifecycle of the bacterium and analyzed the impact of this exposure on growth.

Methods

S. aureus 8325-4 was cultivated in TSB for 5 generations with or without sub-inhibitory concentrations of antibiotics (SICAB) at 37°C to OD_{600} of approx. 0.5. Cultures were then diluted 100 times when transferred to microtiter-wells containing TSB with no antibiotic or rifampicin (RIF), erythromycin (ERM), vancomycin (VAN) at 2×MIC, 1×MIC, 0.5×MIC and 0.1×MIC. Samples were incubated at 37°C for 16 h and OD_{600} was measured every 30 minutes. Samples were run in triplicates.

SICAB was estimated as the highest concentration where the optical density after 1 h of antibiotic treatment was no more than 15% less than the optical density of the control culture. SICAB values for the following antibiotics have been determined: rifampicin (0.008 µg/ml), erythromycin (0.016 µg/ml), ampicillin (0.100 µg/ml), ciprofloxacin (0.05 µg/ml), puromycin (6 µg/ml), gentamycin (0.02 µg/ml) and vancomycin (1 µg/ml).

Table 1: Evaluation of growth rate and final yield of 8325-4, when pre-exposed to SICAB and subsequently grown in media containing RIF, ERM or VAN (2×MIC, MIC, ½×MIC and 1/10×MIC). "+" indicates a higher growth rate and/or final yield compared to cultures not pre-exposed to SICAB.

SICAB	MoA	RIF	ERM	VAN
Rifampicin	Inhibits DNA dependent RNAP	+	+	+
Erythromycin	Binds to 50S	+	+	+
Ampicillin	Inhibits PG-crosslinking	-	-	+
Ciprofloxacin	Inhibits DNA gyrase	(+)	(+)	+
Puromycin	Resembles the 3' end of the aminoacylated tRNA	+	-	+
Gentamycin	Binds to 30S	+	-	+
Vancomycin	Prevents the incorporation of the NAM/NAG-peptide subunits into the peptidoglycan matrix	-	-	-

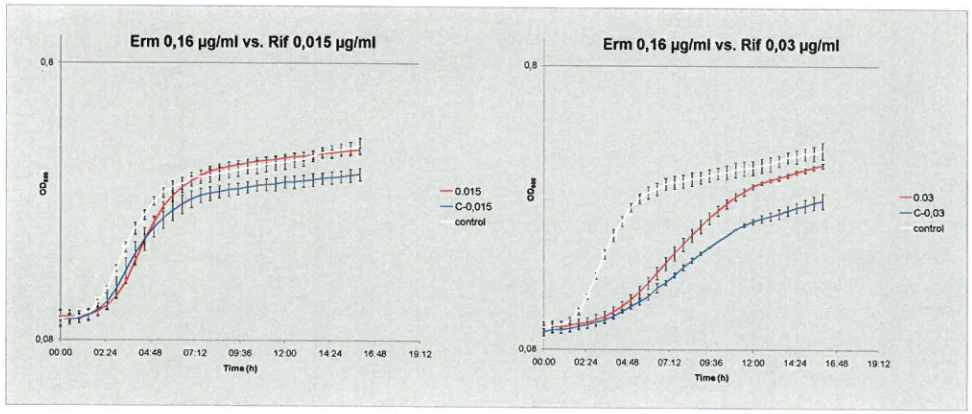

Figure1: Growth of *S. aureus* in TSB with rifampicin. Strains have been pre-cultured for 5 generations in either TSB (blue) or TSB with SIC of erythromycin (red). Control strains (white) have been cultured in TSB. OD_{600} was measured every 30 minutes.

Results

Growth rate and final yield have been evaluated after having pre-exposed *S. aureus* to SICAB belonging to either of 4 different antibiotic classes: inhibitors of translation, transcription, DNA replication and cell wall synthesis, and subsequently cultured the bacteria in TSB with antibiotics from one of 3 different functional classes: inhibitors of transcription, translation and cell wall synthesis (Table 1).

When bacteria were pre-exposed to SIC of translation and transcription inhibitors and subsequently exposed to inhibitory concentrations of rifampicin, erythromycin and vancomycin the cells had in most cases both higher growth rate and final yield compared to cells that were not pre-exposed (Figure 1, Table 1). Increased tolerance to rifampicin and vancomycin was obtained after pre-exposure to all translational and transcriptional inhibitor tested. Increased tolerance to erythromycin was only observed after pre-exposure to erythromycin itself.

In contrast, pre-exposure to SIC of cell wall inhibitors did not increase tolerance to antibiotics. Only when bacteria were pre-exposed to ampicillin we observed an increased tolerance against the cell wall inhibitor vancomycin.

Conclusions

We have demonstrated that pre-exposure to SICABs belonging to translation and transcription inhibitors allows *S. aureus* to increase growth rate and reach a higher cell density upon subsequent exposure to inhibitory concentrations of antibiotics, when compared to cells that receive no pre-SICAB treatment. This suggests that pre-exposure to small doses of certain antibiotics induces a cellular response, which promotes an increased tolerance to antibiotics belonging to not just one but to several classes of antibiotics with very different mode of action. Current work is focused on revealing the underlying mechanisms behind this phenomenon.

References:

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