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P1832 First whole-cell assay to study the time-course of beta-lactams binding to penicillin binding proteins in *Acinetobacter baumannii*

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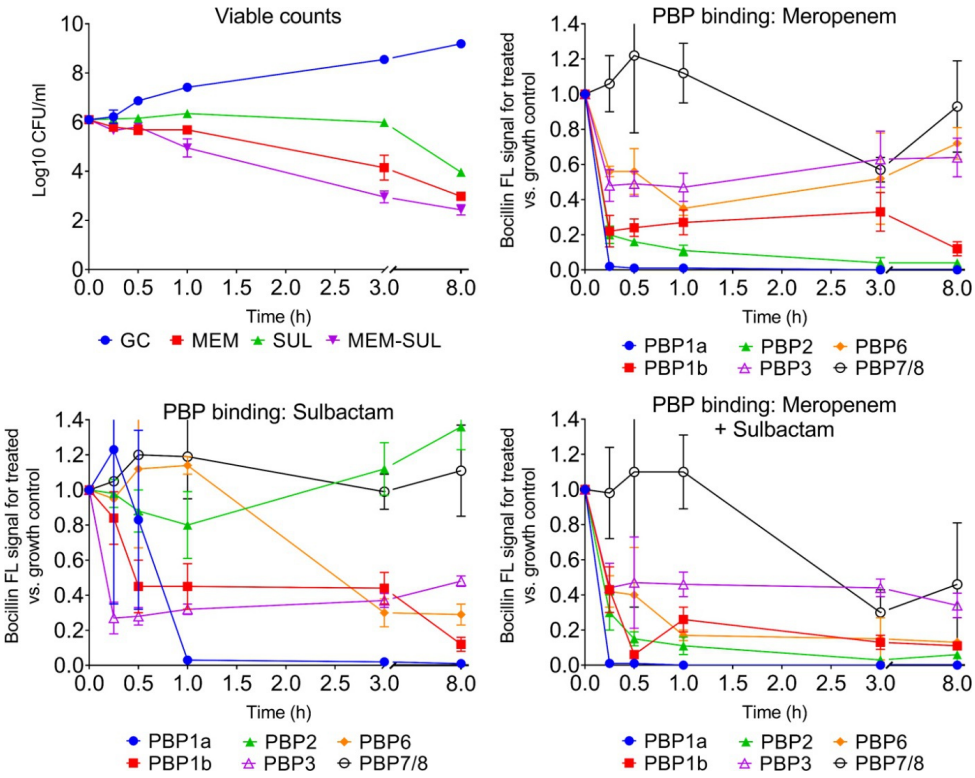
Background: All β-lactam antibiotics bind to and inactivate PBPs as their high affinity target sites. Prior studies presented PBP binding for β-lactams using isolated membranes but none have characterized the time-course of PBP binding in intact cells. We aimed to develop a novel assay for PBP binding by multiple β-lactams in intact AB and to integrate these receptor binding data with the expression of PBPs and β-lactamases.

Materials/methods: AB strain ATCC 19606 at an initial inoculum of 1.1 x 10^6 CFU/mL was incubated in the presence of meropenem (4 mg/L), sulbactam (4 mg/L) and their combination. Viable counts were assessed and antibiotic concentrations quantified via LC-MS/MS. PBP binding in intact cells was determined at 0, 0.25, 0.5, 1, 3, and 8h. Membranes containing the PBPs were labelled with Bocillin FL and analyzed on SDS-PAGE. Binding was reported as the relative Bocillin FL signal for the treatment compared to the signal of the growth control at the same time-point. mRNA expression of PBPs, AmpC and OXA-51 was characterized over time by RT-qPCR. All experiments were performed in triplicate.

Results: The time-course of PBP binding in whole-cell AB showed predominant binding to PBPs 1a and 2 by meropenem, PBPs 1b, 3 and 1a by sulbactam, and extensive binding to all four PBPs by their combination (Figure). Rapid and extensive PBP inactivation was observed for the targeted PBPs of the respective β-lactams in intact AB. The combination inactivated PBPs 1a, 1b and 2 by >80% at 1h and yielded >3.5 $\log_{10}$ bacterial killing at 3h. Antibiotic concentrations remained constant. mRNA expression of PBPs 1a, 1b, 2, and 3 was higher at 0.5 and 1h and lower at 3 and 8h compared to initial expression (t=0) irrespective of treatment. The AmpC and OXA-51 β-lactamase expression showed no significant changes over time.

Conclusions: This whole-cell PBP binding assay is the first to characterize the time-course of PBP inactivation in intact AB. This assay can assess β-lactam monotherapy and double β-lactam combinations and provides novel mechanistic insights which greatly support the development and rational optimization of double β-lactam and β-lactam plus β-lactamase inhibitor combinations.


Viable counts
Log10 CFU/ml
Time (h)
GC
MEM
SUL
MEM-SUL
PBP binding: Meropenem
Bocillin FL signal for treated vs. growth control
Time (h)
PBP1a
PBP1b
PBP2
PBP3
PBP6
PBP7/8
PBP binding: Sulbactam
Bocillin FL signal for treated vs. growth control
Time (h)
PBP1a
PBP1b
PBP2
PBP3
PBP6
PBP7/8
PBP binding: Meropenem + Sulbactam
Bocillin FL signal for treated vs. growth control
Time (h)
PBP1a
PBP1b
PBP2
PBP3
PBP6
PBP7/8

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