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P1530 First data on Penicillin-binding protein (PBP) occupancy patterns of beta-lactams in Klebsiella pneumoniae (KP)

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Background: PBPs are the high affinity target sites for all beta-lactam antibiotics. Although it is known that all β-lactams bind to and inactivate different subsets of PBPs with different affinities, and β-lactams are commonly used against KP, we are not aware of any published PBP binding data in KP. We aimed to generate the first comprehensive PBP binding dataset including a variety of 14 chemically diverse and clinically relevant β-lactams in KP.

Materials/methods: PBP binding by β-lactam antibiotics was determined in KP strain ATCC 43816. Membrane fractions containing PBPs were isolated via ultracentrifugation. Binding reactions were conducted for β-lactams in the concentration range of 0.0075 to 128 mg/L. Membranes were subsequently labelled with BOCILLIN FL and binding affinities (IC50s) were determined. PBP labeled gel fragments were excised and submitted to the mass-spectrometry core to validate the protein sequences. Binding IC50s were reported as the beta-lactam concentration which half-maximally decreased the Bocillin FL signal.

Results: Aztreonam showed high affinity for PBP3, whereas mecillinam and avibactam showed specific affinity for PBP2; the latter at much higher concentrations. Carbapenems bound PBP2 and PBP4 at concentrations <0.02 mg/L and PBP3 and 1a/b at (slightly) higher concentrations. Ceftazidime bound PBP3 at low and PBP1a/b at higher concentrations. In contrast, cefepime bound PBPs 1-4 more evenly. PBP5/6 was bound by imipenem and to a lesser extent by meropenem.

	IC50 (mg/L)							
	IPM	**MEM**	**ATM**	**MEC**	**PIP**	**AVI**	**CAZ**	**FEP**
PBP 1a/b	0.25	2	>128	>128	16	>128	4	1
PBP 2	0.0075	0.0075	16	0.0075	2	2	>128	0.12
PBP 3	8	0.12	0.06	>128	0.06	>128	0.25	0.06
PBP 4	0.0075	0.015	32	>128	64	>128	>128	2
PBP 5/6	1	8	>128	>128	64	>128	>128	>128

IPM, imipenem; MEM, meropenem; ATM, aztreonam; MEC, mecillinam; PIP, piperacillin; AVI, avibactam; CAZ, ceftazidime; FEP, cefepime.

Conclusions: This study provided the first comprehensive PBP binding dataset for 14 beta-lactams in KP. These binding data enable, for the first time, rational optimization of double beta-lactam and beta-lactam / beta-lactamase inhibitor combinations in KP.