29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

P1833 Outer membrane permeability of 15 beta-lactams in multidrug-resistant *Acinetobacter baumannii* (MDR-AB) characterised via LC-MS/MS

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Background: Poor outer membrane (OM) permeability of Gram-negative bacteria presents a critical barrier for antibiotic efficacy. Extremely few studies assessed the OM permeability of β-lactams in MDR-AB. This study characterized the OM permeability of 15 clinically-relevant β-lactams in MDR-AB via LC-MS/MS.

Material/methods: ATCC strain *BAA-2801* was grown to $10^{7.7}$ CFU/mL in broth and washed six times via centrifugation and resuspension in PBS. The supernatant after the last wash was used as control to monitor any remaining extracellular β-lactamase activity. The bacterial suspension was concentrated 10-fold to increase sensitivity. Half of these bacteria were lysed via ultra-sonication and the other half used as intact bacteria. In separate vials, 15 β-lactams were added at 3 mg/L to the supernatant control, intact and lysed bacteria arms. The time-course of extracellular β-lactam concentrations was quantified over up to 120 min via LC-MS/MS (LLOQ: 0.03 mg/L for each beta-lactam). Population modeling was used to estimate the rates of hydrolysis and OM permeability.

Results: For lysed bacteria, β-lactams were directly exposed to β-lactamases and hydrolysis was rapid for all drugs. For intact bacteria, β-lactams had to penetrate the OM and the overall decline of extracellular β-lactam concentrations was permeability limited (Figure). Extracellular β-lactamase activity in controls was minimal. Permeability coefficients were 205±15.6 nm/s for imipenem, 20±7.7 nm/s for meropenem, 15±0.7 nm/s for doripenem, 17±3.1 nm/s for biapenem, 30±1.0 nm/s for sulbactam, 18±0.7 nm/s for tazobactam, 180±38.9 nm/s for aztreonam, 173±36.3 nm/s for carumonam, >1000 nm/s for piperacillin, 91±16.8 nm/s for mecillinam, 256±40.6 nm/s for cefsulodin, 216±10.7 nm/s cefotaxime, 182±14.3 nm/s for cefpirome, 93±1.4 nm/s for ceftazidime, and 80±1.4 nm/s for cefepime.

Conclusions: OM permeability was rapid (>150 nm/s) for imipenem, piperacillin, monobactams, and some cephalosporins, whereas other cephalosporins and mecillinam had intermediate OM permeability (50-150 nm/s). The OM permeability of the other carbapenems, sulbactam and tazobactam was lower (<50 nm/s). While zwitter-ionic β-lactams tend to penetrate the OM of *Pseudomonas aeruginosa* rapidly, this physicochemical property was

not required for rapid OM penetration of MDR-AB. Future studies on the impact of efflux and structure permeability relationship analyses are warranted.