Background: The outer membrane (OM) of Gram-negative bacteria presents a formidable penetration barrier to antibiotics and substantially contributes to bacterial resistance. No data exist on the OM permeability of clinically relevant beta-lactams in Klebsiella pneumoniae. We aimed to develop a novel and efficient cassette assay which can simultaneously quantify the OM permeability of five beta-lactams in KPC-KP.

Materials/methods: KPC-KP at ~10^8.6 CFU/mL in broth was centrifugated and resuspended 6 times in PBS. To quantify any remaining extracellular beta-lactamase activity, the supernatant control was obtained from the sixth washing step. Part of this bacterial suspension was lysed by ultrasonication and the other part concentrated 5 fold to form the intact bacteria group. Imipenem, meropenem, ceftazidime, cefepime and aztreonam were added simultaneously each at concentrations of 1, 3 or 10 mg/L to the three intact bacteria arms. For the five lysed bacteria arms, concentrations of 0.3, 1, 3, 10 or 30 mg/L of each beta-lactam were used; the supernatant control contained 3 mg/L. The time-course of extracellular beta-lactam concentrations was monitored over up to 120 min by a multiplexed LC-MS/MS assay (LLOQ: 0.03 mg/L for each beta-lactam). Population modeling in NONMEM was used to estimate the hydrolysis and permeability parameters.

Results: For lysed bacteria, beta-lactams were directly exposed to beta-lactamases and thus hydrolysis was rapid for all drugs (except ceftazidime). For intact bacteria, beta-lactams had to penetrate through the OM and hydrolysis in the extracellular compartment was permeability limited. Permeability coefficients were 520 nm/s for imipenem, 20.1 nm/s for meropenem, 3.15 nm/s for aztreonam, 2.10 nm/s for cefepime, and <1 nm/s for ceftazidime (Figure). Extracellular beta-lactamase activity in controls was minimal.
**Conclusions:** Imipenem penetrated the OM 26-fold faster than meropenem and at least 165-fold faster than aztreonam, cefepime and ceftazidime in KPC-KP. Our new LC-MS/MS cassette assay simultaneously quantified the permeability of 5 clinically relevant beta-lactams, and was efficient and sensitive. This assay substantially extends the Zimmermann-Rosselet method and provides fundamental new insights for the mass balance of beta-lactams at the periplasmic target site of KPC-KP.