P0987
Poster Session IV
Molecular epidemiology and surveillance of MDR Pseudomonas and Acinetobacter

CHARACTERIZATION OF INTRINSIC BETA-LACTAM RESISTANCE MECHANISMS AMONG CONTEMPORARY CEFTAZIDIME NON-SUSCEPTIBLE PSEUDOMONAS AERUGINOSA FROM USA HOSPITALS

M. Castanheira[1], J.C. Mills[1], R.E. Mendes[1], R.N. Jones[1]

[1]Molecular, JMI Laboratories, North Liberty Iowa, USA

Objective: To evaluate beta-lactam intrinsic resistance mechanisms among 70 ceftazidime non-susceptible (MIC, >8 mg/L) *P. aeruginosa* (PSA) collected during 2012 in USA hospitals, and compare the activity of ceftolozane/tazobactam and five beta-lactams against these isolates. Additionally, 30 PSA with ceftazidime MIC values of 4 and 8 mg/L were analysed.

Methods: 100 PSA were susceptibility tested and selected based on MIC values representative of the MIC distributions for non-beta-lactam agents. Isolates were analysed using quantitative real-time PCR for AmpC, MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM. High quality DNA-free RNA preparations were tested in triplicate and normalised with an internal control gene (*rpsL*) and results compared with PSA PAO1. Isolates were subjected to outer membrane protein extractions and an OprD probe was used to detect this protein by Western blot.

Results: AmpC de-repression and OprD loss were the most prevalent mechanisms: 57.0 and 47.0% of the isolates were considered positive (>=10-fold greater expression when compared to control or decreased/no band, respectively). Elevated expression (>=5-fold) of the efflux pumps MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM were observed in 29.0, 10.0, 0.0 and 33.0% of the isolates, respectively. 21 phenotypes were detected and the most common were AmpC de-repression alone (11 isolates), AmpC de-repression with OprD loss (10) alone orassociated with MexAB-OprM or MexCD-OprJ hyperexpression (10 and 8, respectively). Ten isolates had no detectable intrinsic mechanisms and 8 of those displayed ceftazidime MIC values of 4 or 8 mg/L. Isolates displaying lower ceftazidime MIC values had low levels of expression for AmpC or efflux pumps (5–10-fold increase), but 14/30 displayed OprD loss. OprD loss had a strong correlation with elevated MIC results for imipenem (50 isolates with MIC, >4 mg/L; 46 with OprD loss) and meropenem (50 isolates with MIC, >4 mg/L; 47 with OprD loss), whereas AmpC expression levels were higher in isolates displaying elevated cefepime (62 isolates with MIC, >8 mg/L; 51 with AmpC de-repressed), ceftazidime (62 isolates with MIC, >8 mg/L; 51 with AmpC de-repressed) or piperacillin/tazobactam (67 isolates with MIC, >32 mg/L; 58 with AmpC de-repressed) MIC values. Isolates displaying de-repressed AmpC had ceftolozane/tazobactam MIC values ranging from 1–16 mg/L, but no strong correlation was noted between MIC values for this combination and OprD loss or hyperexpression of efflux systems. Three isolates displaying ceftolozane/tazobactam MIC values of >=32 mg/L displayed MexXY-OprM hyperexpression alone or in combination with OprD loss.

Conclusions: We observed multiple intrinsic resistance mechanisms among contemporary ceftazidime non-susceptible PSA collected from USA hospitals; and these mechanisms were not common among PSA isolates displaying MIC values of 4 and 8 mg/L. Acquired beta-lactamases might also play a role in PSA resistance against these beta-lactams; however, intrinsic mechanisms appear common and display strong correlations with elevated MIC for certain agents.