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O0816 Detecting porin expression in carbapenem-resistant Klebsiella Pneumoniae (CRKP) by flow cytometry (FCM) and reverse Transcriptase-PCR (RT-PCR)

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Background: Loss of outer membrane porins have been implicated in antibiotic resistance in CRKP. Currently, rapid testing for porin expression is unavailable. We assessed the use of FCM and RT-PCR for the rapid detection of OmpK35 and OmpK36 porin in CRKP.

Materials/methods: 71 clinical CRKP isolates were tested. Anti-sera designed against OmpK35 (K35) and OmpK36 (K36) peptide antigens were used for detection of porin in western blot (WB) and FCM. Porin loss in WB was defined as band absence. Expression of porin in FCM was obtained using fluorescence intensity, and normalised as fold-decrease relative to ATCC13883. Similarly, changes in porin gene expression was elucidated using RT-PCR and expressed as fold-decrease relative to ATCC13883. Receiver operating characteristics (ROC) curves for RT-PCR and FCM were used to discriminate between presence and absence of porin compared to WB. AUCs (area under ROC curve), sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were computed.

Results: 38% and 36% of the isolates had K35 and K36 loss in WB respectively. The AUC between RT-PCR and WB for K35 was 0.80 (95% CI, 0.69–0.91) with a sensitivity of 72.4% and specificity of 90.5% (PPV=91.3%; NPV=70.4%). For K36, AUC between RT-PCR and WB is 0.78 (95% CI, 0.68–0.87), with sensitivity and specificity of 63.6% and 90.6% respectively (PPV=84.5%; NPV=81.3%). In comparison, ROC analysis between FCM and WB for K35 revealed a low AUC of 0.69 (95% CI, 0.57–0.82) (sensitivity=51.7%, specificity=61.9%, PPV=65.2%; NPV=48.2%). The K36 anti-serum was not able to detect K36 protein in FCM after two rounds of peptide synthesis, and further testing was discontinued.

Conclusions: Detection of porin expression in FCM was less sensitive and specific compared to RT-PCR. This is likely due to the challenge posed by protein folding and variation in the 3D conformation of proteins, leading to poor binding by K35 anti-sera. Rapid testing for porin loss by RT-PCR is more promising.