O159
Oral Session
Detection of broad spectrum beta-lactamases

DEVELOPMENT AND ANALYSIS OF ASSAYS FOR THE DETECTION OF CARBAPENEMASES IN ENTEROBACTERIACEAE

F.J. Hamilton[1], R. Crispin[1], D. Coyle[1], L. Mulhern[1], A.P. Gibb[1], K.O. Helgason[1], E.C. Class[2], K.E. Templeton[1]

[1]Medical Microbiology, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom ; [2]Medical Microbiology, Leiden University Medical Centre, Leiden, Netherlands

Objectives: Carbapenemase-producing Enterobacteriaceae (CPE) pose a growing clinical threat worldwide. Their ability to hydrolyse carbapenems, and other β-lactams, leave limited therapeutic options, thus making early detection enabling infection control measures a high priority. CPE confirmation is normally performed in national reference centres. However, many new commercial and in-house options could aid rapid detection in local diagnostic laboratories. We compared four methods of carbapenemase detection in Enterobacteriaceae in order to assess which method is most suitable for routine use in our laboratory.

Methods: A total of four methods were used to assess the presence of carbapenemase activity or to detect the genes responsible for carbapenemase production. The Carba NP test, a biochemical assay which detects carbapenemase activity using an incubation step with a carbapenem (imipenem); a modified MALDI-TOF (Bruker-MS) method which includes an incubation step with a carbapenem; an in-house multiplex PCR for the detection of carbapenemase genes (KPC, NDM, OXA-48 and VIM) and the Check-DirectCPE (Check-Point) for BD MAX™ System (Becton Dickinson) which also detects KPC, NDM, OXA-48 and VIM. A total of 43 isolates of Enterobacteriaceae were evaluated, 4 NCTC type specific strains and 39 isolates collected from NHS Lothian which had all been referred to the UK reference centre for carbapenemase detection.

Results: Of the 43 isolates of Enterobacteriaceae included in this study, 22 were confirmed as CPE by the reference laboratory. All 21 of the non-CPE isolates were correctly identified by all methods. Of the 22 CPE, the Carba NP identified 7 as positive, 9 as indeterminate and 6 as negative. Combining positive and indeterminate gave a CPE detection rate of 16/22 (72%). The in-house molecular assay showed positive results in 15/22 isolates (68%). The Checkpoint BDMax assay gave positive results in 20/22 (90%), although in two cases the wrong gene was identified. The MALDI-TOF using the extended spectrum was assessed with prior incubation with imipenem, ertapenem and meropenem. The antibiotics were prone to oxidation and the specific peak to differentiate CPE from non-CPE was only observed when using freshly prepared meropenem, which showed 100% concordance with reference laboratory results.

Conclusion: The in-house Carba NP assay relies on subjective interpretation and gave a large number of indeterminate results. This may be circumvented by using a robust commercial assay. The MALDI-TOF assay was reliably able to detect CPE when using meropenem. The Checkpoint assay showed good detection of CPE but the in-house assay could be improved by including more primers and probes. Overall both the molecular and MALDI-TOF assays provide promising platforms for the development of rapid and accurate CPE detection. Our results suggest that a combination of genetic detection and carbapenemase activity based methods may be the most assured approach for reliable detection of CPE.