

Clostridium difficile

real-time PCR detection of toxin genes in faecal samples is more sensitive compared to toxigenic culture

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Abstract (poster session)

Diagnosis of Clostridium difficile: real-time PCR detection of toxin genes in faecal samples is more sensitive compared to toxigenic culture

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Objective: The aim of this study was to evaluate four DNA-amplification methods detecting toxigenic Clostridium difficile (CD), including CD with PCR ribotype 027 (CD027), directly from faecal samples. Thereby to establish a rapid primary diagnostic test of CD based on the evaluation. Methods: A total of 300 faecal samples from 284 Danish hospitalized patients with diarrhoea were included consecutively from mid February to the beginning of April 2011. CD was detected routinely by anaerobic culture on cycloserine cefoxitin fructose agar for 48hrs. Genotypic toxinprofiling by PCR and PCR ribotyping were performed on CD culture positive samples. In parallel, the samples were analysed for toxigenic CD by a) an "in-house" multiplex Real-time (RT) PCR two-step algorithm and three commercial DNA-amplification methods: b) Illumigene(R) C. difficile [Meridian Bioscience]; c) PCRFast(R) C. difficile [Simoco Diagnostics] and d) Xpert(R) C. difficile [Cepheid]. a) and d) detects presumptive CD027; b) and c) detects tcdA/B only. Results: Assay a) and d) were significantly more sensitive compared to toxigenic culture. The concordance between a) and d) was 97.3% (292/300). Re-culture and/or prolonged incubation time (3-7 days) of the culture negative, but a) and d) CD positive samples, increased the culture positive rate by 29% (from 38 to 49/300). Compared to initial culture as the reference standard, the re-culture data resulted in an increased positive predictive value (> 80%) and specificity (> 95%) for all assays, although a reduced negative predictive value (NPV) (> 90%) and sensitivity (63-95%) of all but d) which had a NPV and sensitivity of 100%. RT PCR inhibition was <1% (0 and 2/300) in a) and d) respectively i.e. in Bovine Serum Albumin (BSA) optimized assays. In contrast, PCR inhibition was 13% (38/300) in c) not containing BSA. Preliminary results from PCR ribotyping showed 94% and 97% concordance of a) and d) respectively for the detection of CD027. Conclusion: Assay a) and d) are sensitive diagnostic methods and their high concordance suggests that a) and d) CD positives, but culture negative faecal samples are true positives. Both methods are usable as rapid primary diagnostic tests for toxigenic CD and for presumptive identification of CD027. The current toxigenic culture method is sub-optimal for primary diagnostics of CD. BSA efficiently reduces PCR inhibition and could be considered included in PCR protocols for detection of CD genes in faecal samples.