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Three years of experience of *Clostridium difficile* infection: diagnosis and clinical associations

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Objectives: *Clostridium difficile* infection (CDI) is a major infectious concern, accounting for substantial morbidity and resource utilization. Advances in microbiological and molecular techniques have resulted in an increasing number of testing options for CDI. The purpose of the present study was to report our experience in the diagnosis of CDI from patients hospitalized with diarrhoea, to investigate pathogenicity and virulence of positive strains and to highlight the clinical features of positive cases.
Methods: Fecal samples from seven hundred forty-four patients were tested at a 883- bed University Hospital in Greece over a period of three years. A commercially available combined Glutamate Dehydrogenase Antigen (GDH) and toxin A/B membrane EIA assay (CDiff Quick Chek Complete, QCC, Techlab, Blacksburg) were performed. Twenty negative [GDH-/ toxin A/B(-)] and all positive samples by QCC test [GDH+/ toxin A/B(+) or GDH+/ toxin A/B(-)] were then tested by PCR (GenoType CDiff, Hain LifeScience, Germany) to perform the molecular genetic identification of *C. difficile* strains.
Results: Thirty stool specimens from consecutive patients (4%) were tested positive with GDH and PCR and six more were only with GDH. The PCR assay showed complete concordance with GDH+/toxin A/B+ (n=30) and GDH-/ toxinA/B- results (n=20). Of the GDH+/ toxinA/B- specimens 25% were negative by PCR. Both A and B toxins were detected in 80% of all PCR-positive samples and binary toxin genes cdtA and cdtB were also detected in 30% of them. The GenoType CDiff assay was able to identify a strain of ribotype 078 with detectable binary toxin but no one strain were found to be hypervirulent (ribotype 027). Males and children were 56.6% and 6.6% respectively and 89.3% of adults aged more than 65 years. Twenty-five cases were hospital onset and the majority of them derived from Medical wards (64%). Twenty-eight cases (93.3%) had received antibiotics in the preceding month, 73.3% had underlying disease and no one had co-infection with another gut pathogen. No mortality was observed. The CDI prolonged their hospitalization despite the good clinical outcome.
Conclusion: CDI is an emerging nosocomial problem in our hospital (2 cases per 10000 patient-days). The antibiotics, increased age and underlying disease are factors associated with high risk of CDI. The combined CDiff Quick Chek Complete test can be used as an initial screening test followed by confirmation with PCR assay of GDH positive and toxin-negative samples. Additionally, PCR-GenoType CDiff allows the differentiation between non-pathogenic, virulent and hypervirulent *C. difficile* strains. Further extended studies to confirm the above-mentioned results are required.