Modification of a PCR Ribotyping Method for Application as a Routine Typing Scheme for Clostridium difficile

G. l. O'Neill, F. T. Ogunsola 1, J. S. Brazier and B. I. Duerden

A modification of a PCR ribotyping procedure based on polymorphisms in the 165-235 intergenic spacer region was evaluated for use as a typing method for Clostridium 1ifficile. This procedure depends on the variation that can occur in the intergenic space between the 165 and 235 rRNA genes of the ribosomal RNA gene complex. The primers used inthis study were chosen by examining the se'lucn<;e of the 165 gene of C. difficile and the 235 gene of C. botulinum. The primers used were: CTG GGG TGA AGT CGT AAC AAG G (positions 1445-1466 in the 165 rRNA gene) and GCG CCC TTT GTA GCT TGA CC (positions 1-20 in the 235 rRNA gene) and the PCR parameters were optimised for this primer pair. To evaluate the discriminatory "power of the method, PCR ribotyping was performed on strains of C. difficile serotyped by Delmee (serogroups A-X and sub-serogroups A2-A10). Each isolate gave multiple DNA bands in PCR ribotyping and a series of products ranging in size from 260 to 585 bp in length was obtained. All of the 19 different serogroups gave different banding patterns and these patterns were reproducible. This 'modification of PCR ribotyping offers several advantages over the original method and appears to hold much promise as a method for typinf; wild isolates of C. difficile.

Anaerobe Reference Unit, Public Health Laboratory and 'Department of Medical Microbiologi;, University Hospital of Wales, Heath Park, Cardiff, CF44XW, U.K. (Received 22 November 1995, accepted in revised form 8 May 1996) Key Words: Clostridium difficile, PCR ribotyping, intergenic spacer