Use of Modified PCR Ribotyping for Direct Detection of *Clostridium difficile* Ribotypes in Stool Samples

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Direct PCR ribotyping from stool samples. A total of 105 stool samples submitted to the Institute of Public Health Maribor (MB laboratory) for routine *C. difficile* testing and 84 samples from the Institute of Public Health Murska Sobota (MS laboratory) were tested. Samples from the MB laboratory were identified as *C. difficile* positive according to positive culture on CLO selective plates (bioMérieux) after ethanol shock. Samples from the MS laboratory were tested using the PCR ribotype of the strain isolated from the same stool sample. This was isolated as described above.

For direct PCR ribotyping from stool samples, we designed new primers located partially within the *C. difficile* 16S-23S rRNA intergenic spacer region (ISR) and partially within the 16S (forward primer) and 23S (reverse primer) rRNA genes. New primers were defined on the basis of DNA sequences of *C. difficile* reference strains were available were designated by standard Cardiff nomenclature (001, 002, …), while others are designated by internal nomenclature (SLO and a 3-digit code).

Altogether, 189 samples were tested; among them, 99 were *C. difficile* positive and 90 were *C. difficile* negative. By using the new primers, direct PCR ribotyping from stool samples was possible in 86 out of 99 *C. difficile*-positive stool samples. In 84 cases (84.8%), the ribotype determined directly from the stool sample was identical to the ribotype of the strain isolated from the same stool sample.

**Clostridium difficile** infections represent a significant burden on the health care system. Although many infections are sporadic, nosocomial transmission is still important and outbreaks are a constant threat in the hospital environment. The ability to detect such outbreaks quickly is critical to infection control.

Several typing techniques, all of them based on having a pure culture of the organism, have been described for *C. difficile* (3). Pulsed-field gel electrophoresis (PFGE) and PCR ribotyping are the methods of choice in North America and in Europe, respectively. Three variations of PCR ribotyping have been described, two of them differing in the primers used, and while two use traditional agarose gel-based analysis (1, 5), the third uses capillary gel electrophoresis-based analysis of the results (2). Here, we describe a modification of PCR ribotyping that can be used for detection of *C. difficile* ribotypes directly in stool samples.

**PCR ribotyping was modified to allow direct detection of *Clostridium difficile* from stool samples.** Direct PCR ribotyping was possible in 86 out of 99 *C. difficile*-positive stool samples, and in 84 cases (84.8%), the ribotype determined directly from the stool sample was identical to the ribotype of the strain isolated from the same stool sample.

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**PCR ribotyping was modified to allow direct detection of *Clostridium difficile* from stool samples.** Direct PCR ribotyping was possible in 86 out of 99 *C. difficile*-positive stool samples, and in 84 cases (84.8%), the ribotype determined directly from the stool sample was identical to the ribotype of the strain isolated from the same stool sample.

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In 24 of the 90 C. difficile-negative samples, direct ribotyping with modified primers generated 1 to 4 nonspecific fragments which were clearly distinct from the common C. difficile ribotyping profiles and could easily be interpreted as C. difficile negative.

Samples positive with direct ribotyping (n = 84) were distributed into 25 PCR ribotypes; the most common were 027 (n = 32; due to an outbreak), 014/020 (n = 7), 070 (n = 4), 023 (n = 4), 002 (n = 4), SLO 011 (n = 3), SLO 006 (n = 3), and 003 (n = 3). Thirteen samples positive by culture for C. difficile but negative or weak on direct ribotyping contained 10 different ribotypes (014/020, 027, 001, 023, 106, 010, SLO 076, SLO 064, SLO 011, and SLO 036). When analyzed by direct ribotyping, none of the C. difficile-positive samples showed band profiles that would indicate the presence of two different ribotypes that is occasionally noticed (4, 6).

Conventional PCR ribotyping performed on total stool DNA. For comparison, Bidet primers were used for standard PCR ribotyping of strains and also for direct ribotyping from 97 of the 189 stool samples. Amplification conditions were as described in Bidet et al. (1). While a ribotype profile was obtained from 37 of 51 (72.5%) C. difficile-positive samples, most of the C. difficile-negative samples (31 out of 46; 67.4%) reacted with this primer pair but with distinctively different fragment profiles. In a majority of cases (33 out of 37; 89.2%), profiles obtained from C. difficile-positive samples could not be assigned to known ribotypes due to the nonspecific bands (data not shown).

Summary. Direct PCR ribotyping gives the information on the presence and type of C. difficile within hours, in contrast to standard culture-dependent methods where typing results can be obtained only after 3 days or more (48 h for culture and 1 day for PCR ribotyping). Direct PCR ribotyping on DNA isolated from stool samples is convenient, rapid, and useful for the detection of specific types of C. difficile in fecal samples.

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