COMPARISON OF DIFFERENT PRIMER PAIRS AND ELECTROPHORESIS PLATFORMS FOR *Clostridium difficile* PCR-RIBOTYPING

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INTRODUCTION

PCR-ribotyping, method based on size variation of intergenic spacer region (ISR) of ribosomal operons, has in recent years become a method of choice for typing of *C. difficile*. Several different primer pairs have been described for *C. difficile* PCR-ribotyping. Most commonly used are primers described by Bidet *et al*. (1999) and Stubbs *et al*. (1999). Recently, we reported modified primers which allow direct ribotyping of *C. difficile* in total DNA from stool samples (Janezic *et al*., 2011). Furthermore, several different electrophoresis platforms have been described to separate amplified spacers. Classical agarose gel electrophoresis-based PCR-ribotyping is, irrespective of the drawbacks such as poor resolution and lack of interlaboratory comparability, still most often used. Recently capillary-sequencer-based PCR-ribotyping suported with a web based software WEBRIBO (http://webribo.ages.at/) for assignments of PCR-ribotypes was described (Indra *et al*., 2008). As an alternative to classical gel electrophoresis commercial automated multicapillary electrophoresis system (QIAxcel, Qiagen) has become available (Figure 1).

In this study we have compared different primer pairs and electrophoresis platforms available for *C. difficile* PCR-ribotyping.

MATERIALS AND METHODS

The ISRs of a well characterized *C. difficile* strains were amplified with three different primer pairs; Bidet *et al*., 1999; Stubbs *et al*., 1999; Janezic *et al*., 2012.

Amplified fragments were resolved by:
- Classical agarose gel electrophoresis; 3% agarose gel (Bio-Rad) in 1x TAE buffer (GE) (Janezic and Rupnik, 2010).
- QIAxcel capillary electrophoresis system (Q-CE) with QX DNA High Resolution Cartridge, using the OM500 method and QX Alignment Marker 15 bp/1 kb and QX Size Marker 50 – 800 bp v2.0 (Qiagen).
- Capillary electrophoresis on automated sequencer using two different apparatus (S-CE):
  CEQ™ 8000 Genetic Analysis System (Beckman Coulter) with gel LPAI and DNA Size Standard 600BP; injection of samples was carried out at 2 kV over 60 s with a running time of 75 min at 4,8 kV at 50°C.
  ABI 3130 DNA Analyser (Applied Biosystems) using POP7 gel with Liz1200 (Chimerx) used as an internal marker for each sample (Indra *et al*., 2008).

PCR-ribotypes were determined with WEBRIBO software (for S-CE-based ribotyping) and by comparison of banding patterns with the internal library using the BioNumerics software v6.10 (for GE- and Q-CE-based ribotyping).

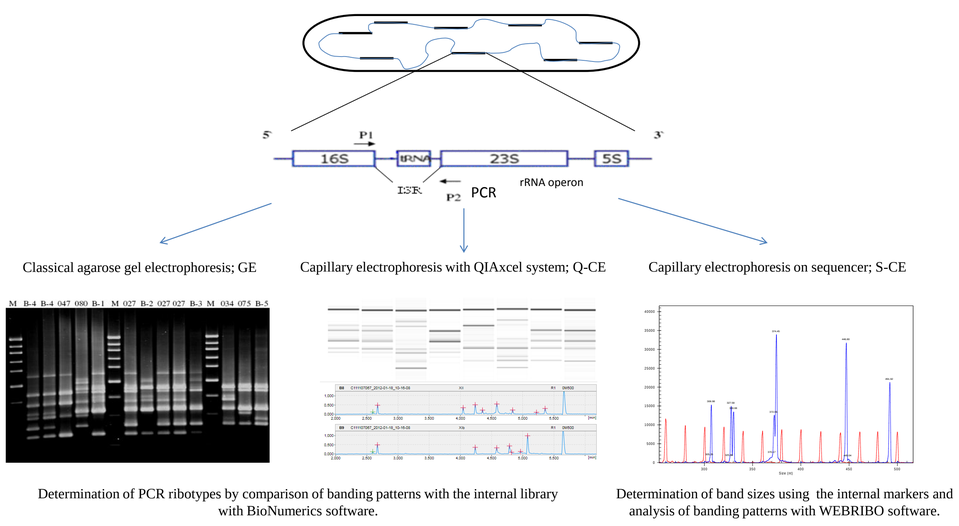


Figure 1. Schematic representation of PCR-ribotyping on different electrophoresis platforms. P1 and P2 - 16S and 23S rRNA primers, respectively.

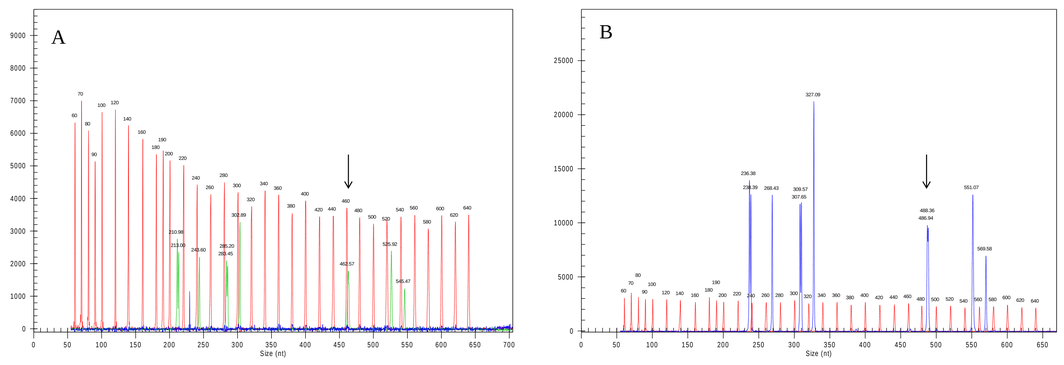


Figure 2. Electropherograms showing difference in PCR-ribotyping profiles (presence/absence of a double peak; arrow) generated with two different primers; Janezic (A) and Bidet (B) for PCR-ribotype 020 strain. The theoretical size difference between bands produced by these two primer pairs is 24 bp.

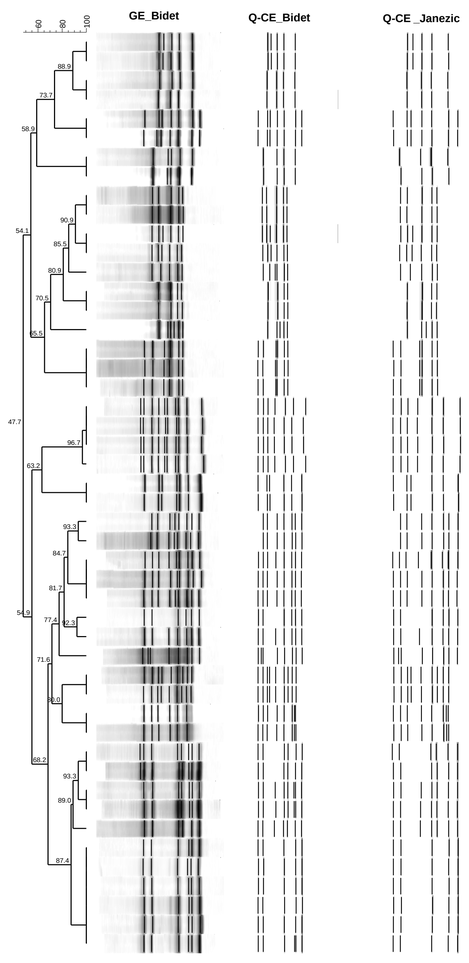
RESULTS AND DISCUSSION

Comparability of different primer sets. Both primer pairs (Bidet *et al*., 1999 and Janezic *et al*., 2011) generated agreeable results. The only difference observed between the banding patterns, in 10 out of 57 cases, was the presence or absence of double peaks (± < 2 bp) generated with one of the primer sets compared to the other (Figure 2). However, in all these cases WEBRIBO identification of the PCR-ribotype was correct regardless of the primers used (Figure 3).

A subset of 48 strains was also typed with capillary sequencer-based PCR-ribotyping using primers described by Stubbs *et al*. (1999) and for all the strains tested correct PCR-ribotypes were determined with WEBRIBO analysis (Figure 3).

Comparison of different electrophoresis platforms. As described before, capillary sequencer based PCR-ribotyping is more discriminatory and enabled us to subtype some of the PCR-ribotypes (Figure 3).

Discriminatory power of Qiaxcel system is comparable to classical agarose gels (Figure 3), however, Q-CE sometimes failed to detect the highest peak leading to incorrect identification of closely related PCR-ribotypes (data not shown).



Strain	GE_Bidet	Q-CE_Bidet	Q-CE_Janezic	S-CE_Bidet	S-CE_Janezic	S-CE_Stubbs
R11402	SLO 060	SLO 060	SLO 060	N4	N4	N4
CD162	SLO 060	SLO 060	SLO 060	N4	N4	N4
IS58	033	033	033	033	033	033
CD219	033	033	033	033	033	033
7325	027	027	027	027	027	027
R12087	027	027	027	027	027	027
57267	063	063	063	063/1	063/1	063/1
39696	063	063	063	063/1	063/1	063/1
CD08-070	126	126	126	126	126	126
RT126	126	126	126	126	126	126
R7605/78	078	078	078	078	078	078
SUC36	078	078	078	078sub	078sub	078sub
R9385	122	122	122	122	122	122
RT045	045	045	045	045	045	045
SE866	045	045	045	045	045	045
R6786	SLO 144	SLO 144	SLO 144	N5	N5	N5
ZZV 1121	070	070	070	070	070	070
ECDC 070	070	070	070	070	070	070
KORZ12-1	070	070	070	070	070	070
RT010	010	010	010	010	010	010
ZZV 8	010	010	010	010	010	010
ZZV 6004	010	010	010	010	010	010
CD169	010	010	010	010	010	010
7459	SLO 036	SLO 036	SLO 036	AI-84	AI-84	AI-84
ZZV 4724	SLO 036	SLO 036	SLO 036	AI-84	AI-84	AI-84
AC008	103	103	103	AI-82sub	AI-82sub	AI-82sub
ZZV 8970	103	103	103	AI-82sub	AI-82sub	AI-82sub
ZZV11-3660	011/049	011/049	011/049	AI-15	AI-15	AI-15
ZZV 3111	011/049	011/049	011/049	049/1	049/1	049/1
8832	011/049	011/049	011/049	049/1	049/1	049/1
10049/10	SLO 163	SLO 163	SLO 163	N3	N3	N3
IS25	056	056	056	N2	N2	N2
UKC129-1	SLO 130	SLO 130	SLO 130	N1	N1	N1
10849	005	005	005	005	005	005
zzv 924	005	005	005	005	005	005
TR13	SLO 009	018	018	018	018	018
ZZV 2496	SLO 009	018	018	018	018	018
ECDC 020	020	014/020	014/020	020	020	020
10111/09	020	014/020	014/020	020	020	020
KO95	014/020	014/020	014/020	014/0	014/0	014/0
E14	014/020	014/020	014/020	014/0	014/0	014/0
ZZV 4922	014/020	014/020	014/020	014/0	014/0	014/0
ZZV 11996	001/072	001/072	001/072	001	001	001
CD07-140	001/072	001/072	001/072	001ecdc	001ecdc	001ecdc
ZZV 660	001/072	001/072	001/072	001	001	001
ZZV 2590	001/072	001/072	001/072	001	001	001
VODZ6-2	001/072	001/072	001/072	001	001	001
E2	001/072	001/072	001/072	001	001	001

Figure 3. Comparison of PCR-ribotyping results on different electrophoresis platforms (classical agarose gel electrophoresis, QIAxcel capillary electrophoresis system (Q-CE) and capillary electrophoresis on an automated sequencer, Beckman Coulter (S-CE)), and three different primer pairs (Bidet, Janezic and Stubbs - only S-CE). PCR-ribotypes for which reference strains were available are designated by standard Cardiff nomenclature (027, 066, etc.), whilst others are designated by internal nomenclature (SLO and a 3-digit code). With N1 – N5 are designated new PCR-ribotypes that did not match with any of the reference ribotypes in WEBRIBO library.

Comparison of PCR-ribotyping on two different automated sequencer (ABI 3130 and CEQ™ 8000). Identical PCR-ribotype was determined for 17 out of 22 strains ribotyped on both sequencers. In other 5 strains the same PCR-ribotype but different subtype was identified (Table 1). Due to the close nature of some PCR-ribotype subtypes and the mathematical approach of comparing samples done with different sequencers WEBRIBO can calculate a slightly different first BM as a user by visual comparison would choose. In all these cases the correct PCR-ribotype was given as one of the best fitted matches and hence would be chosen by the human administrator.

Table 1. Comparison of PCR-ribotyping results from two different sequencers (ABI3130 and CEQ™ 8000).

Strain	ABI_Bidet	Beckman_Bidet	Strain	ABI_Bidet	Beckman_Bidet
S1	036/2	036/2	**S12**	176	176
S2	241	001	**S13**	036/1	036/1
S3	539	539	**S14**	014/6=232	014/0
S4	001/4	001/4	**S15**	014/6=232	014/0
S5	441	441	**S16**	003/2	003/2
S6	413	413	**S17**	AI-52	AI-52
S7	209	209	**S18**	203	203
S8	066/4	066/4	**S19**	005/1	005
S9	076/1	076/1	**S20**	001/5	001ecdc
S10	408	408	**S21**	001ecdc	001ecdc
S11	002/2	002/2	**S22**	AI-12	AI-12

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CONCLUSIONS

Comparability of results with both primer pairs (Janezic *et al*., 2011 and Bidet *et al*., 1999) is very good.

PCR-ribotyping based on S-CE is more discriminatory and accurate than GE and Q-CE. Furthermore, WEBRIBO software enables fast and reliable identification of PCR-ribotypes using any of the three primer sets described for *C. difficile* PCR-ribotyping.