

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med 2005;353:2442-9.

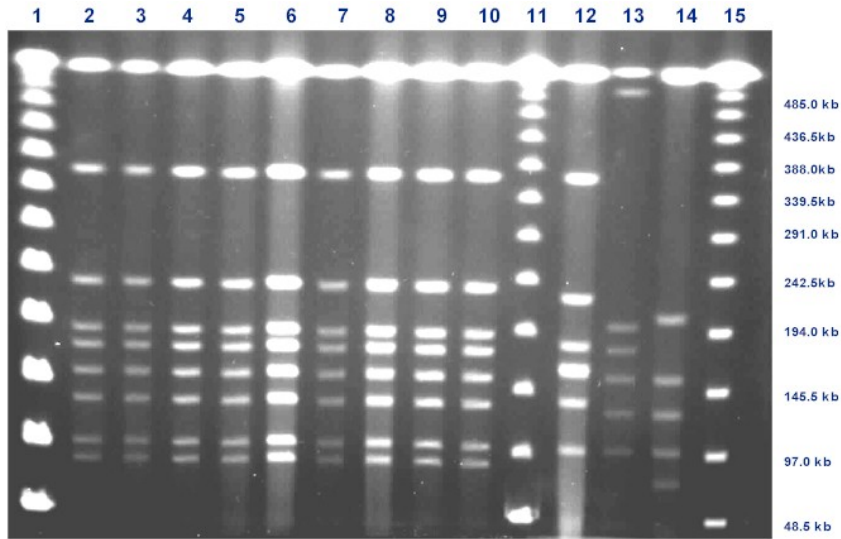


Figure 1. PFGE fingerprints of interhospital *C. difficile* isolates using *Sma*I restriction enzyme digestion. Lanes 1,11 and 15 are concatamerized lambda molecular weight markers; lanes 2-10, isolates representative of the predominant strain obtained from 9 hospitals; lanes 12-14, isolates carrying binary toxin genes and partial deletion of the *tcdC* gene that are of the non-predominant pulsovar, named QCD\_76j23, QCD\_33n64 and QCD\_65s46 respectively.

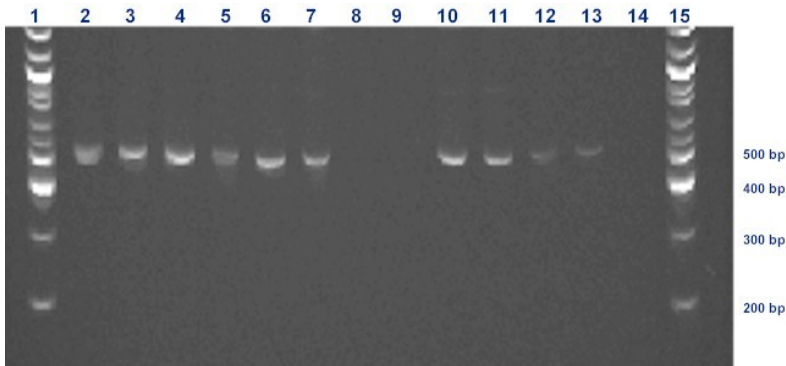


Figure 2A. PCR amplification of partial *cdtB* gene. Lanes 1 and 15, 100 bp ladder; lanes 2-7, isolates representative of the predominant strain obtained from 6 hospitals; lane 8, *S. aureus* ATCC 25923; lane 9, *C. difficile* ATCC 43255 *cdtB* gene negative control; lane 10, *C. difficile* CIP 107932 *cdtB* gene positive control; lanes 11-13, isolates that are of the non-predominant pulsovar, named QCD\_76j23, QCD\_33n64 and QCD\_65s46 respectively; lane 14, water.

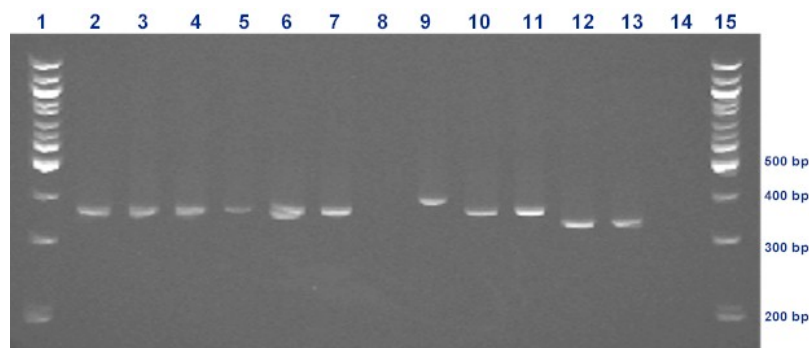


Figure 2B. PCR amplification of partial *tcdC* gene. Lanes 1 and 15, 100 bp ladder; lanes 2-7 clinical isolates representative of the predominant strain obtained from 6 hospitals; lane 8, *S. aureus* ATCC 25923; lane 9, *C. difficile* ATCC 43255 negative control with no partial deletion of the *tcdC* gene; lane 10, *C. difficile* CIP 107932 positive control with partial deletion of the *tcdC* gene; lanes 11-13, isolates that are of the non-predominant pulsovar, named QCD\_76j23, QCD\_33n64 and QCD\_65s46 respectively; lane 14, water.

Isolate	Sequence
ATCC 43255	AAAGCTGAAGAAGCTAAAAAAGCTGAAGAAGCTAAAAAGGCTGAAGAACAACGCAAAAAAGAAGAAGAGGAGAAGAAAGGATAT
CIP 107932	AAAGCTGAAGAAGCT-----AAAAAGGCTGAAGAACAACG <b>T</b> AAAAAAGAAGAAGA <b>A</b> GAGAAGAAAGGATAT
QCD_76j23	AAAGCTGAAGAAGCT-----AAAAAGGCTGAAGAACAACG <b>T</b> AAAAAAGAAGAAGA <b>A</b> GAGAAGAAAGGATAT
QCD_38a67	AAAGCTGAAGAAGCT-----AAAAAGGCTGAAGAACAACG <b>T</b> AAAAAAGAAGAAGA <b>A</b> GAGAAGAAAGGATAT
AJ428941_Pd5	AAAGCTGAAGAAGCT-----AAAAAGGCTGAAGAA-----GAGAAGAAAGGATAT
QCD_33n64	AAAGCTGAAGAAGCT-----AAAAAGGCTGAAGAA-----GAGAAGAAAGGATAT
QCD_65s46	AAAGCTGAAGAAGCT-----AAAAAGGCTGAAGAA-----GAGAAGAAAGGATAT

Figure 2C. DNA Sequence alignment of the *tcdC* gene region harboring 18 bp and 39 bp mutations. *C. difficile* ATCC 43255 control strain with no partial deletion of the *tcdC* gene; *C. difficile* CIP 107932 control strain with 18 bp deletion; Québec isolate QCD\_38a67 is a member of the predominant pulsovar group with 18 bp deletion; Québec isolate QCD\_76j23 is a member of the non-predominant pulsovar group with 18 bp deletion; Québec isolates QCD\_33n64 and QCD\_65s46 are members of the non-predominant pulsovar group with an 18 bp deletion and an additional 21 bp deletion (identical to Genbank submission AJ428941). Mutations different from control strains are highlighted in red.