**Appendix 1. *Clostridium difficile* Ribotyping Methods**

Ribotyping was performed on all isolates positive by enrichment culture. From Banana broth, cultures were streaked for single colonies onto Brusporea supplemented agar and grown anaerobically for 7-10 days at 37ᵒC to generate spore stocks. Eight colonies were selected from each plate and dissolved in 50 µL of ddH2O. 30 µL of each suspension was heated at 98ᵒC for 10 min to extract DNA, vortexed at 1,500 rpm for 5 min, and then centrifuged for 10 min at 4,300 rpm to remove debris. The ribotyping primers used in this study have been described previously and consisted of a 5´-FAM-labeled 16S primer.1 Ribotyping reactions (12 µL) consisting of 2 µL of DNA, 6 µL Type-it Microsatellite PCR Master Mix (Qiagen), 1 µL primer mix (10 pmol/µL) and 3 µL ddH2O were amplified by PCR using the thermocycler conditions: 95ᵒC 5 min, followed by 40 cycles of 95ᵒC 30 sec, 58ᵒC 1 min, 72ᵒC 45 sec, and a final 60ᵒC 30 min elongation. PCR products were diluted 20-fold in ddH2O, then an additional 10-fold in formamide containing GeneScan 600 Liz Size Standard 2.0 (Applied Biosystems). Samples were heated at 98ᵒC for 5 min prior to analysis by capillary gel electrophoresis on an ABI 3130*xl* Genetic Analyzer at 60ᵒC with a 50 cm capillary loaded with a POP7 gel (Applied Biosystems).2 Ribotypes were identified using WEBRIBO online software (https://webribo.ages.at/).

**References**

1. Bidet P, Barbut F, Lalande V, Burghoffer B, Petit JC. Development of a new PCR-ribotyping method for Clostridium difficile based on ribosomal RNA gene sequencing. *FEMS Microbiol Lett*. 1999;175:261-266.

2. Indra A, Huhulescu S, Schneeweis M, et al. Characterization of Clostridium difficile isolates using capillary gel electrophoresis-based PCR ribotyping. *J Med Microbiol*. 2008;57:1377-1382.

**Appendix 2. Estimated environmental sample *C. difficile* positivity and quantity based on quantitative PCR (16s target and Toxin B target) and enrichment culture from environmental samples collected at a tertiary hospital**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Positivity (N, %) | Estimated spore count (geometric mean) |
|  | N | 16s qPCR  | Toxin B qPCR  | Enrichment culture | 16s qPCR  | Toxin B qPCR  |
| Total | 48 | 31 (64.6) | 19 (39.6) | 21 (43.8) | 13.8 | 1.9 |
|  |  |  |  |  |  |  |
| Room risk of contamination\* |  |  |  |  |  |  |
|  Low | 20 | 10 (50) | 5 (25) | 7 (35) | 4.3 | 1.3 |
|  Small | 10 | 4 (40) | 1 (10) | 3 (30) | 2.4 | 0.8 |
|  Large | 10 | 6 (60) | 4 (40) | 4 (40) | 7.9 | 2.1 |
|  Medium  | 8 | 4 (50) | 3 (37.5) | 1 (12.5) | 5.0 | 1.7 |
|  Small | 4 | 2 (50) | 1 (25) | 0 (0) | 3.1 | 1.1 |
|  Large | 4 | 2 (50) | 2 (50) | 1 (25) | 8.2 | 2.6 |
|  High  | 20 | 17 (85) | 11 (55) | 13 (65) | 65.6 | 3.0 |
|  Small | 10 | 8 (80) | 5 (50) | 6 (60) | 29.0 | 2.0 |
|  Large | 10 | 9 (90) | 6 (60) | 7 (70) | 148.5 | 4.6 |

qPCR, quantitative PCR

\* Low = occupant had no receipt of antibiotics in prior 14 days, medium = occupant had receipt of antibiotics in prior 14 days, high = occupant had confirmed *C. difficile* infection.