

Differentiating virulent 027 and non-027 *Clostridium difficile* strains by molecular methods

Introduction

Hypervirulent ribotype 027 (BI/NAP1) strains were first believed to be genetically homogenous clade, but has later been shown to include not only several lineages of ribotype 027 but also other ribotypes, e.g. 176 in Poland and Czech Republic, and ribotype 244 in Australia. We present data on additional non-027 strains, identified as presumptive 027 by two commercial molecular *C. difficile* assays and compare the results with in-house PCR assay for presence of toxin genes, and ribotype profiles.

Materials and methods

A set of 18 non-027 *C. difficile* isolates were carefully selected from the national reference laboratory collection on the basis of similarities in their ribotype and/or toxin gene profile with to those of ribotype 027, i.e. Paloc variants (Table 1). The used tests with 2-level detection, firstly for toxigenic *C. difficile* and secondly for presumptive ribotype 027 identification, were Xpert®*C. difficile*/Epi (Cepheid, USA)

and AmpliDiag™ *C. difficile*+027 (Mobidiag, Finland).

GeneXpert detects toxin B, binary toxin and 1-bp deletion at nucleotide 117 in *tcdC*. AmpliDiag has three target genes: toxin B, +027 positive marker (*pct*), and -027 negative marker (*hydR*).

Results

Xpert®*C. difficile*/Epi assay misclassified five ribotypes (016, 019, 080, 176 and variant of type 046) as presumptive 027 and AmpliDiag™ *C. difficile*+027 assay two ribotypes (016, 176).

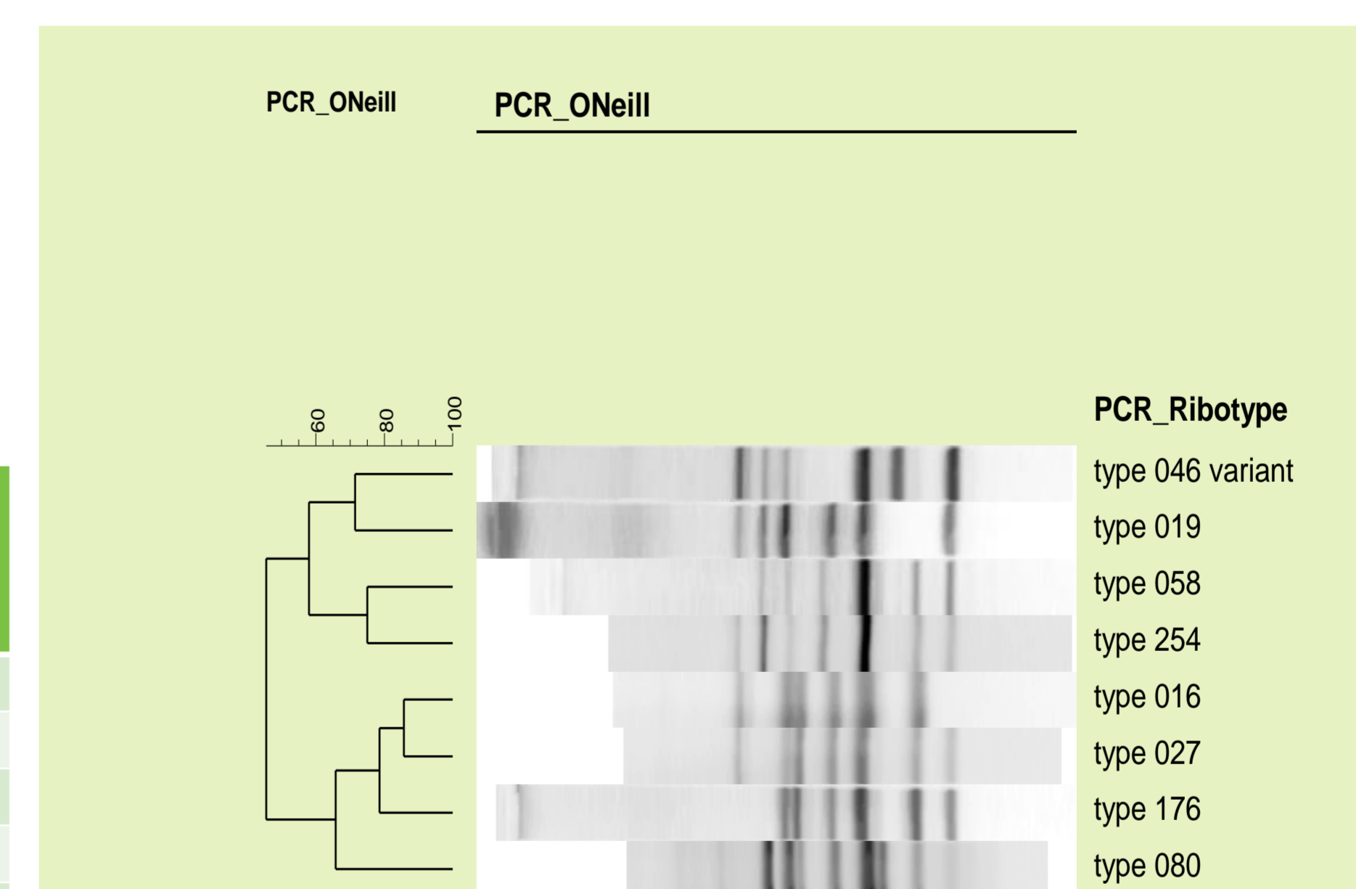
The ribotype profiles of types 016 and 176 differ from 027 by a single band, and 080, 019 and variant 046 by 3-5 bands (Figure 1).

Misclassified strains were rare, covering 1.6% of national reference laboratory strain collection, while type 027 accounted for 23%.

Conclusions

- Our findings confirm the concept that there are closely related outliers to hypervirulent 027 clones
- Outliers can be misclassified as 027
- Outliers comprise of an increasing number of ribotypes, including previously reported four ribotypes (198, 176, 244, 019), and additional three (016, variant 046, 080) identified in the present study.
- Tests with presumptive 027 identification are useful in assessing hypervirulence, and in classifying or grouping strains.
- Though individual Paloc variants are rare, they should not be overlooked since their relative pathogenicity is likely to be high.

Figure 1. PCR ribotype profiles on agarose gel of ribotype 027 and types that were misidentified as 027 by commercial molecular tests.



Ribotype	In-house PCR <i>cdu2</i>	Tox A	Tox B	Binary	<i>tcdC</i> deletion	GeneXpert Tox B	Binary	<i>tcdC</i> deletion	Presumptive 027	AmpliDiag Tox B	Presumptive 027
027	POS	A	B	BIN	18 bp	B	BIN	POS	POS	B	POS
016	POS	A	B	BIN	18 bp	B	BIN	POS	POS	B	POS
176	POS	A	B	BIN	18 bp	B	BIN	POS	POS	B	POS
variant 046	POS	A	B	BIN	18 bp	B	BIN	POS	POS	B	NEG
019	POS	A	B	BIN	18 bp	B	BIN	POS	POS	B	NEG
080	POS	A	B	BIN	18 bp	B	BIN	POS	POS	B	NEG
063	POS	A	B	BIN	18 bp	B	BIN	NEG	NEG	B	NEG
122	POS	A	B	BIN	18 bp	B	BIN	NEG	NEG	B	NEG
075	POS	A	B	BIN	18 bp	B	BIN	NEG	NEG	B	NEG
131	POS	A	B	BIN	18 bp	B	BIN	NEG	NEG	B	NEG
308	POS	A	B	NEG	18 bp	NT	NT	NT	NT	B	NEG
015	POS	A	B	NEG	18 bp	NT	NT	NT	NT	B	NEG ^a
variant 063	NEG	A	B	BIN	39 bp	NT	NT	NT	NT	B	NEG
045	POS	A	B	BIN	39 bp	B	BIN	NEG ^b	NEG	B	NEG
111	NEG	A	B	BIN	39 bp	B	BIN	NEG	NEG	B	NEG
126	NEG	A	B	BIN	39 bp	NT	NT	NT	NT	B	NEG
254	POS	A	B	BIN	54 bp	NT	NT	NT	NT	B	NEG
058	POS	A	B	BIN	54 bp	NT	NT	NT	NT	B	NEG
023	POS	A	B	BIN	54 bp	B	BIN	NEG	NEG	B	NEG

Table 1. Comparison of results for 19 *C. difficile* isolates using in-house PCR, GeneXpert and AmpliDiag. Variant 046 (Finnish type un67) differs from reference strain by producing binary toxin and having a small deletion in *tcdC* and variant 063 (Finnish type un122) differs by being negative for *cdu2* and by having a bigger deletion in *tcdC*.

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² Mobidiag Ltd., Espoo, Finland.

