Australia — home of *Clostridium difficile* clade 5?

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BACKGROUND

Although *C. difficile* is recognised as an important nosocomial pathogen and an emerging cause of infections in the community, very little data exist on its molecular epidemiology outside of Europe and North America. Phylogenetic analysis of *C. difficile* isolates has revealed at least five clades, some containing subclades.[1-3] Although clade 1 is the largest and most heterogeneous, all five clades contain toxigenic and clinically important strains.[2] Clade 5 is the lineage containing PCR-ribotype (RT) 078, (multilocus sequence type [MLST] ST11), an important animal strain which recently emerged as a common human pathogen, particularly in Europe.[4-5] This clade is genetically divergent from the other four clades and unusually homogeneous.[6]

METHODS

A total of 44 Australian *C. difficile* isolates were chosen to be representative of known genetic diversity in Australia. PCR ribotyping, toxinotyping, and toxin PCR assays were performed as previously described.[7-11]

Whole genome sequences were determined using an Illumina HiSeq2000 at the Wellcome Trust Centre for Human Genetics. MLST alleles were extracted *in silico* and designations obtained by querying the PubMLST database (http://pubmlst.org/cdifficile/).[12]

Novel STs and those already in the existing *C. difficile* PubMLST database were analysed using MEGA v. 5.[13] The Neighbor-joining method was used for all ungapped sites, using a Tamura-Nei substitution model with gamma distributed rates, and 1000 bootstrap resamplings.[14-15] Trees were visualised using FigTree v1.3.1.[16]

RESULTS & DISCUSSION

Half of the 44 Australian isolates (50%) in this study were members of clade 5.

Strains belonging to clade 5 were isolated in four Australian states (WA, NSW, QLD, VIC) from urban and rural areas, and from human and animal (porcine, equine and bovine) hosts.

Of the 22 clade 5 isolates, six (27.3%) were ST11, while the remaining 16 (72.7%) represented eight newly identified STs: ST163 (*n*=2), ST164 (*n*=2), ST166 (*n*=2), ST167 (*n*=5), ST168 (*n*=1), ST169 (*n*=1), ST173 (*n*=1) and ST174 (*n*=1).

All of the novel STs that were identified more than once contained multiple ribotypes.

Two STs (ST164 and ST167) contained both toxigenic (A^-B^+) and non-toxigenic strains (A^-B^-).

Of the six STs that contained A^-B^+ strains, four contained toxinotype XXXI strains and one contained toxinotype XXXI strains.[17]

All of the clade 5 isolates were binary toxin-positive, including the LCT-negative (A^-B^-) strains.

CONCLUSIONS

C. difficile phylogenetic clade 5 is much more heterogeneous than previously thought, both with respect to ST and toxigenic profile.

The diversity of clade 5 within Australia highlights the importance of investigating the molecular epidemiology of _C. difficile_ in hitherto neglected regions if we are to properly understand the evolution of this pathogen.

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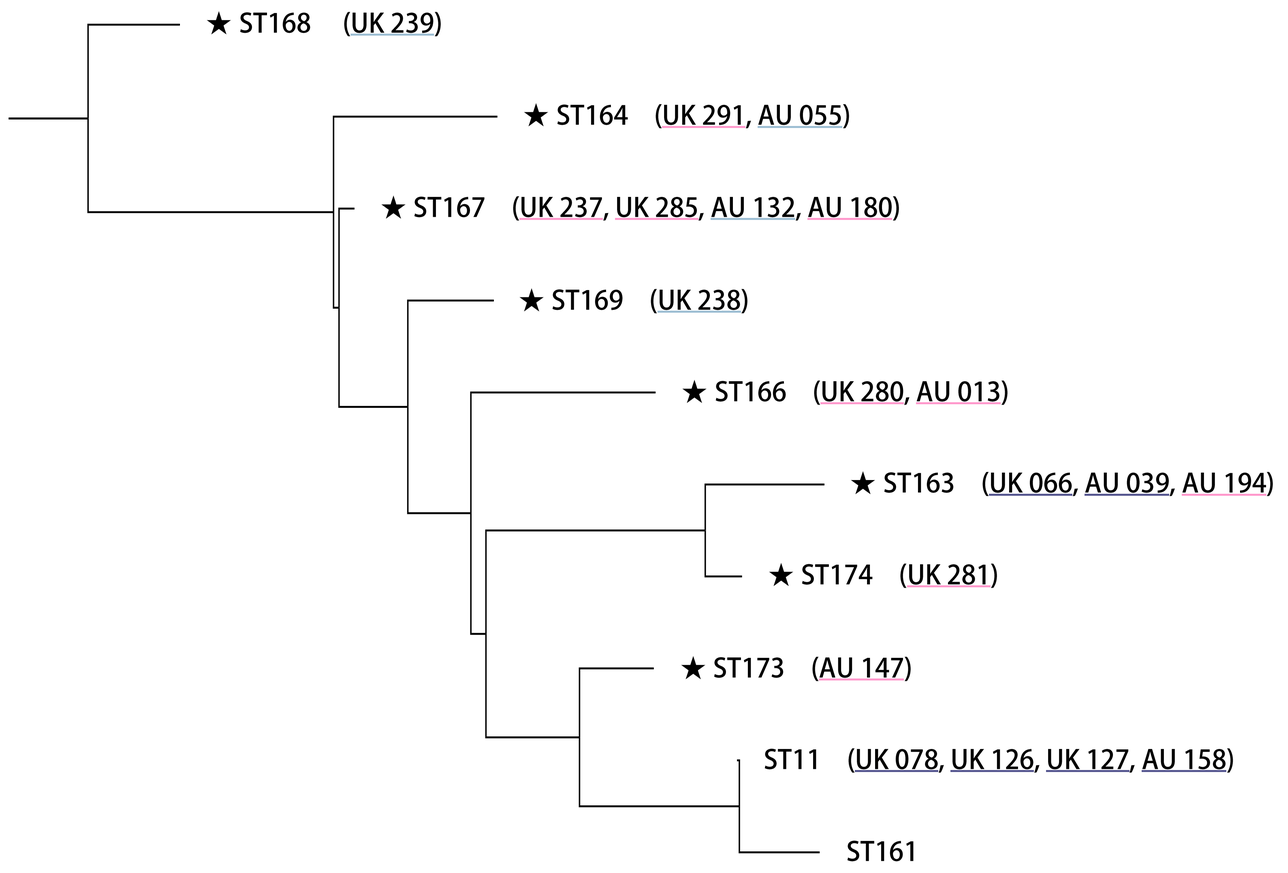

Fig. 1– Neighbor joining tree constructed using the concatenated MLST loci of clade 5 isolates

New sequence types identified during this study are indicated by a star; PCR ribotype is indicated in brackets; the colour underlining the ribotype represents the toxin profile ($A^+B^+CDT^+$, $A^-B^+CDT^+$, $A^-B^-CDT^+$)

Table 1. Characteristics of Australian clade 5 isolates

Isolate code	Origin	Host	ST	Toxin Profile	Toxinotype	Ribotype
ES 210	NSW	Human	ST11	$A^+B^+CDT^+$	–	AU 158
WA94	WA	Human	ST11	$A^+B^+CDT^+$	V	UK 078
ES 280	VIC	Human	ST11	$A^+B^+CDT^+$	–	UK 078
RPH123	WA	Human	ST11	$A^+B^+CDT^+$	–	UK 078
ES 153	NSW	Human	ST11	$A^+B^+CDT^+$	–	UK 126
WA27	WA	Human	ST11	$A^+B^+CDT^+$	VI	UK 127
WA16	WA	Human	ST163	$A^+B^+CDT^+$	–	AU 039
AI155	NSW	Equine	ST163	$A^+B^+CDT^+$	V	UK 066
ES 223	NSW	Human	ST163	$A^-B^+CDT^+$	–	AU 194
WA13	WA	Human	ST164	$A^-B^+CDT^+$	XXX	UK 291
HCD52	WA	Human	ST164	$A^-B^-CDT^+$	–	AU 137
PMH25	WA	Human	ST166	$A^-B^+CDT^+$	XXX	AU 013
ES 130	NSW	Human	ST166	$A^-B^+CDT^+$	XXX	UK 280
AI178	WA	Porcine	ST167	$A^-B^+CDT^+$	XXXI	AU 180
WA151	WA	Human	ST167	$A^-B^+CDT^+$	XXXI	UK 237
AI15	WA	Porcine	ST167	$A^-B^+CDT^+$	XXXI	UK 237
AI152	WA	Porcine	ST167	$A^-B^+CDT^+$	XXXI	UK 285
Q6	QLD	Human	ST167	$A^-B^-CDT^{+(LK-)}$	NT	AU 132
WA12	WA	Human	ST168	$A^-B^-CDT^{+(LK-)}$	NT	UK 239
AI16	WA	Porcine	ST169	$A^-B^-CDT^+$	NT	UK 238
AI27	NSW	Bovine	ST173	$A^-B^+CDT^+$	XXX	AU 147
ES 166	VIC	Human	ST174	$A^-B^+CDT^+$	XXX	UK 281

LK^- (Lok1/Lok3 PCR-negative)

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