High prevalence of *Clostridium difficile* in home gardens in Western Australia

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INTRODUCTION

Clostridium (*Clostridioides*) *difficile* is a Gram-positive, spore-forming, rod-shaped anaerobic bacterium that is found in the environment and the gastrointestinal tracts of young individuals (both human and non-human). It is the causative agent of pseudomembranous colitis and a predominant cause of life-threatening antimicrobial-associated diarrhoea worldwide [1]. *C. difficile* infection (CDI) has been thought of as a healthcare-associated infection, and its increasing incidence, severity and recurrence make it a major healthcare threat [2]. In recent years, community-acquired CDI (CA-CDI) has emerged as a significant health problem, accounting for ~50% of all CDI cases [3]. Possible sources of CA-CDI are soil, food and water contaminated with strains of *C. difficile* from animals. More broadly, contaminated home gardens and shoe soles could contribute to the dissemination of *C. difficile* spores in the community.

AIM: To assess the prevalence of *C. difficile* in home gardens, including soil, compost, manure and shoe soles samples collected from community home gardens in Western Australia (WA).

MATERIALS AND METHODS

In total, 97 samples consisting of soil (n=48), compost (n=15), manure (n=12) and shoe sole swabs (n= 22) were collected from 23 homes in 22 suburbs of Perth, WA, during 2018. Approximately 5 g of soil/manure/compost samples were enriched in 90 mL brain heart infusion broth (BHIB) (supplemented with 1 g/L taurocholate, cefoxitin (10 mg/L) and cycloserine (200 mg/L) which was pre-reduced for 4 h in an anaerobic chamber, and incubated at 35°C for 5 days with the lids loose, followed by alcohol shock and plating onto ChromID agar plates (bioMerieux). Viable counts of *C. difficile* from soil, compost and manure were determined from a subset of samples. The total number of colony forming units (CFU) in 100 µL were converted to CFU/mL and multiplied by the dilution factor to determine the concentration in the initial sample. Each shoe sole was swabbed with a sterile pre-moistened PolyWipe® sponge (Medical Wire and Equipment Co Ltd, UK) to enumerate *C. difficile* according to methods previously described [4]. All isolates were characterised by toxin gene PCR and PCR ribotyping.

RESULTS

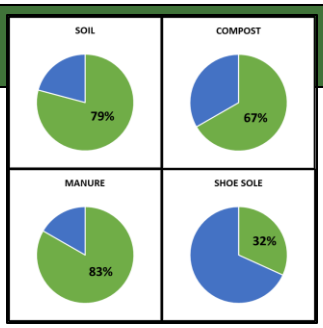
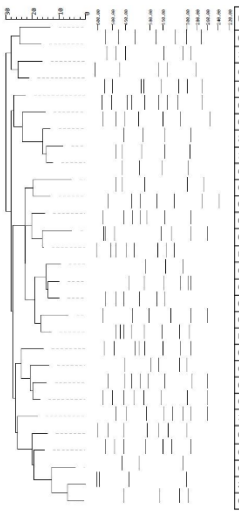


FIG 1 The prevalence of *C difficile* positive specimens (Green = positives)

- Two-thirds (67%, 65/97) of home garden samples were positive for *C. difficile* (Fig 1), yielding 87 isolates.
- Among them, 38% (33/87) of the isolates were toxigenic (A+B+CDT+, n=1; A+B+CDT-, n=27; A-B-CDT+, n=1; A+B-CDT-, n=4).
- 26 different PCR ribotypes (RTs) were identified. The toxigenic *C. difficile* strain, UK 014/020, was the most prevalent (20.7%, 18/87) (Fig 2).
- RT 014/020 was also the only RT found in all sources (soil, compost, manure and shoe soles).
- Of note, 21.8% (19/87) of the isolates produced white colonies on ChromID plates, indicating that they lack esculin hydrolysis enzyme. Of these, four were toxigenic but produced only toxin A (A+B-CDT-) and 5 isolates were UK 125 (A-B-CDT-).
- Both esculin hydrolysis negative and positive colonies were recovered from the same samples with mixed RTs (RT 014/020, RT 014 and RT 010).
- Through direct culture method, 41% (40/97) of *C. difficile* were recovered (the rest recovered only after a culture enrichment procedure).
- The average maximum *C. difficile* viable counts were in compost (2028 CFU/g) followed by manure (619 CFU/g) and soil (554 CFU/g).



Ribotype	Toxin profile	Sample No.				No. (%)	Post codes
		Soil	Compost	Manure	Shoes		
023*	A+B+CDT+	NA	NA	NA	NA	NA	NA
106	A+B+CDT-	1	-	-	-	1 (1.1)	6066
QX 638	A-B-CDT-	1	-	-	-	1 (1.1)	6009
056	A+B+CDT-	1	-	-	1	2 (2.3)	6014, 6018
039	A-B-CDT-	1	-	-	-	1 (1.1)	6010
010	A-B-CDT-	4	2	2	0	8 (9.2)	6062, 6059, 6006, 6009, 6150, 6029
286	A-B-CDT-	2	-	-	-	2 (2.3)	6059, 6054
125	A-B-CDT-	6	7	2	-	15 (17.2)	6059, 6163, 6010, 6054, 6066, 6014, 6018, 6020, 6151, 6024, 6101
QX 597	A-B-CDT-	4	-	-	3	7 (8.0)	6006, 6054, 6024
QX 141	A-B-CDT-	1	-	-	-	1 (1.1)	6066
027*	A+B+CDT+	NA	NA	NA	NA	NA	NA
054	A+B+CDT-	2	1	-	1	4 (4.6)	6152, 6076, 6018
014/020	A+B+CDT-	10	1	5	2	18 (20.7)	6062, 6059, 6006, 6054, 6066, 6076, 6020, 6151, 6112, 6024, 6026
Novel	A-B-CDT+	1	-	-	-	1 (1.1)	6076
QX 639	A-B-CDT-	2	-	-	-	2 (2.3)	6010, 6009
QX 637	A-B-CDT-	3	-	-	-	3 (3.4)	6152
078*	A+B+CDT+	NA	NA	NA	NA	NA	NA
017*	A+B+CDT-	NA	NA	NA	NA	NA	NA
Novel	A-B-CDT-	1	-	-	-	1 (1.1)	6152
051	A-B-CDT-	3	1	1	-	5 (5.7)	6020, 6026, 6163, 6101
QX400	A-B-CDT-	1	-	-	-	1 (1.1)	6152
081	A+B+CDT-	1	-	-	-	1 (1.1)	6026
QX 076	A+B+CDT-		-	1	-	1 (1.1)	6014
103	A+B+CDT-		-	-	1	1 (1.1)	6006
012*	A+B+CDT-	NA	NA	NA	NA	NA	NA
QX 077	A-B-CDT-	2	-	-	-	2 (2.3)	6163, 6019
QX 601	A-B-CDT-	1	-	-	-	1 (1.1)	6009
287	A-B-CDT-	3	-	-	-	3 (3.4)	6062, 6150
QX 189	A-B-CDT-	1	-	-	1	2 (2.3)	6014, 6163

FIG 2 Summary of prevalence, ribotype and toxin gene profile for 87 *C. difficile* isolated from home gardens in Perth, WA.
PCR ribotype pattern analysis was performed by creating a neighbour-joining tree, using the Pearson correlation (optimization, 5%; curve smoothing, 1%).
*Reference strain; NA – Not applicable.

CONCLUSIONS

- A high prevalence of *C. difficile* in home gardens and the RTs 014/020, 056, 054, 010 and 103 identified from this study had been isolated from humans with CDI in WA clearly indicated that home gardens may be a possible reservoir for the dissemination of *C. difficile* spores in the environment which subsequently cause CA-CDI.
- Our findings highlight the importance of a "One Health" approach to dealing with CDI.
- Further investigation is required to compare the relatedness of these isolates with isolates from CA-CDI cases by whole genome sequencing.

REFERENCES

[1] Rupnik, M. *et al.* (2009). DOI:10.1038/nrmicro2164
[2] Khanna, S. *et al.* (2011). DOI:10.1038/ajg.2011.398
[3] Guh, A.Y. *et al.* (2020). DOI: 10.1056/NEJMoa1910215
[4] Knight, D.R. *et al.* (2016). DOI:10.1016/j.cmi.2015.11.017