Comparative genomics and antimicrobial susceptibility of *Clostridium difficile* PCR ribotype 014 isolated from humans and piglets in Australia

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BACKGROUND AND STUDY AIM

Clostridium difficile is a formidable enteric pathogen of humans and production animals, and *C. difficile* infection (CDI) has emerged in recent years as an important One Health issue. Concomitantly, in North America and Europe there are an increasing number of reports of toxigenic *C. difficile* being isolated from food of animal origin highlighting a potential for zoonotic transmission. PCR ribotype (RT) 014 is an extremely successful lineage of *C. difficile*, being the most common RT found in humans worldwide but also recently identified as the most prominent RT in neonatal pigs in Australia. In this study, we sought to investigate the genomic similarities and differences between strains of RT014 isolated from humans and pigs in Australia.

METHODS

RT014 isolates were obtained from humans with CDI (*n*=9, public and private labs) and diarrhoeic piglets aged <7 days (*n*=9, 2 farms, 5 litters) from Victoria, Australia, in 2013. Whole genome shotgun sequencing (WGS) was performed using an illumina MiSeq platform (2x250bp paired-end chemistry) as previously described [1]. Raw sequence data was interrogated bioinformatically for multi-locus sequence type (MLST) and antimicrobial resistance genes using the pubMLST [2,3] and ARG-Annot databases [4], respectively, and compiled within the command line software SRST2 [5]. Sequence data was also assembled *de novo* using SPAdes [6] and contigs interrogated using pubMLST.org for detection of toxin and S-layer allele-types [2,3]. Using BLASTn [7] and a custom recombinase library, sequences were interrogated for detection and characterisation of transposons. Predictions of bacteriophage content were made using PHAST [8]. Minimum inhibitory concentration (MIC) was determined for all isolates against 14 antimicrobials using CLSI agar methodology and CLSI and EUCAST breakpoints [9-11].

RESULTS & DISCUSSION

Comparative phylogenetic, genomic and antibiogram analysis of RT014 isolated from humans (*n*=9) and piglets (*n*=9) in Victoria, Australian 2013

KEY: present ● absent ○

					Toxin genes				AMR genes						Tn			Bacteriophages								*slpA* allele		MIC [mg/L] / Susceptible (S), Intermediate (I), Resistant (R), no breakpoint available (*nr*)										
Isolate ID	Host species	RT	ST	MLST Clade	*tcdA*	*tcdB*	*cdtLoc*	Δ*tcdC*	*ermB*	*tetW*	*tetM*	*aph3'-III*	*sat4*	*ant6*	Tn*6194*	Tn*5397*	Tn*B1230-like*	ΦCD27	ΦC2	ΦCDMH1	ΦCD38-2	ΦCD6356	ΦMMP02	Bacillus G	Clost_c_st	7	9	Vancomycin	Metronidazole	Clindamycin	Erythromycin	Augmentin	Ceftriaxone	Meropenem	Moxifloxacin	Tetracycline	Pip/tazo	Trimethoprim
ESP146	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	○	●	●	●	●	○	○	○	●	●	○	○	○	●	1 S	0.25 S	>32 R	>256 R	0.25 S	16 R	2 S	1 S	32 R	4 S	32 *nr*
ESP148	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	●	●	●	●	○	○	○	●	●	○	○	○	○	●	1 S	0.25 S	>32 R	>256 R	0.25 S	16 R	2 S	1 S	32 R	4 S	32 *nr*
ESP150	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	○	●	●	●	○	●	○	○	●	○	○	○	○	●	1 S	0.25 S	>32 R	>256 R	0.25 S	16 R	2 S	0.5 S	32 R	4 S	32 *nr*
ESP152	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	○	●	●	●	●	○	●	○	●	○	○	○	○	●	1 S	0.25 S	>32 R	>256 R	0.25 S	16 R	2 S	1 S	32 R	4 S	32 *nr*
ESP154	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	○	●	●	●	○	○	○	○	●	○	●	○	○	●	1 S	0.25 S	>32 R	>256 R	0.25 S	16 R	2 S	1 S	32 R	4 S	32 *nr*
ESP156	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	○	●	●	●	○	●	●	○	●	○	○	○	○	●	1 S	0.5 S	>32 R	>256 R	0.5 S	32 R	2 S	1 S	32 R	4 S	32 *nr*
ESP158	Porcine	14	49	1	●	●	○	○	●	●	●	●	●	○	●	●	●	○	○	○	○	●	●	●	○	○	●	1 S	0.25 S	>32 R	>256 R	0.12 S	16 R	2 S	0.5 S	32 R	4 S	32 *nr*
ESP160	Porcine	14	13	1	●	●	○	○	○	●	○	●	○	○	○	○	●	○	○	○	○	○	○	○	○	○	●	1 S	0.25 S	2 S	0.25 S	0.25 S	16 R	2 S	1 S	1 S	4 S	32 *nr*
ESP162	Porcine	14	13	1	●	●	○	○	○	●	○	●	○	○	○	○	●	○	○	○	○	○	○	○	○	○	●	1 S	0.5 S	2 S	0.25 S	0.25 S	16 R	2 S	1 S	1 S	4 S	32 *nr*
SQ0120	Human	14	13	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	●	○	●	1 S	0.5 S	2 S	0.5 S	0.12 S	8 S	0.5 S	1 S	0.06 S	4 S	32 *nr*
SQ0114	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.12 S	0.5 S	0.12 S	0.12 S	8 S	0.5 S	0.5 S	0.06 S	2 S	16 *nr*
SQ0116	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.25 S	0.5 S	0.25 S	0.12 S	4 S	1 S	0.5 S	0.12 S	4 S	32 *nr*
SQ0118	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	●	○	○	○	○	○	●	○	1 S	0.5 S	4 I	0.5 S	0.25 S	16 R	2 S	2 S	0.12 S	4 S	32 *nr*
SQ0126	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.5 S	4 I	0.5 S	0.12 S	32 R	0.5 S	2 S	0.06 S	4 S	16 *nr*
SQ0341	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.25 S	1S	0.12 S	0.12 S	16 R	0.5 S	1 S	0.06 S	4 S	32 *nr*
SQ0342	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.25 S	0.5S	0.5 S	0.12 S	4 S	0.5 S	1 S	0.12 S	4 S	8 *nr*
SQ0348	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.5 S	0.5S	0.5 S	0.25 S	4 S	2 S	1 S	0.12 S	4 S	32 *nr*
SQ0352	Human	14	2	1	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	●	○	1 S	0.5 S	1S	0.25 S	0.5 S	16 R	2 S	1 S	0.12 S	4 S	32 *nr*

Phylogenetic analysis

- Three different STs were identified (ST2, ST13 and ST49), belonging to the heterogeneous MLST clade I, congruent with RT014.
- STs differed from each other by 1–2 single loci variants (*dxr* and *tpi*) and microevolutionary analysis by ClonalFrame showed ST2 and ST49 were genetically highly similar sharing a recent common ancestor (data not shown).
- Notably, distribution of STs 2 and 49 appeared host species specific: ST2 was found exclusively in humans (8/9 isolates) and ST49 exclusively in pigs (7/9) ($p<0.05$). ST13 was present in both humans (1/9) and pigs (2/9).

Antibiogram

- Resistance to erythromycin, clindamycin and tetracycline was observed in all isolates of ST49, but absent from STs 2 and 13.
- No resistance or raised MIC to key therapeutic agents vancomycin and metronidazole was observed and all isolates were susceptible to moxifloxacin, meropenem, augmentin and pip/tazo.
- High MICs were observed for trimethoprim and all isolates were inherently resistant to aminoglycosides gentamicin, tobramycin and spectinomycin (data not shown).

Comparative genomics

- All isolates possessed genes encoding large clostridial cytotoxins TcdA and TcdB, but were negative for binary toxin and mutations in *tcdC*.
- Significant diversity was observed between STs in the bacterial S-layer and in phages belonging to *Siphoviridae* and *Myoviridae* including those shown to influence *C. difficile* pathogenesis (ΦCD27, ΦC2, ΦCDMH1 and ΦMMP02).
- *ermB* and *tetM* were identified in all isolates of ST49 carried on conjugative transposons Tn*6194* and Tn*5397*, respectively and are in agreement with the antibiogram data. To our knowledge this is the first report of Tn*6194* in *C. difficile* isolated from livestock. Tn*6194* is the most common *ermB* containing element in clinical isolates in European hospitals and contributed to the dissemination of FQR1 lineage of epidemic RT027 [12,13]. Moreover, Tn*6194* is capable of inter- and intraspecies (*E. faecalis*) transfer [14] demonstrating the capacity for horizontal gene transfer between *C. difficile* RT014 (ST49) and other genera within the shared niche of animal gastrointestinal tracts.
- STs 49 and ST13 (porcine only) also possessed *tetW* - an uncommon tetracycline resistance element, previously described in a very small number of clinical isolates in Europe and in swine in North America [15,16]. *tetW* was located on a Tn*B1230*-like element originating from ruminal anaerobe *B. fibrisolvens* [17]. However, in the case of ST13 (where *tetM* was absent) the presence of *tetW* did not confer a resistance phenotype and may indicate upstream promoter regions are absent.
- Components of an aminoglycoside-streptothricin resistance cassette (*ant6-sat4-aph3'-III)* previously described in staphylococci and viridans streptococci [18] were identified in all porcine isolates.

Summary

- **Significant diversity was seen in ST, antibiogram and several clinically relevant loci including the bacterial S-layer, antimicrobial resistance genes, transposons and bacteriophages. These differences appear to be host species specific, possibly reflecting anthropogenic and host selection pressures in piggery and hospital environments.**
- **These data suggest there is limited genetic overlap between RT014 from porcine and human *C. difficile* in this particular snapshot, however animal strains of *C. difficile* RT 014 are clearly a reservoir of antimicrobial resistance genes of clinical importance.**

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