Antibiogram patterns of non-toxigenic, CDT producing *Clostridium difficile* ribotypes

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Background

- Antimicrobial resistance (AMR) is commonly found in *Clostridium difficile,* a leading cause of antibiotic associated infectious diarrhoea, and is a major driver of strain evolution[1].
- Antimicrobial susceptibility of *C. difficile* strains that produce large clostridial toxins A and B (LCT) have been determined periodically.
- *C. difficile* strains that lack the LCTs and only produce binary toxin (CDT), a third toxin produced by some *C. difficile* strains, are currently considered clinically irrelevant, mainly because the importance of CDT in *C. difficile* Infection (CDI) is unknown.
- These strains are predominantly isolated from colonised (and in some instances diarrhoeic) food animals, however, have also recently been detected in patients with idiopathic diarrhoea[2].
- We tested the *in vitro* activities of 11 antimicrobials against a diverse collection of *C. difficile* strains which only produce CDT [$A^-B^-CDT^+$].

Methods

- Genomic DNA extraction, PCR ribotyping, toxin gene profiling and antimicrobial susceptibility testing [agar dilution method] was performed as previously described[3]. The sample population comprised $A^-B^-CDT^+$ *C. difficile* isolated from humans (*n*=29), bovine (*n*=54), porcine (*n*=39), food (*n*=1), and effluents (*n*=26).
- Whole genome sequencing (WGS) was performed on a subset of these isolates (*n*=52) using the Illumina MiSeq platform as previously described[4]. Acquired AMR genes were detected *in silico* from short reads using the ARG-ANNOT database[5] compiled in SRST2 v0.1.8[6]. Chromosomal resistance loci were investigated using Artemis as previously described[3].

Results

- All of the tested isolates were susceptible to vancomycin, metronidazole and fidaxomicin, agents currently considered first line treatments for CDI.
- AMR was observed in 14 isolates [environmental *n*=11, human *n*=3] to tetracycline [TetR, MIC=16mg/L], moxifloxacin [MxfR, MIC=16mg/L], erythromycin [EryR, MIC ≥128mg/L] and clindamycin [CliR, MIC=8mg/L].
- The MxfR strain possessed mutations in *gyrA/B* whilst the TetR strain contained a *tetM* gene carried on the conjugative transposon Tn*6190.*
- All EryR and CliR strains were negative for the methylase *erm* genes, suggesting a possible alternative mechanism of resistance.
- Slight differences in phenotypes between PCR ribotypes were observed (Fig 2) but were minimal and corroborated previous studies[3].
- Manual curation of the $A^-B^-CDT^+$ *C. difficile* genomes detected AMR genes [*blaR* and *cme*] in RT033 and RT288 strains, loci which have been previously reported in other *C. difficile* ribotypes (Table 2)[7] .
- A single RT033 strain harboured a vanB2 resistance gene cluster carried on conjugative transposon Tn*1549*, the first such finding for this species.
- This work illustrates the presence of multiple AMR genes in various $A^-B^-CDT^+$ *C. difficile* ribotypes.

Table 1: Susceptibility of $A^-B^-CDT^+$ *C. difficile* strains against 11 antimicrobial agents

Antimicrobial Agent	MIC Range [mg/L]	MIC50 [mg/L]	MIC90 [mg/L]	Clinical breakpoints			$A^-B^-CDT^+$ isolates		
				S	I	R	S	I	R
Fidaxomicin[a]	0.004 - 0.12	0.03	0.12	-	-	≥1	-	-	0%
Vancomycin[b]	1.0 - 2.0	1	1	≤2	-	>2	100%	0%	0%
Metronidazole[b]	0.12 - 1.0	0.5	1	≤2	-	>2	100%	0%	0%
Rifaximin[c]	0.004 - 2	0.004	0.015	-	-	≥32	-	-	0%
Clindamycin[d]	0.03 - 8	0.5	4	≤2	4	≥8	92.6%	6%	1.4%
Erythromycin[d]	0.12 - 128	1	4	-	-	>8	-	-	7.4%
Amox-clavulanate[d]	0.25 - 2	0.5	1	≤4	8	≥16	100%	0%	0%
Ceftriaxone[d]	1.0 - 64	32	32	≤16	32	≥64	46.3%	53%	0.7%
Moxifloxacin[d]	1.0 - 2.0	1	1	≤2	4	≥8	98.6%	0.7%	0.7%
Meropenem[d]	2.0 - 4.0	2	2	≤4	8	≥16	100%	0%	0%
Tetracycline[d]	0.06 - 8	0.12	2	≤4	8	≥16	96%	3%	1%

[a]Resistance ≥1mg/L[8]; [b]EUCAST breakpoints[9]; [c]Resistance ≥ 32mg/L [10]; [d]CLSI breakpoints[11]

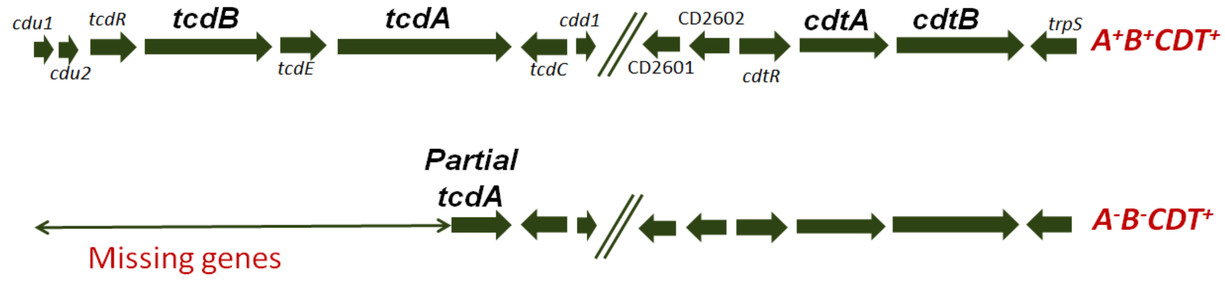

Fig 1. Comparison of LCT/CDT producing *C. difficile* ($A^+B^+CDT^+$) strain versus CDT only producing *C. difficile* strain [$A^-B^-CDT^+$] . $A^-B^-CDT^+$ strains lack LCT genes in general, however, some $A^-B^-CDT^+$ retain a non-functional fragment of *tcdA* gene.

Table 2: AMR genes detected from raw sequence reads of $A^-B^-CDT^+$ strains

Phenotype	Gene	Ribotype	Toxin profile	Source
Aminoglycoside resistance [a]	*Aph3-III-Sat4A*	UK 033	$A^-B^-CDT^+$	Human, *n=1,* Porcine, *n=3* and Effluent, *n=4*
	Aph3-III-Sat4A-Npm	UK 033	$A^-B^-CDT^+$	Porcine, *n=1*
β-lactamase resistance [b]	*blaR cme*	UK 033	$A^-B^-CDT^+$	Human, *n=19,* Bovine, *n=2,* Porcine, *n=3,* Effluent, *n=4* and Food, *n=1*
		UK 288	$A^-B^-CDT^+$	Human, *n=1* and Bovine, *n=3*
Fluoroquinolone resistance	*gyrA (Lys413Asn) gyrB (Gln160His, Ser366Val, Ser416Ala,Asp426Asn)*	UK 033	$A^-B^-CDT^+$	Human, *n=1*
Glycoprotein resistance	*Van B2* operon	UK 033	$A^-B^-CDT^+$	Bovine, *n=1*
Tetracycline resistance	*TetM*	UK 033	$A^-B^-CDT^+$	Human, *n=1*

[a]All genomes positive for aminoglycoside resistance genes *aph3-III* and *sat4A* harboured a 7269bp fragment of a resistance gene cassette from the ruminant anaerobe species *Erysipelothrix rhusiopathiae* (99% seq ID to KP339868.1).[b] Results obtained by manual curation of genomes.

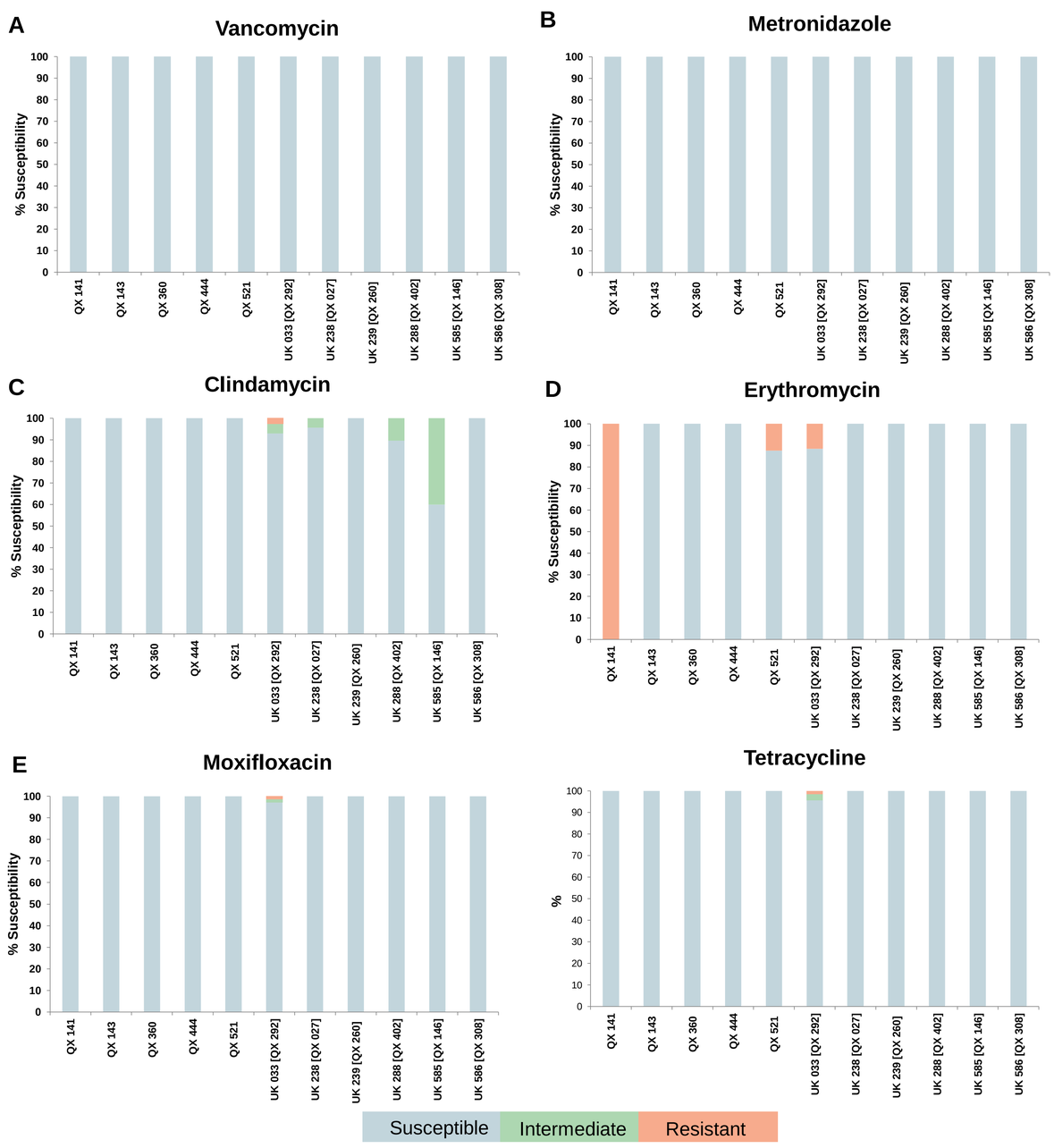

Fig 2. Percentage susceptibility data for vancomycin, metronidazole clindamycin, erythromycin, moxifloxacin and tetracycline, grouped by ribotypes. The resistant isolates belonged to RT033, QX 521 and QX141.

Conclusions

- AMR is an exemplary One Health issue that highlights the importance of the association between human health, animal health and the environment.
- While the role of $A^-B^-CDT^+$ *C. difficile* strains in idiopathic diarrhea is still unclear, they remain common in food animals (veal calves, piglets) and could be potential transmission agents of AMR genes.
- This study provides a comprehensive analysis of antibiotic profiles of various $A^-B^-CDT^+$ *C. difficile* strains isolated from humans, animals, food and environmental sources.

References

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