Antimicrobial Susceptibility in *Clostridium difficile* Varies According to European Region and Isolate source

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Abstract

Background: *C. difficile* (CD) epidemiology continues to evolve: recent studies have identified emerging and resistant ribotypes (RT) that are country-associated. The COMBACTE-CDI project provides an ideal opportunity to examine CD epidemiology and resistance across Europe.

Method:
All diarrhoeal faecal samples sent to 119 recruited testing facilities from 12 European countries, on two sampling days, were collected. All samples were cultured for CD on CCEY agar; isolates were typed by PCR-ribotyping and toxinotyping. Contemporaneous CD isolates were collected from animals in the same countries. Metronidazole, vancomycin, fidaxomicin, moxifloxacin, clindamycin, imipenem, tigecycline and rifampicin MICs for 215 clinical and 44 animal isolates were determined by Wilkins-Chalgren agar dilution. MICs for each antimicrobial were scored as sensitive=0; intermediate=1; resistant=2 for each isolate according to published breakpoints and added to generate a cumulative resistance score (CRS).

Results: Fidaxomicin was the most active treatment agent (geometric mean for both clinical and animal isolates=0.03mg/L) but reduced susceptibility was observed in n=2 (RT181 & RT066) clinical isolates (≥1mg/L). Geometric mean metronidazole MICs (clinical isolates) were 0.3mg/L, but were elevated among predominating epidemic RT027 (2.17mg/L) and Eastern European-associated RT181 (1.03mg/L). RT027 and RT181 also had elevated geometric mean moxifloxacin MICs (16.95mg/L) and 14.75mg/L); clindamycin (9.6mg/L and 10.83mg/L) and rifampicin (20.877mg/L and 0.40mg/L). Two -isolates (RT016 and RT002) were metronidazole resistant (MIC=8mg/L) and 9 (8 RT027; 1 RT198) had intermediate resistance (4mg/L).

Elevated metronidazole MICs were not observed in animal isolates from Eastern Europe, and no location-linked predominating RTs were observed. Increased geometric mean vancomycin MICs were observed in RT078s, which were more commonly isolated from animals than humans (22 vs 12 respectively), but there was no resistance (MIC≥4mg/L). Moxifloxacin and clindamycin resistance was seen in both clinical and animal isolates of multiple RTs. No resistance to imipenem or tigecycline was observed. Average (mean) and median CRS showed that resistance levels among clinical (but not animal) isolates were highest in Eastern Europe.

Conclusions:
Epidemiology and resistance differs between clinical and animal CD isolates and by geographic location. Epidemic CD RT027 and highly-related emerging RT181 have increased levels of antimicrobial resistance and are associated with CD infections in Eastern Europe.

Introduction

Antimicrobial resistance in *C. difficile* a growing concern. As a coloniser of the gastro-intestinal tract of humans and animals, *C. difficile* may be exposed to selection pressures from multiple or sequential antimicrobial courses. Previous studies have identified multiple antimicrobial resistance markers in *C. difficile* PCR ribotypes, such as epidemic PCR ribotype 027 and highlighted emerging antimicrobial resistant PCR ribotypes within specific geographical locations.[1]

There is increasing interest in animal and environmental sources of *C. difficile* and wider concerns about antimicrobial resistance within the food-chain.[2,3] The COMBACTE-CDI project is an IMI2 Horizon2020 framework project that aims to develop a detailed understanding of the epidemiology and clinical impact of CDI across Europe (https://www.combacte.com/about/combacte-cdi-understanding-of-the-epidemiology-and-clinical-impact-of-clostridium-difficile-infection/). This afforded an excellent opportunity to examine the epidemiology of human and animal derived strains collected as part of this study to provide detailed antimicrobial resistance data that can be matched against the genotypic (WGS) data generated during COMBACTE-CD. Here we present the results of phenotypic antimicrobial susceptibility testing.

Methods

All diarrhoeal faecal samples sent to 119 recruited testing facilities from 12 European countries, on two sampling days, during 2018 were collected. All samples were cultured for *C. difficile* on CCEY agar and isolates were subsequently typed by PCR-ribotyping (CDRN, Leeds, UK) and toxinotyping (NLZOH. Maribor, Slovenia). Contemporaneous *C. difficile* isolates were collected from animals in the same countries.

Methods

The antimicrobial testing panel was selected to provide data that can be compared with previous baseline data on anti- *C. difficile* agents (vancomycin, metronidazole, fidaxomicin) and those with known resistance in *C. difficile* (clindamycin, moxifloxacin, imipenem, rifampicin), while including other relevant (linezolid and tigecycline) antimicrobials.

Metronidazole, vancomycin, fidaxomicin, moxifloxacin, clindamycin, imipenem, tigecycline and rifampicin MICs for 215 clinical and 44 animal isolates were determined by Wilkins-Chalgren agar dilution as previously described. [1] MICs for each antimicrobial were scored as sensitive=0; intermediate=1; resistant=2 for each isolate according to published breakpoints (ref) and added to generate a cumulative resistance score (CRS).

Results

Clinical isolates (n=215) comprised 71 different isolates, while animal isolates (n=44) comprised 13 different ribotypes. PCR ribotype distributions differed considerably between clinical and animal sources, despite being taken from the same geographic locations. PCR ribotype 181 was the most commonly isolated clinical ribotype (11% of isolates) and, followed by RT 027 (8%) and 014 (7%). In contrast, among animal isolates PCR ribotype 078 accounted for over 50% of isolates, followed by RT 045 (10%) and 005 (7%).

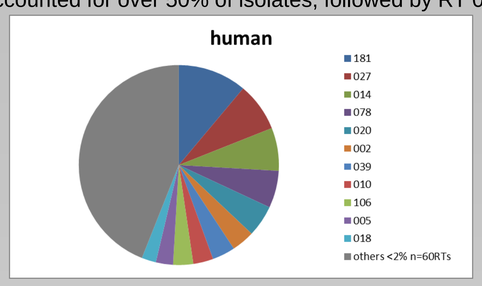

Figure 1. Distribution of PCR ribotypes among human clinical isolates and animal isolates recovered

Fidaxomicin was the most active treatment agent (geometric mean for both clinical and animal isolates=0.03mg/L) but reduced susceptibility was observed in n=2 (RT181 & RT066) clinical isolates (≥1mg/L).
Geometric mean metronidazole MICs (clinical isolates) were 0.3mg/L, but were elevated among predominating epidemic RT027 (2.17mg/L) and Eastern European-associated RT181 (1.09mg/L). RT027 and RT181 also had elevated geometric mean moxifloxacin MICs (14.75mg/L and 17.39mg/L); clindamycin (10.93mg/L and 10.83mg/L) and rifampicin (15.99mg/L and 0.40mg/L). Two -isolates (RT016 and RT002) were metronidazole resistant (MIC=8mg/L) and 9 (8 RT027; 1 RT198) had intermediate resistance (4mg/L).

	MET	VAN	FDX	MXF	IMI	CLINDA	TIGE	RIF
human (n=215)	0.30	0.63	0.03	3.02	2.84	7.21	0.04	0.008
animal (n=44)	0.17	0.92	0.03	2.48	2.17	9.75	0.05	0.001

Figure 2. Geometric mean MICs for all antibiotics tested against human clinical and animal isolates

RT Human clinical isola	N=	MET	VAN	FDX	MXF	IMI	CLINDA	TIGE	RIF
181	24	1.09	0.52	0.03	17.39	4.72	10.93	0.03	15.989
027	17	2.17	0.77	0.03	14.75	4.34	10.83	0.03	0.395
014	15	0.17	0.56	0.02	1.10	1.59	8.00	0.05	0.001
078	13	0.17	0.89	0.03	2.48	2.00	5.04	0.04	0.001
020	11	0.19	0.55	0.03	1.66	2.27	5.66	0.05	0.001
002	8	0.25	0.56	0.04	0.92	1.83	8.00	0.03	0.001
039	8	0.32	0.55	0.03	3.67	4.00	16.00	0.04	0.001
010	7	0.45	0.50	0.02	1.35	2.00	73.52	0.03	0.001
106	7	0.23	0.71	0.04	3.62	4.00	4.49	0.04	0.001
005	6	0.35	0.44	0.03	2.24	2.24	4.18	0.04	0.001
018	5	0.18	0.50	0.01	9.19	3.03	8.00	0.03	0.439
RT Animal	N=	MET	VAN	FDX	MXF	IMI	CLINDA	TIGE	RIF
078	22	0.18	1.03	0.04	2.49	2.20	5.66	0.05	0.001

Figure 3. Geometric mean MICs for all antibiotics tested against human clinical and animal isolates from common ribotypes (n>5)

Elevated metronidazole MICs were not observed in animal isolates from Eastern Europe, and no location-linked predominating RTs were observed. There were little diffferences in MICs, except for vancomycin, which showed a slightly higher geometric mean MIC in animal isolates than in human clinical isolates (1.03mg/L vs 0.89mg/L). RT078 was more commonly isolated from animals than humans (22 vs 12 respectively), but there was no resistance (MIC≥4mg/L). RT078 was the only ribotype represented in number >10 in both human clinical and animal isolate collections in the COMBACTE-CDI study.).

RT	N=	MET	VAN	FDX	MXF	IMI	CLINDA	TIGE	RIF
078 human clinical	13	0.17	0.89	0.03	2.48	2.00	5.04	0.04	0.001
078 animal	22	0.18	1.03	0.04	2.49	2.20	5.66	0.05	0.001

Figure 4. Geometric mean MICs for all antibiotics tested against human clinical and animal RT078 isolates

Moxifloxacin and clindamycin resistance was seen in both clinical and animal isolates of multiple RTs. Geometric mean clindamycin MICs were highest in animal isolates in North, West, and East Europe. Rifampicin MICs were elevated only in human clinical isolates from Eastern Europe. No resistance to imipenem or tigecycline was observed.

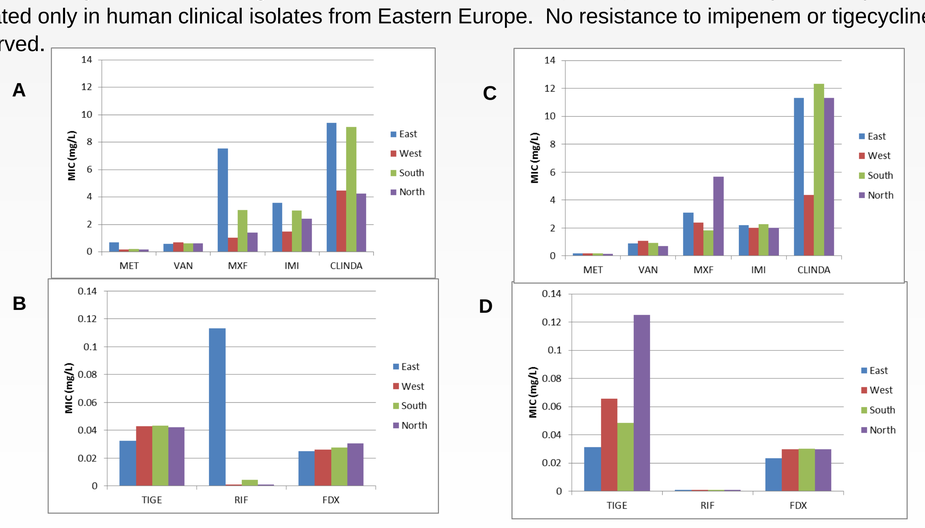

Figure 5. Geometric mean MICs for all antibiotics tested against human clinical (A,B) and animal (C,D) isolates by region

Results

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Average (mean) and median CRS showed that resistance levels among clinical (but not animal) isolates were highest in Eastern Europe.

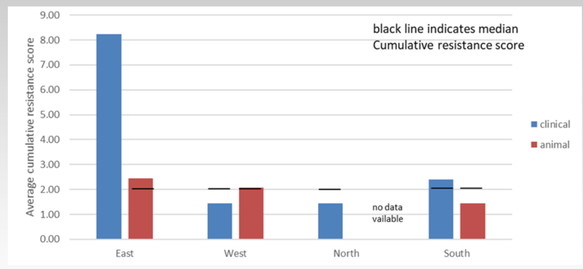

Figure 6. Antimicrobial resistance in clinical human and animal *C. difficile* isolates by European region

Discussion

There is increasing interest in the “one-health” approach to healthcare. The WHO particularly highlight the one-health approach as a potentially highly effective tool in combatting the spread of antimicrobial resistance (https://www.who.int/westernpacific/news/q-a-detail/one-health). The distribution of ribotypes among human clinical and animal isolates differed substantially, with ribotype 078 predominating in animals (>50% of isolates) and ribotype 181 in human clinical isolates. The relatively high proportion of RT181 was influenced by the emergence of this RT in Eastern Europe (Romania). Similar high levels of emerging RTs (176 and 198) were previously described in Eastern European countries (Czech Republic and Hungary, respectively in a previous Pan European study of antimicrobial resistance. [1,4] In both these cases, the emergent RTs also showed increased levels of antimicrobial resistance, and were highly related to RT027.
In the present study, the opportunity to examine animal isolates from the same locations (albeit in smaller numbers 38 clinical vs 5 animal), showed that while RT181 accounted for 68% of human clinical isolates from Romania, this ribotype was not represented at all in the animal collection.

Geometric MICs for clinical isolates were reflective of those observed in the large pan-European study by Freeman et al. and geometric mean MICs for animal isolates, were also similar.[1] However, when general levels of resistance (to all antimicrobials) were assessed according to a cumulative resistance score, these were higher in clinical isolates, and in particular those from Eastern Europe. This is likely driven by the emergent antibiotic resistant RT181 in Romania, accounting for 40% of human clincal isolates submitted from Eastern Europe.

RT078 was the only ribotype present in both human clinical and animal collection in numbers >10 isolates, in line with prevsioulty studies highlighting the association of this ribotype with both humans and animals.[2,3]
While there were moxifloxacin and clindamycin resistant isolates in both these collections, neither exhibited high level resistance (>128mg/L) such as that previously reported in epidemic human-associated RTs such as RT027 and 001.[1] While geometric mean vancomycin MICs in animal-derived RT078 isolates were slightly higher (1.03 mg/L), none displayed vancomycin resistance, which is uncommon amongst C. difficile[1].

This highlights the rapid emergence of antimicrobial resistant clinical isolates, in particular geographic locations, and may be linked to local antimicrobial prescribing policies. Analysis of genome sequence data will further enhance our knowledge of C. difficile resistance emergence and epidemiology

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