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Highlights

- All the *C. difficile* strains from manure and digestate harbored *tcdA* and *tcdB* genes
- 83.3% of the isolated strains belonged to PCR ribotypes 078 or 126
- All the strains were susceptible to vancomycin and metronidazole

Abstract

Clostridioides difficile strains were isolated from manure and digestate samples from five biogas plants in France. The objective of this study was to characterize these isolates using PCR ribotyping, wgMLST, a multiplex PCR targeting genes encoding for the main virulence factors, i.e. *tcdA*, *tcdB*, *cdtA* and *cdtB,* and antimicrobial susceptibility assays. The 54 strains characterized were all positive for *tcdA* and *tcdB* and 83% (45/54) were positive for the binary toxin genes. PCR ribotypes 126 (59%) and 078 (37%) were predominant, and wgMLST analysis of 18 isolates showed close proximity of strains within a single biogas plant. Samples from the biogas plant supplied with cattle and poultry manure displayed the largest variety in PCR ribotypes. The *in vitro* activities of nine antimicrobial agents were determined. All the strains were susceptible to vancomycin and metronidazole, which are currently considered first-line treatments for *C. difficile* infection in humans. All the strains were resistant to clindamycin. The results of this study show that a high percentage of *C. difficile* strains present in the French biogas plants investigated are toxigenic strains from PCR ribotypes also commonly found in humans.

Keywords: *Clostridioides difficile*, PCR ribotype 126 and 078, wgMLST, biogas plant, pig, cattle

Introduction

Clostridioides difficile is a Gram-positive, anaerobic, spore-forming bacterium considered as an emerging pathogen responsible for diarrhea and colitis in both humans and animals. *C. difficile* infection (CDI) is the major cause of nosocomial diarrhea in adults, and has been increasingly reported in the community in the past decade or so [1]. Nowadays, the incidence of community-acquired CDI in some regions accounts for up to a quarter of all cases [2, 3].

Food, animals and the environment are considered potential reservoirs of *C. difficile* strains [4]. Indeed, animal and food PCR ribotypes (RT) have been correlated with those found in humans. Although the zoonotic status of *C. difficile* is still debated [5], recent data highlight a potential risk of transmission from animals or environmental reservoirs to humans [6]. CDI should therefore be managed through a One Health approach.

It has recently been demonstrated that *C. difficile* can be detected in digestate from agricultural biogas plants (BPs) and is able to survive anaerobic digestion [7, 8]. The conversion of livestock manure into biogas through anaerobic digestion in BPs has become increasingly popular in the past few decades in some European countries. However, the *C. difficile* strains isolated from agricultural BPs have never been characterized up to now. The PCR ribotypes of these strains and their virulence gene contents are unknown, yet this information is of prime importance to be able to evaluate the risks associated with its detection in digestate, which can be spread over fields after its storage or post-digestion and may constitute a reservoir of *C. difficile* strains.

66 The aim of our study was to characterize *C. difficile* isolates collected from manure
67 and raw digestate in five different agricultural BPs so as to determine which kind of *C.*
68 *difficile* strains can survive anaerobic digestion.

Materials and methods

Strain collection

A total of 54 *C. difficile* isolates were collected during a study evaluating the presence of various pathogens in manure and digestate samples from five agricultural BPs [8]. Briefly, 1 g of each sample was 10-fold diluted in Brain Heart Infusion (BHI; BioMérieux, Craponne, France) supplemented with 0.1% taurocholate (Sigma Aldrich, Lyon, France), cefoxitin (8 mg/l) and cycloserine (250 mg/l) (Oxoid, Dardilly, France). Tubes were incubated at 37°C in an anaerobic chamber (A35; Don Whitley distributed by BioMérieux, Bruz, France) filled with anaerobic gas (10% H2, 10% CO2, 80% N2). After 7 days of incubation, 10 µl of broth was plated on ChromID *C. difficile* agar (BioMérieux, Craponne, France). The plates were incubated for 48 hours at 37°C in the anaerobic chamber. *C. difficile* colonies were suspected by their specific black color and/or shape and two colonies from each positive sample were re-isolated on BHI agar. These plates were incubated for 48 hours at 37°C in the anaerobic chamber. The isolates were then stored at -80°C. The strains were identified using PCR as described below.

For each BP, three replicate samples of manure and three of digestate were collected on the same day. Two different colonies were stored from each positive replicate. Fifty-four strains were collected from the five BPs as depicted in Table 1, either from manure (25) or from digestate (29).

DNA extraction

DNA for PCR and PCR ribotyping was extracted from colonies grown on Brucella agar (BioMérieux, Craponne, France) after anaerobic incubation for 48 h. After centrifuging a suspension of bacterial colony in sterile water, the supernatant was

removed and the bacterial pellet re-suspended with 200 µL of InstaGene™ matrix (Bio-Rad®, Marnes-la-Coquette, France). This suspension was incubated at 56°C for 20 min, then 96°C for 8 min. The supernatant containing the bacterial DNA was recovered after centrifugation and stored at -20°C.

Characterization of *C. difficile* and detection of toxin genes

A multiplex PCR according to Barbut et al. [1] was used to detect the PaLoc genes (*tcdA, tcdB, tcdC*) and CDTLoc genes *(cdtA* and *cdtB*). It also detected the 117 bp fragment (lok) present in non-toxigenic strains and the *tpi* (triose phosphate isomerase) gene fragment used to identify *C. difficile*. The amplified products were analyzed after a 1/25 dilution by capillary electrophoresis with an ABI 3500 sequencer. Migration profiles were analyzed with the GeneMapper® software (Applied Biosystems®, Foster City, USA) by comparing the size of the fragments (peaks) obtained with the expected sizes for each specific gene fragment.

PCR ribotyping

Capillary gel electrophoresis PCR ribotyping was performed as previously described by Bidet et al. [9]. After DNA amplification, 1 µL of a 1/200 dilution of each PCR product was mixed with 10.5 µL formamide and 0.5 µL GeneScan LIZ600 (Applied Biosystems®, Foster City, USA) as an internal marker. After 30 s of denaturation at 90°C, capillary electrophoresis was performed on an 8-capillary 3500 Genetic Analyzer (Applied Biosystems®, Foster City, USA). The GeneMapper software (Thermo Fisher Scientific, Villebon-sur-Yvette, France) was used to analyze the banding patterns. PCR ribotypes (RTs) were identified using the webribo software (https://webribo.ages.at/).

Whole genome sequencing (WGS)

WGS was performed on 18 strains (two isolates from manure and two from digestate for BP1, BP3, BP4 and BP5, and one of each for BP2, based on the PCR-ribotyping and PCR results). DNA was extracted as described in [10]. Sequencing was performed as per the ICM institute using the NovaSeq 6000 Illumina technology (2x150 paired-end sequencing, Nextera XT DNA Sample Prep Kit, Illumina). Genome sequences generated as part of this study were deposited in SRA (PRJNA599117).

The quality of reads was evaluated using BioNumerics 7.6.3 (Applied Maths NV, Sint-Martens-Latem, Belgium): isolates with an average quality below 30 and estimated coverage below 30 were excluded from subsequent analysis.

Whole genome multilocus sequence typing (wgMLST) analyses

The wgMLST scheme used contains 8,745 coding loci, representing a pan-genome of *C. difficile* identified from 259 previously-published genomes. WgMLST analyses were performed using BioNumerics 7.6.3 as described in [11]. Briefly, the genetic relationship between two isolates was assessed by calculating the number of different alleles for wgMLST. We defined two isolates as genetically related or belonging to the same clonal complex (CC) when they had an allelic difference ≤ 200 or ≤ 20 respectively.

Antimicrobial susceptibility

Antimicrobial susceptibility to vancomycin (30 µg), metronidazole (4 µg [Sanofi Diagnostics Pasteur, Marnes la Coquette, France]), erythromycin (15 IU), clindamycin(2 IU), moxifloxaxin (5 µg), chloramphenicol (30 µg), imipenem (10 µg) and tetracycline (30 IU) was determined by the disk diffusion method on pre-reduced

Brucella agar plates as described elsewhere [12]. The results were interpreted according to the French Society of Microbiology's "Comité de l'Antibiogramme" (2013, available at https://resapath.anses.fr/resapath_uploadfiles/files/Documents/2013_CASFM.pdf). Strains were considered susceptible if the inhibition diameters for vancomycin (VA), metronidazole (MZ), erythromycin (ERY), clindamycin (CM), moxifloxacin (MXF), chloramphenicol (C), imipenem (IMI) and tetracycline (TE) were greater than or equal to 17mm, 21 mm, 22 mm, 15 mm, 23 mm, 23 mm, 24 mm and 23 mm respectively.

Results

All the results are fully presented in Table 1.

Identification of *C. difficile* and detection of toxin genes

All the strains were positive for *tpi*, thus confirming the identification of *C. difficile*. All also harbored the *tcdA* and *tcdB* genes. Forty-five (83.3%) contained *cdtA* and *cdtB* genes coding for the binary toxin and had a -39 bp deletion in regulator gene *tcdC*. Nine strains (16.7%) harbored only the *cdtA* gene and had no deletion in regulator gene *tcdC*.

PCR ribotyping

Among the five BPs investigated, only six different RTs (Fig. 1A) were identified, with a predominance of RT 078 (37%) and 126 (46.3%) in manure and digestate. Other RTs included 005 (7.4%), 003 (3.7%), 014 (3.7%) and 106 (1.8%) (Fig. 1A). Isolates from BP2 that contained bovine and poultry manure had the widest RT diversity (Fig. 1B). On the contrary, there was little variability among the RTs: two RTs in BP1 and BP5 (see Fig. 1B) and only one in BP3 and BP4 (see Fig. 1B).

wgMLST

The minimum spanning tree of wgMLST typing is shown in Fig. 2. wgMLST analysis revealed that isolates clustered by BP, with fewer than five alleles of difference within a BP except for strains from RT 005 isolated from BP2. wgMLST results confirm results from PCR ribotyping, with little variability within the same BP except for BP2.

Antimicrobial susceptibility

The antimicrobial susceptibility of *C. difficile* strains was evaluated for nine antibiotics. All the strains were susceptible to vancomycin, metronidazole, imipenem and chloramphenicol. Resistance rates to erythromycin, clindamycin, moxifloxacin, and tetracycline were 85.2%, 98.2%, 24.1%, and 83.3% respectively. Strains from BP1, BP2 and BP3 were susceptible to moxifloxacin, whereas all the isolates from BP4 and two out of the ten isolates from BP5 were resistant. Strains from BP3, BP4 and BP5 were resistant to erythromycin and 85.3% were resistant to tetracycline. All but one of the isolates from BP2 were susceptible to erythromycin and all were susceptible to tetracycline.

Discussion

As previously reported [7], *C. difficile* strains were easily detected and collected from the five agricultural BPs investigated during this study. Manure samples collected from BP2 had a lower positive rate (only one positive sample out of the three analyzed [8]) than the others, but it was still possible to isolate eight strains from this BP (two from manure and six from digestate). Ten isolates were collected from BP5 instead of 12 because one isolate from a manure sample and one from a digestate sample were *tpi*-negative by PCR so were not included in this study. To our knowledge, this study is the first to characterize *C. difficile* strains isolated from agricultural BPs.

As already stated in previous studies [13], there is no standard method for the detection and isolation of *C. difficile* in animal samples. Our study collected isolates using supplemented BHI followed by isolation on ChromID. Two isolates from the same sample were characterized to evaluate the diversity of strains and profiles that could be encountered within a single sample. Considering together the results presented here from PCR ribotyping, multiplex PCR and antibiotic susceptibility assays, it appears that isolates were very similar when pig manure was present, and indeed were similar both in manure and digestate samples. Considering this result, two isolates from manure and two from digestate for BP1, BP3, BP4 and BP5 were analyzed using wgMLST. Isolates from BP3, BP4 and BP5 analyzed by wgMLST had fewer than one allelic difference, and those from BP1 fewer than five allelic differences, confirming the very close proximity of strains. This seems to show the low impact of anaerobic digestion on a potential selection of strains during the process. Greater diversity in isolated strains was observed in BP2 however, where both cattle and poultry manure was used as input, even for isolates from the same

RT analyzed using wgMLST and showing 41 allelic differences (Fig. 2). This difference in strain variability between pigs, cattle or poultry has already been described [14]. According to available studies, a single RT is expected on pig farms, while greater variability is reported on cattle farms and the greatest on poultry and rabbit farms [14]. This variability could have been missed if only one isolate had been characterized per matrix or per BP.

Organic co-substrates are used to supply the BPs, [8] but it was not possible to analyze them during the study mainly because of their diversity from one plant to the next and their variability within the same BP, depending what matrices are available. As mentioned above, the characteristics of strains isolated from BPs supplied with pig manure are very similar, suggesting that *C. difficile* strains isolated from digestate originated from manure. Conclusions are more difficult to draw for BP2 considering that the PCR ribotypes from the manure and digestate are not the same, and that the two RT 005 strains isolated from manure or digestate differ by 41 alleles. It is necessary to detect and characterize *C. difficile* strains in co-substrates or monitor *C. difficile* profiles in manure and digestate over a period of time in order to determine the origin of *C. difficile* strains recovered from digestate.

RT 078 and RT 126 were the most common ribotypes identified in manure and digestate from the BPs investigated. This finding is in accordance with studies conducted on pigs, as RT 078 is the most reported PCR ribotype in pigs worldwide [13]. Except in BP2, all the manure in this study contained at least pig manure, which may explain this result. RT 126 is part of the ribotype 078 lineage that includes RT 078, 045, 066, 126 and 127 [15, 16] and is a fluoroquinolone-resistant descendant of RT 078 [17]. It has also been isolated from pigs in previous studies worldwide [18-21].

RT 078 is considered to be an emerging cause of community-acquired CDI in various countries or regions around the world (the Netherlands, England, Scotland, North America and Europe [22-25]). The zoonotic potential of RT 078 from Sequence Type 11 has been recently demonstrated by genomic analyses, [4] and it is hypothesized that the food chain and/or the environment are its reservoirs [5]. By detecting this RT in manure and digestate, our study appears to confirm this hypothesis.

RT 014, identified in BP2, has been previously detected in wastewater effluents and influents, in human patients, shellfish, and sewage sludge [26]. RT 014 is the third most prevalent ribotype associated with CDI in Europe [27] and the USA [28]. RT 005 has also been detected in wastewater effluents and RT 106 from shellfish [26]. They have been associated with CDI in France but to a lesser extent than RT 014 or RT 078/126 [29]. However, an increase in the prevalence of RT 106 in cases of CDI was observed in the USA between 2011 and 2017 [28].

C. difficile strains are known to be resistant to certain antibiotics, such as quinolones, erythromycin and clindamycin [14]. Resistance to erythromycin has in particular been reported among isolates of PCR ribotype 078 in pigs and cattle [30]. RT 126/078 has been reported to be highly resistant to tetracycline, moxifloxacin, clindamycin, and erythromycin [4]. Our results are thus in accordance with previous studies. Only strains from BP4 and one isolate from BP5 were resistant to moxifloxacin. Fluoroquinolones represented 0.24% of the tonnage of active ingredients sold in the veterinarian field in 2017 in France [31]. It was not possible to obtain information regarding the antimicrobial treatments of animals whose manure was input into the BPs investigated in this study, and no link between rearing practices and antimicrobial resistance could therefore be evaluated.

Tetracycline is the most widely-used antimicrobial for the treatment, control, and prevention of infections in animals [32]. Tetracycline represented around 38% of veterinary antimicrobial sales in France in 2017 and is the main antimicrobial used in pig and bovine production [31]. The high level of decreased-susceptibility strains to this antimicrobial (83.3% with an inhibition diameter below 23) in our study is not therefore surprising. The resistance of RT078 and RT126 to tetracycline may also play a role in its high prevalence in pigs by providing these strains with a selective advantage in comparison with susceptible strains.

The resistances to antimicrobials reported here are similar to those observed in strains involved in human CDI [29]. Fortunately, no resistance to antibiotics such as vancomycin or metronidazole, used to treat human *C. difficile* infections, was detected in the strains isolated from the BPs studied.

Conclusion

This study shows that toxigenic *C. difficile* strains resistant to a number of antimicrobials can be isolated from manure and digestate. Such matrices represent a reservoir of *C. difficile* strains whose RTs are similar to those found in patients with CDI. This illustrates the relevance of the One Health approach to this issue and our results show that, in addition to monitoring the strains involved in CDI, *C. difficile* strains from animal and environmental reservoirs should be monitored to identify circulating strains so as to be able to detect at an early stage any emergence that could impact human health.

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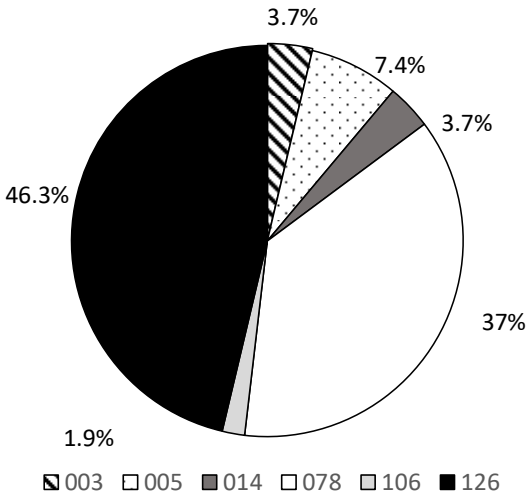
Figures:

Fig. 1: Distribution of isolates according to PCR ribotypes (RTs). A: Distribution of the 54 isolates per RT; B: Distribution of RTs per BP (the percentage of each RT per BP is indicated) (RT126 in black, RT078 in white, RT005 with hatched lines, RT014/020/077 in dark gray, RT106 in light gray, RT003 with dots)

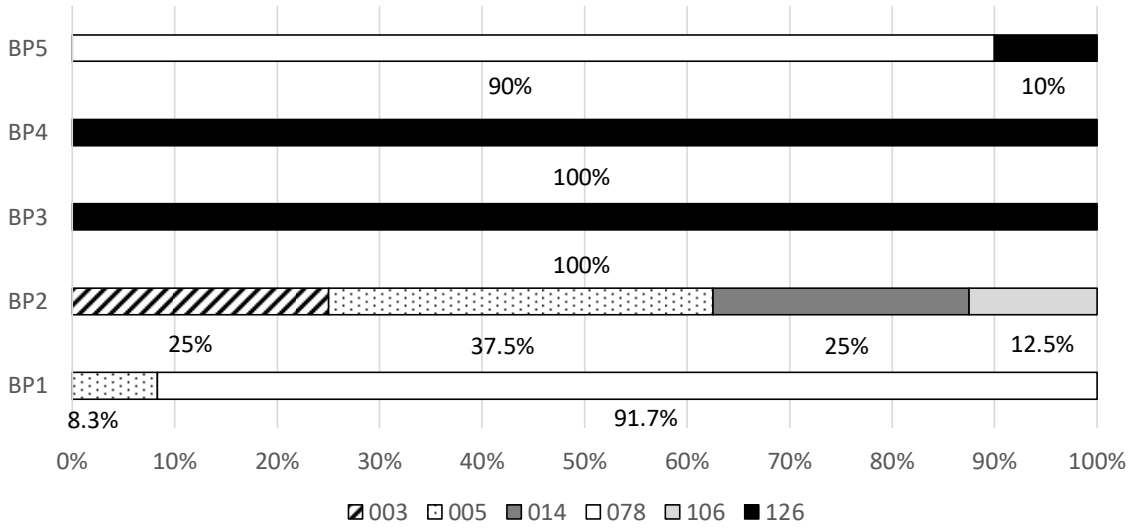
Fig. 2: wgMLST analysis with minimum spanning tree of 18 strains isolated from BP1 to BP5. Each circle represents a single wgMLST, the size of the circle being proportional to the number of isolates included. The numbers between the circles correspond to the number of alleles between the wgMLST types. The shaded areas represent clusters, grouping the genetically-linked strains. Blue circles encompass strains from the same BP.

413 Figure 1

A



B



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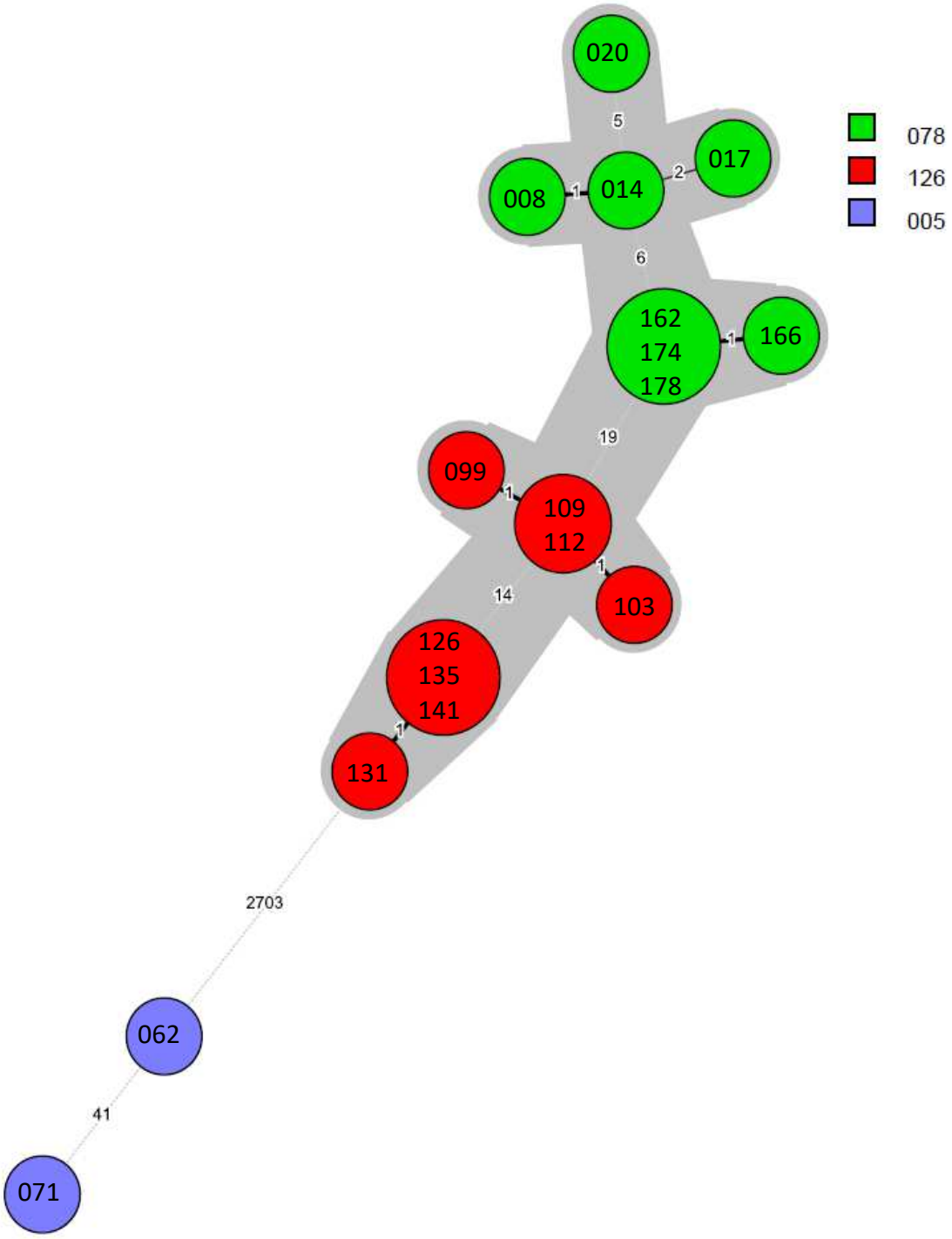
Figur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429 Tables

430 Table 1: PCR ribotypes, toxin gene profiles and antimicrobial susceptibility of *Clostridioides difficile* isolated from manure and
431 digestate samples collected from French agricultural biogas plants (BPs).

432 Strains in bold were sequenced; BP: Biogas Plant; ERY: Erythromyci; CN: Clindamycin; MOXI: Moxifloxaxin; MZ: Metronidazole;
433 VA: Vancomycin; TE: Tetracyclin; IMP: Imipenem; C: Chloramphenicol; Tox A: Gene encoding for Toxin A; Tox B: Gene encoding
434 for Toxin B; LOK: gene encoding for the lok gene, characteristic of non-toxic *C. difficile;* tpi: gene fragment used to identify *C.*
435 *difficile;* Binary Tox: cdtA and cdtB genes encoding for the binary toxin; del. tcdC: détection of deletions in *tcdC* gene; +: detected; -:
436 not detected

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Strain reference	Sample	Type	BP	Production	Antimicrobial								Strain gene content						
					ERY	CN	MOXI	MZ	VA	TE	IMP	C	Tox A	Tox B	LOK	tpi	Binary Tox	del. tcdC	Ribotype
D17MD08	17MD01-01-1	Manure	BP1	Pig	6	6	26	36	30	16	32	27	+	+	-	+	+	-39	078
D17MD09	17MD01-01-1	Manure	BP1	Pig	6	6	26	36	31	16	31	28	+	+	-	+	+	-39	078
D17MD11	17MD01-01-2	Manure	BP1	Pig	6	6	26	40	32	16	28	30	+	+	-	+	+	-39	078
D17MD12	17MD01-01-2	Manure	BP1	Pig	32	16	31	46	34	50	29	36	+	+	-	+	Truncated	0	005
D17MD14	17MD01-01-3	Manure	BP1	Pig	6	7	26	32	32	15	27	32	+	+	-	+	+	-39	078
D17MD15	17MD01-01-3	Manure	BP1	Pig	6	6	26	33	28	15	30	27	+	+	-	+	+	-39	078
D17MD17	17MD01-01-4	Digestate	BP1	Pig	6	7	29	38	33	16	30	33	+	+	-	+	+	-39	078
D17MD18	17MD01-01-4	Digestate	BP1	Pig	6	8	28	34	31	16	32	31	+	+	-	+	+	-39	078

D17MD20	17MD01-01-5	Digestate	BP1	Pig	6	6	26	34	31	20	31	31	+	+	-	+	+	-39	078
D17MD21	17MD01-01-5	Digestate	BP1	Pig	6	6	26	32	31	16	29	30	+	+	-	+	+	-39	078
D17MD23	17MD01-01-6	Digestate	BP1	Pig	6	7	26	35	32	14	30	30	+	+	-	+	+	-39	078
D17MD25	17MD01-01-6	Digestate	BP1	Pig	6	7	28	34	31	15	31	32	+	+	-	+	+	-39	078
D17MD62	17MD01-02-9	Manure	BP2	Bovine, Poultry	28	8	24	32	34	40	30	36	+	+	-	+	Truncated	0	005
D17MD64	17MD01-02-9	Manure	BP2	Bovine, Poultry	28	6	25	34	32	44	27	33	+	+	-	+	Truncated	0	005
D17MD65	17MD01-02-10	Digestate	BP2	Bovine, Poultry	36	6	23	36	31	50	28	34	+	+	-	+	Truncated	0	014
D17MD66	17MD01-02-10	Digestate	BP2	Bovine, Poultry	6	9	26	40	34	25	31	35	+	+	-	+	Truncated	0	003
D17MD69	17MD01-02-11	Digestate	BP2	Bovine, Poultry	26	6	23	34	31	44	24	28	+	+	-	+	Truncated	0	003
D17MD70	17MD01-02-11	Digestate	BP2	Bovine, Poultry	36	6	27	41	35	56	30	38	+	+	-	+	Truncated	0	014
D17MD71	17MD01-02-12	Digestate	BP2	Bovine, Poultry	24	6	24	36	32	40	27	23	+	+	-	+	Truncated	0	005
D17MD73	17MD01-02-12	Digestate	BP2	Bovine, Poultry	30	10	30	40	40	60	50	38	+	+	-	+	Truncated	0	106
D17MD99	17MD01-03-13	Manure	BP3	Pig	6	11	29	40	34	20	30	36	+	+	-	+	+	-39	126
D17MD100	17MD01-03-13	Manure	BP3	Pig	6	7	28	38	35	16	33	34	+	+	-	+	+	-39	126
D17MD102	17MD01-03-14	Manure	BP3	Pig	6	7	29	34	35	15	30	37	+	+	-	+	+	-39	126
D17MD103	17MD01-03-14	Manure	BP3	Pig	6	8	31	35	36	15	32	36	+	+	-	+	+	-39	126
D17MD105	17MD01-03-15	Manure	BP3	Pig	6	8	29	39	34	16	30	35	+	+	-	+	+	-39	126
D17MD106	17MD01-03-15	Manure	BP3	Pig	6	6	30	38	35	15	32	35	+	+	-	+	+	-39	126
D17MD108	17MD01-03-16	Digestate	BP3	Pig	6	8	30	39	34	13	30	36	+	+	-	+	+	-39	126
D17MD109	17MD01-03-16	Digestate	BP3	Pig	6	12	30	44	36	19	33	37	+	+	-	+	+	-39	126
D17MD111	17MD01-03-17	Digestate	BP3	Pig	6	6	28	36	34	15	31	33	+	+	-	+	+	-39	126
D17MD112	17MD01-03-17	Digestate	BP3	Pig	6	7	30	34	34	10	34	34	+	+	-	+	+	-39	126
D17MD114	17MD01-03-18	Digestate	BP3	Pig	6	7	30	38	35	15	29	36	+	+	-	+	+	-39	126
D17MD115	17MD01-03-18	Digestate	BP3	Pig	6	6	28	42	34	17	33	35	+	+	-	+	+	-39	126
D17MD125	17MD01-04-19	Manure	BP4	Pig, Bovine, Poultry	6	6	6	44	33	18	33	32	+	+	-	+	+	-39	126
D17MD126	17MD01-04-19	Manure	BP4	Pig, Bovine, Poultry	6	6	6	42	32	19	30	34	+	+	-	+	+	-39	126
D17MD128	17MD01-04-20	Manure	BP4	Pig, Bovine, Poultry	6	6	6	42	33	15	30	34	+	+	-	+	+	-39	126
D17MD129	17MD01-04-20	Manure	BP4	Pig, Bovine, Poultry	6	8	6	43	35	16	31	35	+	+	-	+	+	-39	126
D17MD131	17MD01-04-21	Manure	BP4	Pig, Bovine, Poultry	6	6	6	41	31	14	34	34	+	+	-	+	+	-39	126

D17MD132	17MD01-04-21	Manure	BP4	Pig, Bovine, Poultry	6	6	6	35	31	14	26	30	+	+	-	+	+	-39	126
D17MD134	17MD01-04-22	Digestate	BP4	Pig, Bovine, Poultry	6	6	6	40	34	14	30	36	+	+	-	+	+	-39	126
D17MD135	17MD01-04-22	Digestate	BP4	Pig, Bovine, Poultry	6	10	6	40	35	17	32	36	+	+	-	+	+	-39	126
D17MD137	17MD01-04-23	Digestate	BP4	Pig, Bovine, Poultry	6	6	6	36	32	16	28	31	+	+	-	+	+	-39	126
D17MD138	17MD01-04-23	Digestate	BP4	Pig, Bovine, Poultry	6	8	6	37	31	16	29	31	+	+	-	+	+	-39	126
D17MD140	17MD01-04-24	Digestate	BP4	Pig, Bovine, Poultry	6	7	6	37	34	17	33	35	+	+	-	+	+	-39	126
D17MD141	17MD01-04-24	Digestate	BP4	Pig, Bovine, Poultry	6	6	6	37	31	11	27	27	+	+	-	+	+	-39	126
D17MD162	17MD01-05-25	Manure	BP5	Pig, Bovine	6	6	28	31	29	17	26	27	+	+	-	+	+	-39	078
D17MD165	17MD01-05-26	Manure	BP5	Pig, Bovine	6	6	27	30	30	17	27	27	+	+	-	+	+	-39	078
D17MD166	17MD01-05-26	Manure	BP5	Pig, Bovine	6	6	28	34	31	17	28	31	+	+	-	+	+	-39	078
D17MD168	17MD01-05-27	Manure	BP5	Pig, Bovine	6	6	28	33	31	16	28	31	+	+	-	+	+	-39	078
D17MD169	17MD01-05-27	Manure	BP5	Pig, Bovine	6	6	26	32	30	18	26	30	+	+	-	+	+	-39	078
D17MD171	17MD01-05-28	Digestate	BP5	Pig, Bovine	6	6	26	29	31	19	26	29	+	+	-	+	+	-39	078
D17MD174	17MD01-05-29	Digestate	BP5	Pig, Bovine	6	6	26	31	31	18	26	28	+	+	-	+	+	-39	078
D17MD175	17MD01-05-29	Digestate	BP5	Pig, Bovine	6	6	6	39	31	14	28	28	+	+	-	+	+	-39	126
D17MD177	17MD01-05-30	Digestate	BP5	Pig, Bovine	6	6	26	30	28	16	26	27	+	+	-	+	+	-39	078
D17MD178	17MD01-05-30	Digestate	BP5	Pig, Bovine	6	6	27	32	30	19	27	29	+	+	-	+	+	-39	078

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