MOLECULAR CHARACTERISATION AND ANTIMICROBIAL RESISTANCE PATTERNS IN *C. difficile* ISOLATED FROM THE ENVIRONMENT, HUMANS, AND OTHER ANIMAL SPECIES ORIGINATED FROM THE IBERIAN PENINSULA

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INTRODUCTION

Clostridium difficile is a microorganism that can infect several animal species among which are human beings. It is a spore-forming bacterium, thus it can be isolated from different environmental sources as well [1]. The epidemiology of this microorganism has changed and it cannot be considered an exclusively nosocomial pathogen anymore. In the last years, the literature related with its presence in different animal species and environmental niches has grown, as well as the studies about the identification of close genetically related strains in humans and animals [2,3]. Hence, it started to be considered a zoonotic agent [4].

As concerns about community-acquired *C. difficile* infections are growing, new *C. difficile* sources have been proposed in the community e.g. pets and livestock. Thus, the aim of this study was to assess the presence of *C. difficile* and its molecular and antimicrobial resistance diversity isolated from different sources located in the Iberian Peninsula.

MATERIAL & METHODS

- Different type of samples were included coming from different locations within the Iberian Peninsula: fecal samples from different animal species including humans, and environmental samples .
- Bacterial isolation protocol: sample pre-enrichment in selective broth, ethylic shock, and platting onto selective agar [5].
- *Clostridium difficile* identification was carried out by species-specific PCR (*tpi* gene); conventional PCR was employed for toxin genes detection as well (A, B, CDT toxins); all isolated strains were characterised by toxinotyping and PCR-ribotyping, and a sub-set of isolates were characterised by MLST [5].
- The minimal inhibitory concentration (MIC) was determined by Etest for all the isolates obtained [5].

RESULTS AND DISCUSSION

- A total of 734 samples were analysed: 40% originated from different animal species (faecal samples), 18.7% from humans (faecal samples), and 41.3% from the environment (faecal and animal food samples) (**Table 1**).
- A total of 64 *C. difficile* isolates were obtained.
- Globally, 22 different PCR-ribotypes (RTs) and 3 toxinotypes (TXs) were detected (**Table 2**) which exhibits its heterogeneous epidemiology.
- RT078 and RT010 were isolated from all kind of samples and at high frequencies (**Figs. 1 and 2**) showing their ability to colonize different hosts and environments.
- Overall, resistance percentages to tetracycline, erythromycin, and clindamycin ("old" resistances) were 10 times higher than to vancomycin, metronidazole, and moxifloxacin ("new" resistances, PR= 0.06) which reflects already described trends [6].
- 60% of the non-toxigenic strains were MDR compared to 28.6% among toxigenic isolates (p= 0.026) highlighting their importance as source of antimicrobial resistance determinants for toxigenic strains.
- Three isolates were resistant (stable) to MZ (animal and human origin), belonging 2 of them to the non-toxigenic RT010; the third one was identified as RT014 (**Table 2**). These results might reflect its MZR increasing trend.
- Most of the isolates obtained in this study belonged to Clade 1 (**Fig. 3**) which confirm previous observations that it is the most heterogeneous clade.

Table 1 Number and type of samples analysed, number and percentage of positive samples, and toxigenic *C. difficile* isolates distribution per category

Sample type	Sample %	Positive sample %	Toxigenic isolate %
Rodents	10.8	15.2	91.7
Pigeons	2.2	12.5	100
Dogs	12.3	6.7	66.7
Rabbits	6.5	18.7	66.7
Exotics	3.3	4.2	0
Grouse	5	0	0
Pigs	28.6	3.3	85.7
Rodents	5	24.3	77.8
Birds	5	5.4	100
Pig food	2.6	10.5	100
Humans	18.7	10.2	64.3
Total	**734**	**8.7**	**76.6**

Blue and purple, faecal samples; pink, environmental samples.

Table 2 *Clostridium difficile* toxinotype (TX) and ribotype (RT) distribution according to the type of sample analysed; multidrug resistance (MDR) and metronidazole resistance (MZR) distribution according to TX and RT

TX	RT	Isolate % (n= 64)	Animal isolate %	Environ. isolate %	Human isolate %	MDR isolate %	MZR Isolate %
0	005, 012, 014, 020, 106, 110, 154, 295, 358, new	35.9	33.3	30	50	39.1	33.3
V	078, 126	39.1	43.3	55	7.1	17.4	
VIII	017	1.6	0	0	7.1	4.3	
NT	010, 204, 029, 035, 039, 051, 073, 123, new	23.4	23.3	15	35.7	39.1	66.7

NT, no toxigenic.

Fig. 3 Maximum likelihood tree (concatenated MLST alleles). *Clostridium difficile* isolates corresponding to our collection are showed with a coloured circle. ST, sequence type; H, human isolate; RC, rat intestinal content isolate; 5754, sow vagina isolate; RF, environmental rat faeces isolate; E, exotic animal isolate. Purple, clade 1; pink, clade 4; blue, clade 5; green and red, unknown clades

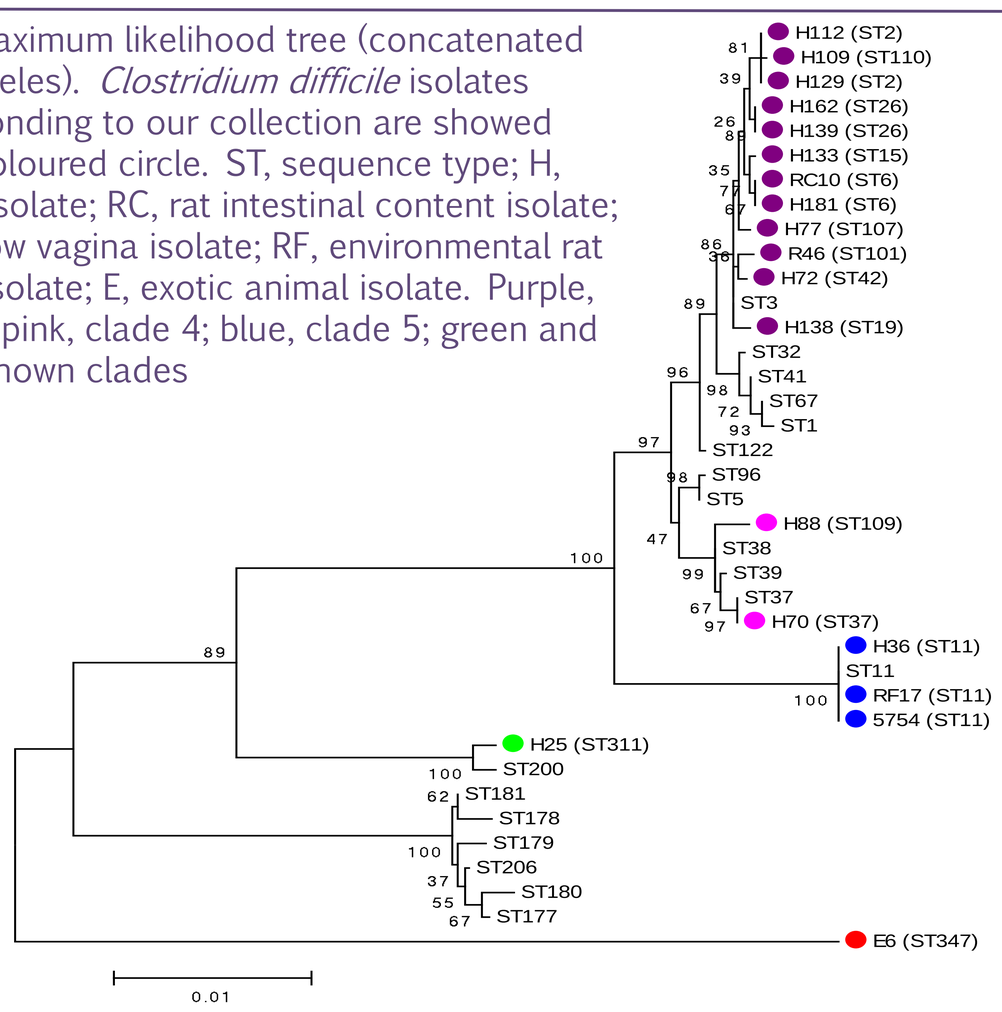


Fig. 1 Overall PCR-ribotype distribution among the isolates obtained

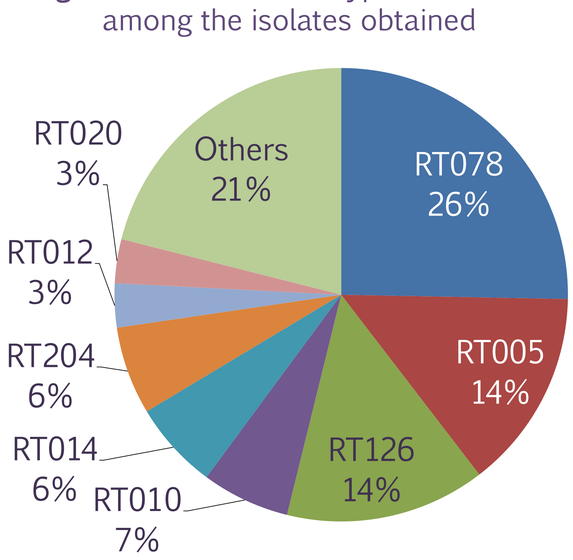
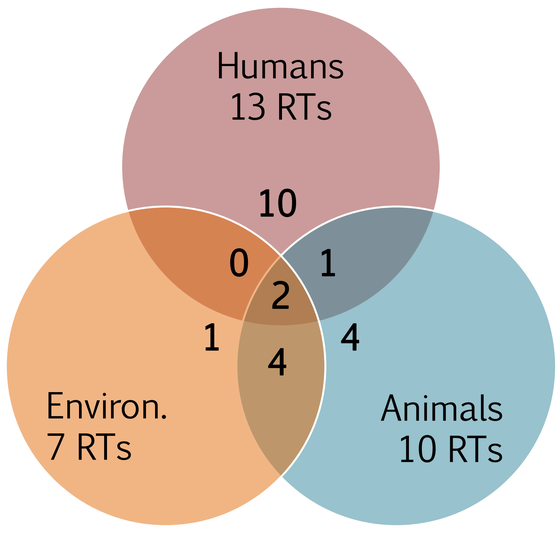


Fig. 2 *Clostridium difficile* RTs distribution



CONCLUSIONS

- The presence of the same *C. difficile* RTs in different animal species and humans could be due to the transmission of this bacterial species among them.
- Rodents, pigeons, and rabbits could act as transmission vectors of toxigenic and antimicrobial-resistant *C. difficile* strains to humans.
- The RT078 once again showed its versatility to colonise different niches and its adaptability to different environments.
- Antimicrobial resistance surveillance programs should be periodically established in order to monitor important antimicrobial trends as MZ resistance and/or the presence of significant rates of non-toxigenic strains as resistance determinant reservoirs.

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