

# ABSENCE OF A METRONIDAZOLE RESISTANCE MARKER IN *Clostridioides difficile* PCR-RIBOTYPE 078 ISOLATES FROM PORCINE ORIGIN

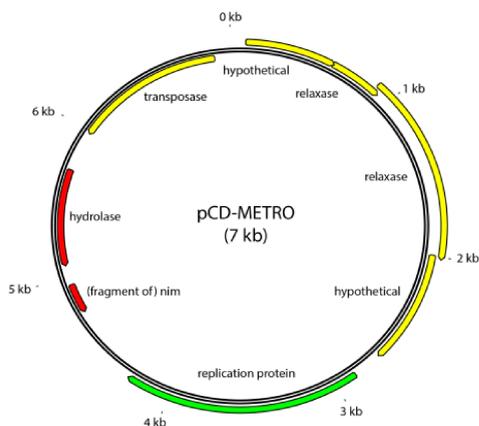
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## INTRODUCTION

- Metronidazole is currently used as a first-line agent in the treatment of *Clostridioides difficile* infection (CDI) in humans and animals. However, reduced susceptibility to this antimicrobial agent has emerged in *C. difficile* and several mechanisms have been proposed to be involved (1-3).
- Recently, we identified a plasmid in both toxigenic and non-toxigenic human and animal *C. difficile* strains, mostly belonging to ribotype 010 with an intermediate level of metronidazole resistance (MIC = 2-8 mg/L) and that is absent from susceptible isolates. The presence of the so-called pCD-metro plasmid in other ribotypes is still unknown.
- Food-producing animals are regarded as a potential reservoir of *C. difficile*, and may also harbor multidrug resistant strains. Even though foodborne transmission of this pathogen is not yet established, the role of animals in human CDI and in dissemination of antimicrobial resistant strains should be of concern (4).



## OBJECTIVE

The aim of this study was to evaluate the presence of pCD-metro in *C. difficile* strains belonging to the “hypervirulent” PCR-ribotype 078 and isolated from pig farms.

## MATERIAL & METHODS

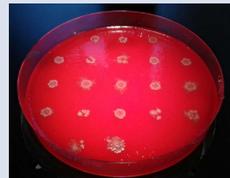
### Sampling

All *C. difficile* isolates analyzed in this study (N=394) were identified as PCR-ribotype 078 by capillary ribotyping (5). All strains were isolated from either animal (67.8%) or environmental (32.2%) samples collected in The Netherlands and Spain between 2009 and 2017.



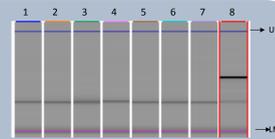
### AST

Metronidazole susceptibility testing was performed according to the CLSI recommended agar dilution method using the breakpoint defined by EUCAST (ECOFF >2 mg/L).



### Plasmid detection

Bacterial DNA was extracted from pure cultures using QIAamp Mini Spin Columns (Qiagen). The detection of the plasmid was performed by conventional PCR using DNA from a characterized plasmid-positive strain as a control.



Visualisation of products from the PCR targeting a pCD-metro fragment (157 bp) on a MultiNA electrophoresis system. (1-6) *C. difficile* PCR-ribotype 078 strains from porcine origin; (7) negative control; (8) positive control; (UM) upper marker; (LM) lower marker.

## RESULTS

- MIC<sub>50</sub>/MIC<sub>90</sub> = **0.25/0.25 mg/L** (n=82)  
No metronidazole resistance was observed
- None** of the 394 isolates tested by PCR was positive for the marker.

## References

- Lynch T, Chong P, Zhang J, et al. Characterization of a stable, metronidazole-resistant *Clostridium difficile* clinical isolate. PLoS ONE. 2013;8(1):e53757.
- Moura I, Monot M, Tani C, et al. Multidisciplinary analysis of a nontoxigenic *Clostridium difficile* strain with stable resistance to metronidazole. Antimicrobial Agents and Chemotherapy. 2014;58(8):4957-4960.
- Chong PM, Lynch T, McCorrister S, et al. Proteomic analysis of a NAP1 *Clostridium difficile* clinical isolate resistant to metronidazole. PLoS ONE 2014;9(1):e82622.
- Smits WK, Lyras D, Lacy DB, et al. *Clostridium difficile* infection. Nature reviews Disease primers. 2016;2:16020.
- Fawley WN, Knetsch CW, MacCannell DR, et al. Development and Validation of an Internationally-Standardized, High-Resolution Capillary Gel-Based Electrophoresis PCR-Ribotyping Protocol for *Clostridium difficile*. PLoS ONE 2015;10(2):e0118150.

## CONCLUSIONS

- ❖ Our data show that the **metronidazole resistance** marker, and metronidazole resistance, are **not very common** in *C. difficile* **PCR-ribotype 078** isolates from **porcine** origin.
- ❖ **No plasmid-positive** strains were found among phenotypically **susceptible** *C. difficile* isolates.
- ❖ Nevertheless, **surveillance** of metronidazole susceptibility and resistance mechanisms is needed in order to improve the management of CDI.