Metagenomic Screening for Antibiotic-Resistant *Clostridioides difficile* in the Gut Microbiomes of Asymptomatic Humans and Animals

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INTRODUCTION

- *Clostridioides difficile* is a spore-forming, anaerobic bacteria that causes *C. difficile* infection (CDI), a leading cause of healthcare–associated diarrhea[1, 2].
- First-line therapies include antibiotics such as Metronidazole (MTZ), Vancomycin (VAN), Fidaxomicin (FDX) and in few cases, Rifaximin (RIF)[3,4,5].
- Resistance to these CDI antibiotics and Fluroquinolones (FQs), has emerged due to development of single nucleotide polymorphisms (SNPs) in key resistance genes[6-7].

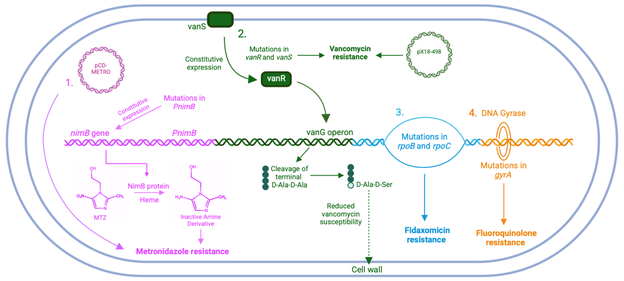

Fig. 1 Mechanisms of antibiotic resistance in *C. difficile*

- While most genomic studies focus on hospitalized CDI patients, *C. difficile* also colonizes asymptotic individuals, with 3-5% healthy humans carrying it in the gut[8].
- However, the prevalence and potential role in the spread of *C. difficile* strains resistant to CDI antibiotics among asymptomatic individuals remains largely unknown, unlike in CDI patients, where resistance typically arises due to antibiotic exposure[9].
- Animals (Livestock and pets) are carriers and reservoirs of *C. difficile*, but the prevalence of strains resistant to key CDI antibiotics remains less studied[10].

OBJECTIVE

To assess the presence of *C. difficile* resistant to key antibiotics used to treat CDI across human (healthy, and non-CDI individuals) and animal in gut metagenomes.

METHODS

Retrieved SRA datasets containing possible *C. difficile*

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Filtered SRA datasets to include only human and animal gut samples

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Contig datasets assembled with Logan

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Identified six resistance-associated genes in SRA datasets via BLAST using gene-specific thresholds

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MAFFT used for multiple sequence alignment (MSA) of gene matches

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Resistant SRAs were confirmed as *C. difficile* by BLAST and their metadata extracted

RESULTS

1. Retrieval of Datasets From Human and Animal Guts Metagenomes

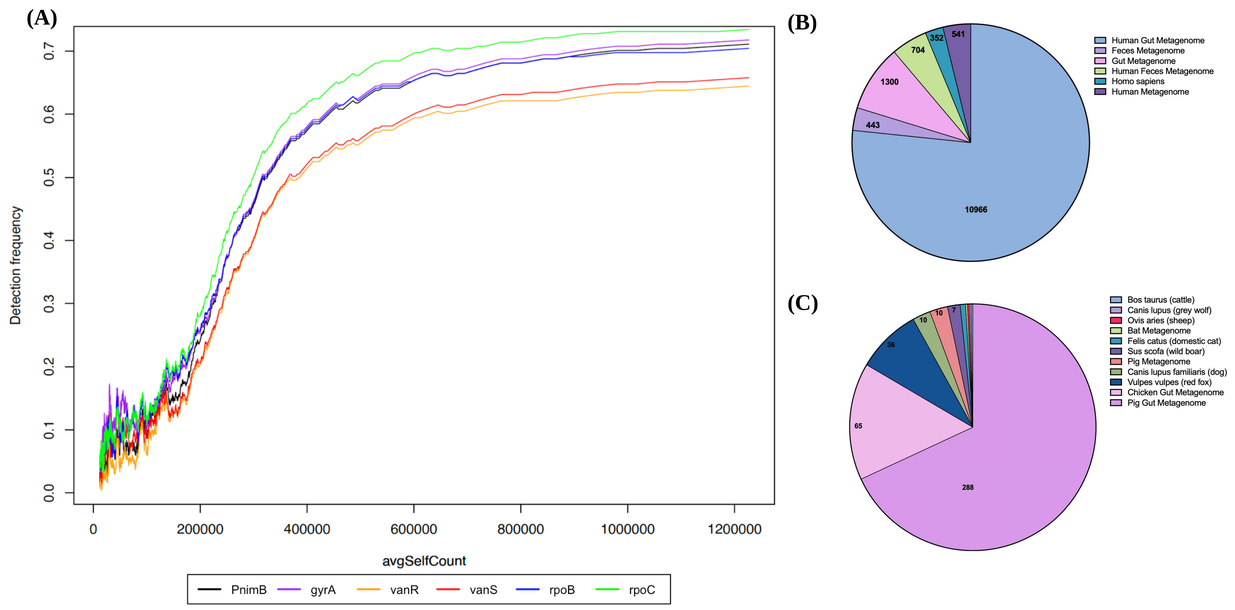

Fig. 2 Detection and selection of human and animal gut metagenomes for resistance analysis. (A) Detection frequencies of six resistance-associated genes across k-mer abundance thresholds in human gut metagenomes, with ≥11,467 selected as the cutoff. (B) From 47,197 total human-associated datasets, 14,306 gut-/feces-related samples met the sufficient k-mer abundance threshold (≥11,467) and were used for downstream analysis. (C) For animal-associated datasets, 423 gut-/feces-related samples met the sufficient threshold (≥11,467).

2. Resistome Analysis

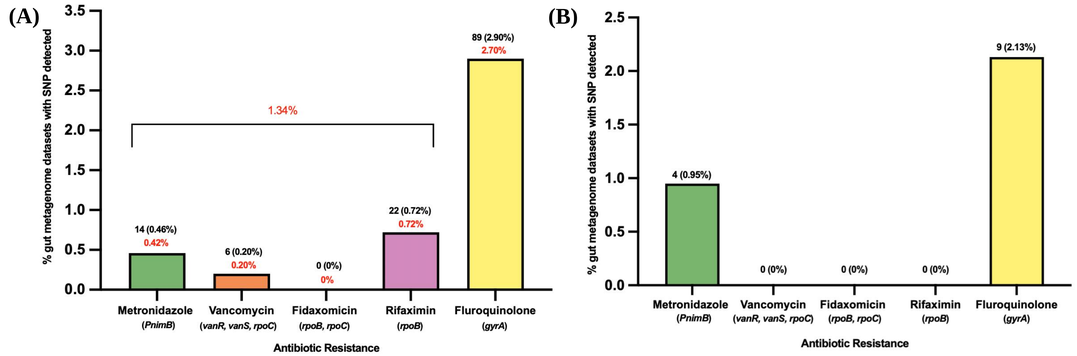

Fig. 3 Prevalence of antibiotic-resistance SNPs in (A) Human- and (B) Animal- associated datasets. (A) In humans, SNPs conferring resistance to CDI antibiotics were detected for MTZ (0.46%), VAN (0.20%), and RIF (0.72%) as well as for epidemic strain marker FQ (2.70%). Percentages in red indicate datasets from asymptomatic carriers containing antibiotic-resistant SNPs. (B) In animals, SNPs were identified in chicken and pig gut metagenomes, with resistant to MTZ (0.95%) and FQ (2.13%).

3. Selected Profiles of Gut Samples (non-CDI) Harboring Resistant *C. difficile*

Table. 1 Representative human and animal gut samples with antibiotic-resistant *C. difficile*.

Gut Sample	Age Category	Country of Sample	Healthy/Patient	SNP Present	Antibiotic Resistance
Human	Adult	USA	Non-CDI Patient (Chronic Liver Disease)	P*nimB*^{G}, *gyrA* (T82I) & *vanR* (T115A)	Metronidazole, Fluroquinolone and Vancomycin Resistance
Human	Infant	Spain	Healthy	*vanS* (S313F)	Vancomycin Resistance
Human	Infant	USA	Healthy	*gyrA* (T82I)	Fluroquinolone Resistance
Human	Adult	China	Non-CDI Patient (Hypertension)	*gyrA* (T82I) & *rpoB* (H502N, R505K)	Fluroquinolone and Rifaximin Resistance
Human	Not-Known	South Korea	Non-CDI Patient (Chronic obstructive Pulmonary Disease)	P*nimB*^{G} & *gyrA* (T82I)	Metronidazole and Fluroquinolone Resistance
Chicken	NA	Belgium	NA	P*nimB*^{G} & *gyrA* (T82I)	Metronidazole and Fluroquinolone Resistance

(NA – Not Applicable)

DISCUSSION

- A total of 123 antibiotic resistance-associated SNPs (4.04%) were identified in asymptomatic and non-CDI human samples, indicating the presence of antibiotic-resistant *C. difficile* outside of clinical infection .
- Notably, antibiotic-resistant *C. difficile* strains were detected in asymptomatic individuals and non-CDI patients, where these drugs were not in use. This suggests that these individuals may act as carriers and that CDI patients can acquire resistant strains prior to treatment, challenging the view that resistance develops mainly from antibiotic exposure.
- A total of 13 resistance-associated SNPs (3.08%) were identified in livestock animals (chicken and pigs) suggesting that animals could also serve as a potential source of transmission.

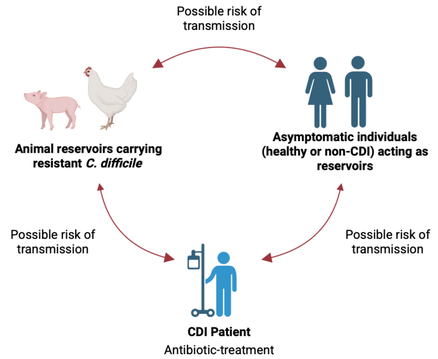

Fig. 4 Potential Transmission Pathways and Clinical Impact of Resistant *C. difficile*

- These findings suggest that both humans and animals may serve as asymptomatic carriers of antimicrobial-resistant *C. difficile* strains, potentially acting as reservoirs that increase the risk of transmission.

REFERENCES

1. Czepiel, J. et al. Eur. J. Clin. Microbiol. Infect. 2019. 38:1211.
2. Finn, E. et al. BMC Infect. 2021. 21:456.
3. Krutova, M et al. Int. J. Infect. 2022. 124:118–123
4. Eubank, T. A. et al. Antibiotics. 2022. 11: 258.
5. O'Connor, J. R. et al. Antimicrob. Agents Chemother. 2008. 52:2813–2817.
6. Kolte, B. et al. J. Antimicrob. Chemother. 2024. 79:1320–1328
7. Olaitan, A. O. et al. Nat. Commun. 2023. 14:4130.
8. Mada, P. K. et al. StatPearls Publishing, Treasure Island (FL). 2025.
9. Gonzales-Luna, A. J. et al. Open Forum Infect. 2021. 8 ofab365
10. Kachrimanidou, M. et al. Microorganisms. 2019. 7:667.

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