Species-Wide Genetic Atlas of Antimicrobial Resistance in *Clostridioides difficile*

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Background and Objectives

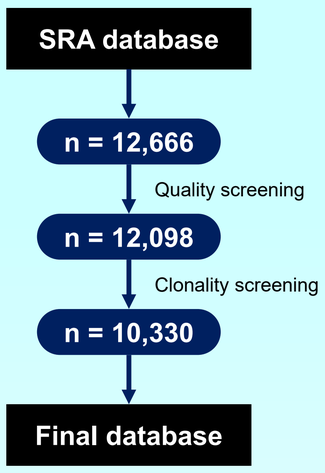
Antimicrobial resistance (AMR) plays an important role in the spread and pathogenesis of *Clostridioides difficile* infection (CDI). An association between AMR and CDI outbreaks has been identified[1,2,3], however, such studies have been limited to a few strains in limited geographical regions.

This study aimed to investigate the prevalence of AMR genotypes in the global population of *C. difficile*.

Methods

1. All publicly available read files were downloaded.
2. All non-redundant illumina read files were screen for quality and contamination using kraken2[4].
3. Read files of epidemic strains (sequence types (STs) 1, 2, 11 and 37) were screened for clonality (nucleotide identity > 99.98%) using SRST2[5] and Sketch[6], respectively.
4. All remaining read files were interrogated against several AMR databases.[#]

Sample size



Gene databases[#]

Gene alleles

- MLST alleles[7]
- *gyrA/ gyrB*[7] (Fluoroquinolones)
- *rpoB*[7](Rifamycins)
- *pbp1* & *pbp3** (Carbapenems)

Accessory genes

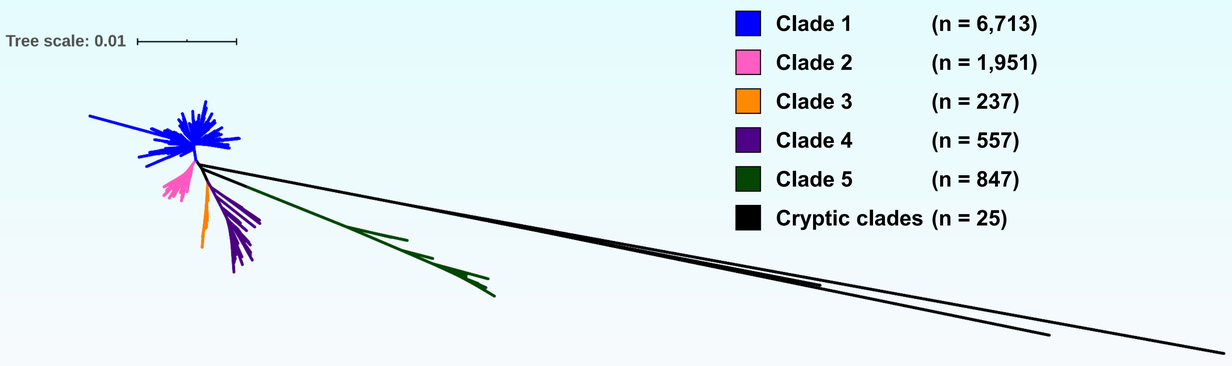
- ARGannot[8]
- Resfinder[9]

* customised databases

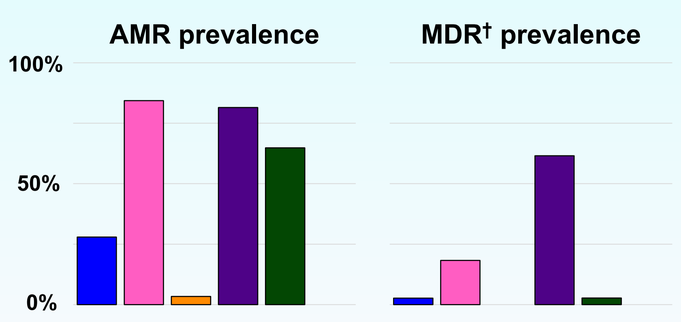
Detailed methods available here **QR**

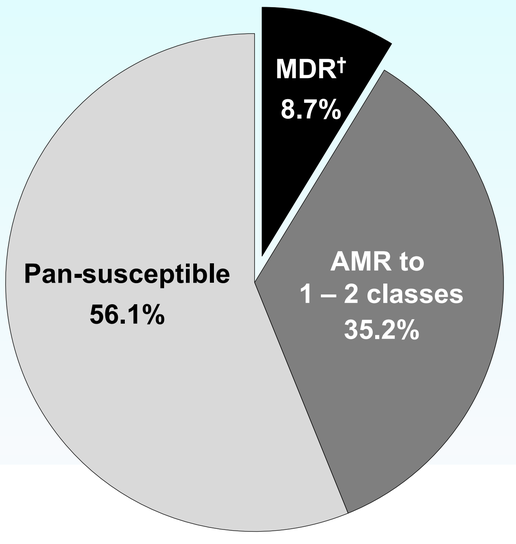
Results

1. Population structure of *C. difficile*



2. Overall AMR prevalence





† MDR: resistant to three or more drug classes

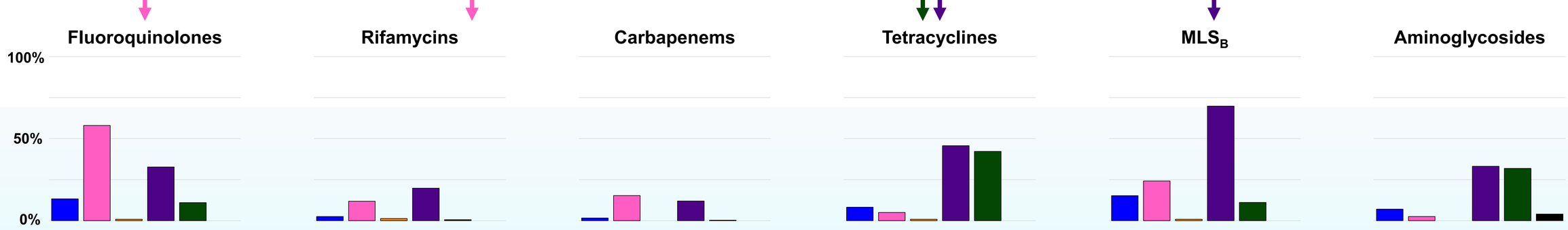
Epidemic *C. difficile* STs

ST1 (clade 2)
Affected regions: North America & Europe
Associated drug classes: Fluoroquinolones & Rifamycins

ST11 (clade 5)
Affected regions: Europe
Associated drug classes: Tetracyclines

ST37 (clade 4)
Affected regions: Global
Associated drug classes: MLS_B, Tetracyclines & MDR

Resistance prevalence by drug classes



Conclusion

There was a higher prevalence of AMR among epidemic *C. difficile* lineages. Despite its intrinsic resistance, some *C. difficile* strains carried aminoglycoside resistance genes, suggesting its role as a reservoir of AMR genes.

References

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Re-Defining *Clostridioides difficile* Using Global Phylogenomic Analyses

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Background and Objectives

Bacterial species[1] = unique genotype + unique phenotypes (two or more independent phenotypes)

DNA-DNA Hybridisation[2]
Species cut-off: 70%
+ Current gold standard
- Labour- and time-intensive

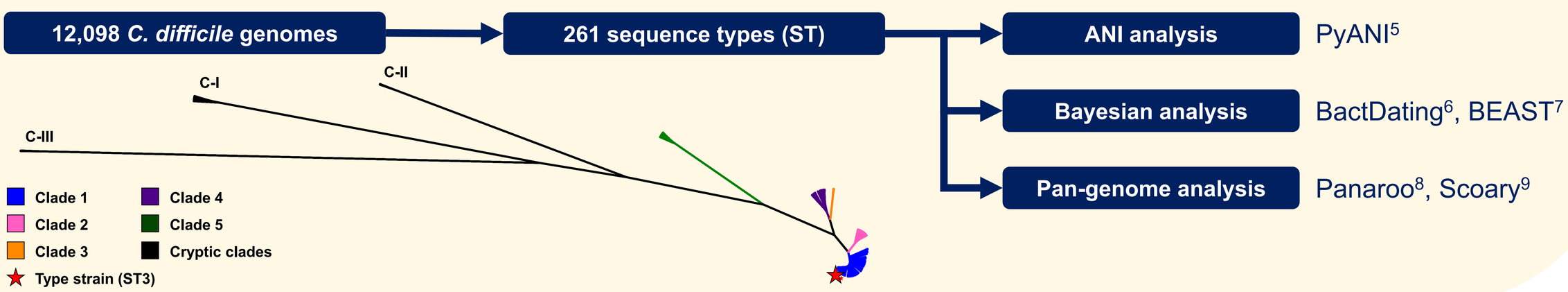
16s rRNA sequence[3]
Species cut-off: 99%
+ Simple
- Utilises < 0.1% of genome
- Limited discriminatory power

Average nucleotide identity[4]
Species cut-off: 96%
+ Simple
+ Utilises the entire genome
+ A promising next-gen standard

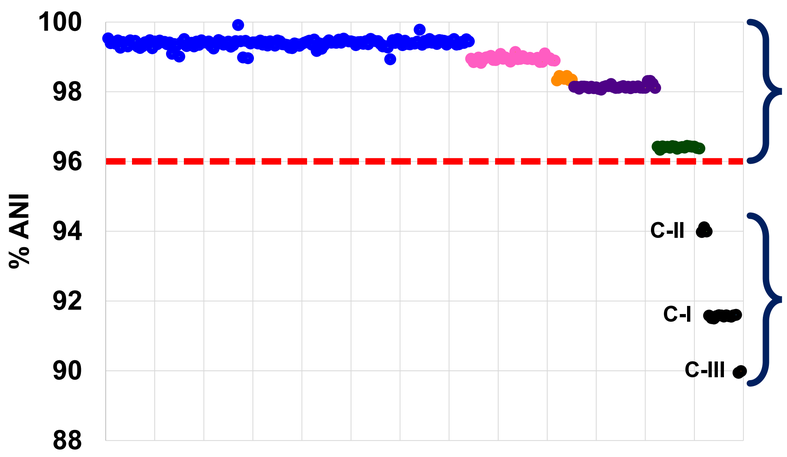
***Clostridioides difficile* species problem:** A diverse population classified into a single species based on 16s rRNA

Study Objective: Use ANI to re-evaluate the species definition of *C. difficile*

Methods



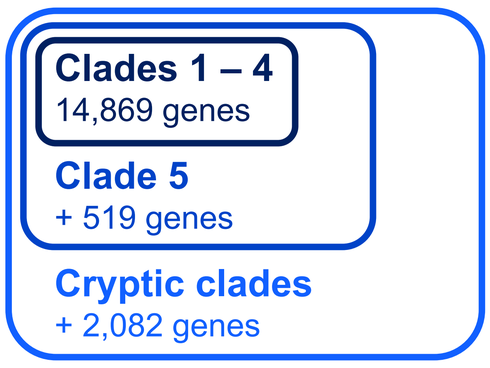
Results



C. difficile

New genomospecies
- Three different species
- Genus *Clostridioides*

1. ANI analysis reveals major discontinuity in *C. difficile* taxonomy

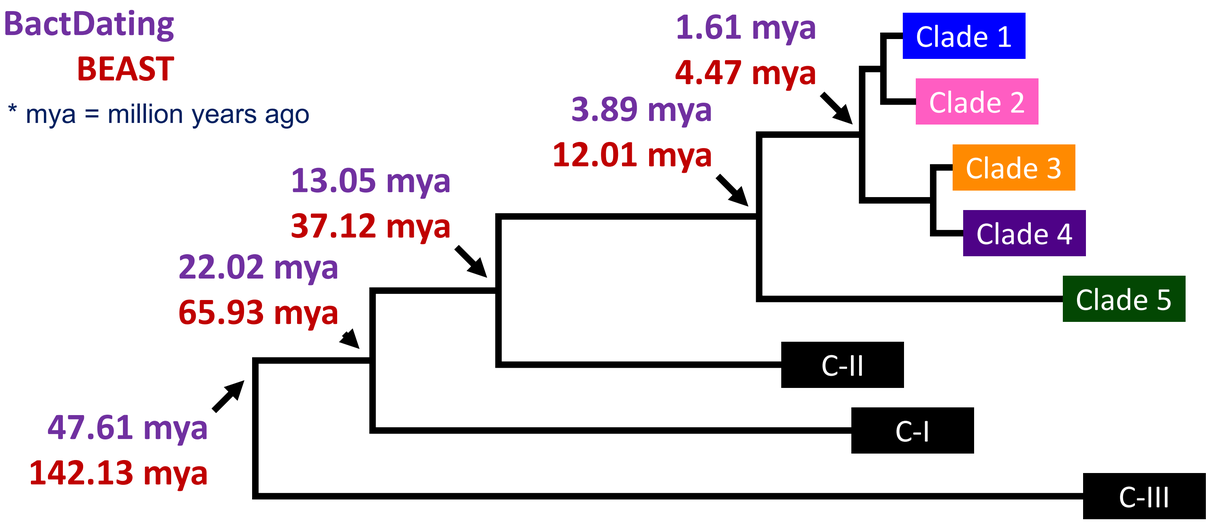


Total gene repertoire
17,470 genes

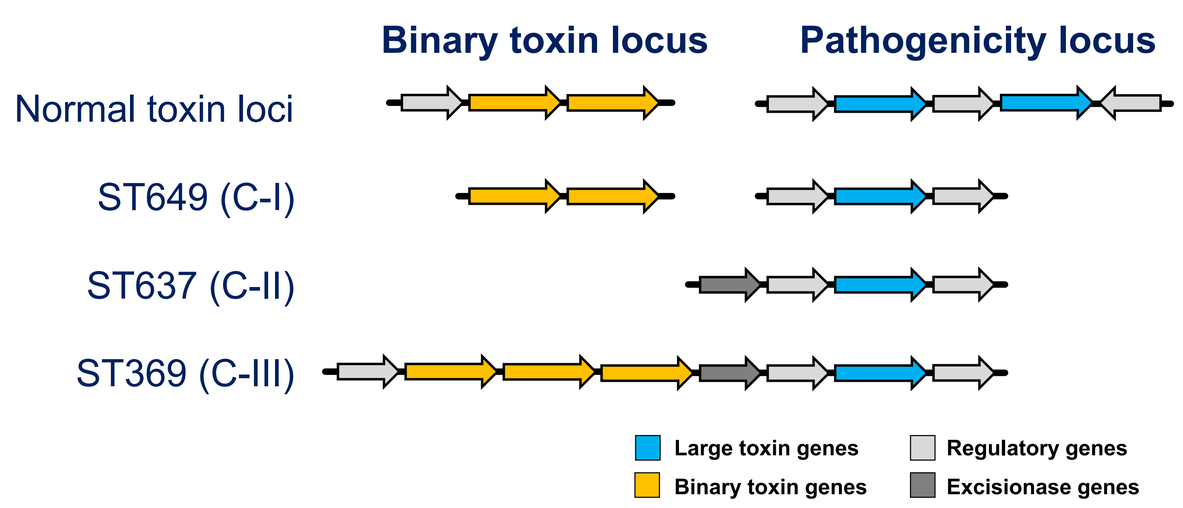
Core gene
2,232 genes (12.8%)

Potential specific phenotypes
C-I - Fructosamine utilisation
C-II - EDTA resistance
C-III - Ethanolamine catabolism
- Fructosamine utilisation
- Polyamine biosynthesis

3. Pan-genome analysis identifies clade specific genes and traits



2. Bayesian evolutionary analysis reveals cryptic clades are ancient species



4. Cryptic clades harbor novel and highly divergent toxin gene architecture identifies

Conclusions

There was a clear species boundary separating *C. difficile* from the 3 novel genomospecies. Several potential phenotypic differences were identified. Difference in toxin gene architecture and its divergence may complicate the diagnosis of *C. difficile* infection.

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