Associations between *Clostridium difficile gyrA* and *gyrB* mutations and 30-day mortality

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INTRODUCTION & AIM

Clostridium difficile infection (CDI) has important associations with morbidity and mortality in the elderly. Risk factors for CDI include older age, antibiotic exposure, care home residence, comorbidities, surgical and non-surgical procedures and length of stay. Exposure to fluoroquinolones in particular has been associated with increased risk of CDI and fluoroquinolone resistance has been a hallmark of hyper-virulent strains of *C. difficile* [1-3]. Mutations in *gyrA* and *gyrB* genes confers fluoroquinolone resistance, with the most important reported mutations at amino acid positions p71, p82 and p118 in *gyrA*, and p426 and p447 in *gyrB* [4].

Investigation of the molecular epidemiology of CDI in Scotland is a interdisciplinary collaboration between various institutions involving patient level routine data, whole genome sequencing of *C. difficile,* and anonymised record linkage between the bacterial genome data and patient data. **This aim of this study was to analyse mutations in *gyrA* and *gyrB* genes and identify any associations with patients' 30 day mortality using logistic regression models.**

METHODS

PATIENT DATA AND GENE SEQUENCE

Scottish National Microbiology Reference Laboratory
Identified isolates

→ **University of Glasgow**
Isolates genotyped

→ **Electronic Data Research and Innovation Service (eDRIS), NHS National Services Scotland**
Obtained patient data

→ **Health Informatics (HIC), University of Dundee**
Genotype and patient data linked

→ **Final Dataset**

DATA ANALYSIS

Mutation Identification
Reference genome used to classify mutated/wild type

→ **Quality Control & Filtering**
Variants with minor allele frequency of >1% used

→ **Logistic Regression**
Univariate and multivariate* models used to identify significant variants

→ **Model Fit Assessment**
The Hosmer-Lemeshow statistics & Area Under the Receiver Operating Characteristic (AUROC) curve used

→ **RESULTS OR (95% CI)**

*Multivariate model adjusted for age, sex, care home residence, healthcare versus community associated (HA/CA), fluoroquinolone use, community prescriptions (1 year prior) and Charlson Comorbidity Index.

1. Appelbaum PC, Hunter PA. The fluoroquinolone antibacterials: past, present and future perspectives. *Int J Antimicrob Agents* 2000; 16:5–15.
2. Bartlett JG. Narrative review: the new epidemic of *Clostridium difficile-associated enteric disease. Ann Intern Med* 2006; *145:758–764.*
3. Knight DR, *et. al.,* Diversity and Evolution in the Genome of *Clostridium difficile. Clinical microbiology reviews* 2015; 28(3): 721-41.
4. Spigaglia P, *et. al.,* Molecular analysis of the gyrA and gyrB quinolone resistance-determining regions of fluoroquinolone-resistant *Clostridium difficile* mutants selected in vitro. *Antimicrob agents and chemother* 2009; 53(6): 2463-8.

RESULTS

Figure 1: Forest plot of odds ratios (95%CI) for *gyrA* and *gyrB* variants significantly (p<0.05) associated with 30 day mortality from **multivariate** logistic regression models

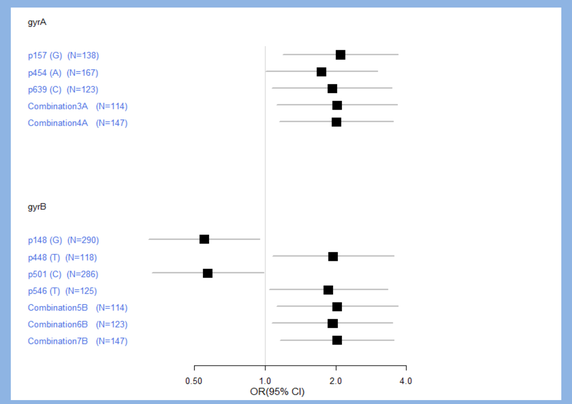
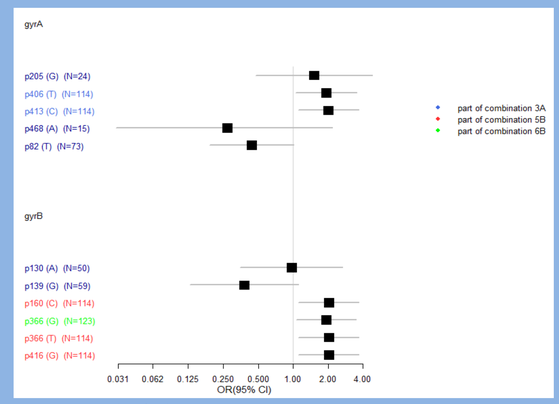


Fig 2: Forest plot of odds ratios (95%CI) for associations between non-synonymous *gyrA* and *gyrB* variants and 30 day mortality from **multivariate** logistic regression models



*Multivariate model adjusted for age, sex, care home residence, healthcare versus community associated (HA/CA), fluoroquinolone use, community prescriptions (1 year prior) and Charlson Comorbidity Index.

- The analysis was conducted on 462 episodes (>3 months apart) from 445 individuals.
- There were 70 mutated *gyrA* and 82 mutated *gyrB* variants, including 5 sets of *gyrA* and 7 sets of gyrB variants, where multiple variants occurred in set groups (of 2-34 variants) across several strains in the dataset.
- Five mutations in *gyrA* and 6 in *gyrB* that are associated with altered amino acid sequences (non-synonymous mutations) were identified.
- For *gyrA*, among 18 unique mutations/sets, 5 were significantly associated with mortality in multivariate analysis (Figure 1).
- For *gyrB*, among 20 unique mutations/sets, 7 were significantly associated with mortality in multivariate analysis and 2 of these associated with decreased likelihood of death (Figure 1).
- Previously described patient factors (e.g. increased age and comorbidity) were significantly associated with increased risk of death in multivariate models (data not shown).
- Six non-synonymous mutations were significantly (p<0.05) associated with increased likelihood of death. Notably the p82 non-synonymous variant in *gyrA* that is known to be associated with increased fluoroquinolone resistance was not significantly associated with mortality in this dataset.

CONCLUSIONS

To the best of our knowledge, this is the first study investigating associations between *gyrA* and *gyrB* variants and patient outcomes. We have successfully used bacterial whole genome sequencing linked to routine patient data and have identified mutations associated with increased likelihood of death.
Our dataset contained a high proportion of ribotype 078 *C. difficile* and the significant variants were over-represented among 078 isolates, which requires further investigation.