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# Influence of gyrA and gyrB mutations in Clostridium difficile isolates on 30-day mortality

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**Background:** Prior antimicrobial use increases the risk of *Clostridium difficile* (*C. difficile*) infection (CDI) which is associated with significant morbidity and mortality, especially among older people. Mechanisms conferring virulence are unclear but have been attributed to the production of toxin A, toxin B and binary toxin, and antibiotic resistance, which are features of hypervirulent ribotypes such as 023, 027, 066, 078 and 244. Exposure to fluoroquinolones has specifically been reported to increase susceptibility to CDI involving ribotypes 027, and mutations in *C. difficile gyrA* and *gyrB* genes are associated with fluoroquinolone resistance with important variants identified at positions p71, p82 and p118 in *gyrA*, and p426 and p447 in *gyrB*. In this study we examined associations between *gyrA* and *gyrB* mutations and 30-day mortality among 461 clinical episodes of CDI in Scotland from which we have sequenced the causative *C. difficile* genomes.

**Material/methods:** Mutations in *gyrA* and *gyrB* were identified by comparison with a reference genome. Genome data were linked to demographic and healthcare data on the source patient at the Health Informatics Centre, University of Dundee. Associations between *gyrA* and *gyrB* mutations (with minor allele frequency >1%), demographic characteristics and 30 day mortality were tested in univariate and multivariate logistic regression models. The demographic characteristics examined

were age (continuous variable), sex, fluoroquinolone use in the prior 6 months, Charlson comorbidity, care home residence, CDI association (healthcare versus community) and the number of different prescriptions in the year prior to CDI (as a comorbidity indicator). Mutations that only occurred in combination with others were included in models as a combination rather than individually.

**Results:** Overall 30 day mortality was 15%. In *gyrA* there were five individual mutations and two combinations, and in *gyrB* four individual and three combinations, that were significantly associated with increased risk of 30-day mortality after adjustment for demographic variables (Table). Among these, there were six non-synonymous mutations (i.e. mutations which lead to a changes in amino acid); two in *gyrA* (p406(T) (OR ) and p413) and four in *gyrB* (p160, p366(T), p366(G), p416). Ribotype 078 was over-represented in our cohort (due to case selection methods prior to genome sequencing) and had a high frequency of significant mutations (e.g. 106 (93%) of all p406(T) mutations were in 078 strains).

**Conclusions:** These analyses have uncovered mutations associated with 30-day mortality in the *gyrA* and *gyrB C. difficile* genes. Testing of these associations in a cohort with a different ribotype distribution is required to determine whether these changes might confer virulence or they are just a characteristic trait of 078 strains. If the same associations are observed, laboratory tests are required to test potential functional importance of these mutations.

Table. Logistic regression results of significant *gyrA* variants associated with 30-day mortality.

| Mutation position (nucleotide), N | 30 day mortality N (%) | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| | | OR (95% CI) | p value | OR (95% CI) | p value |
| p157 (G), 138 | 32 (23) | 2.21 (1.31-3.72) | 0.003 | 2.09 (1.19-3.69) | 0.01 |
| φp271 (A), 114 | 28 (25) | 2.55 (1.45-4.47) | 0.001 | 2.10 (1.13-3.91) | 0.02 |
| φ p271 (C), 61 | 10 (16) | 1.50 (0.69-3.25) | 0.30 | 1.21 (0.52-2.78) | 0.66 |
| * p406 (T), 114 | 28 (25) | 2.26 (1.32-3.88) | 0.003 | 1.93 (1.06-3.52) | 0.03 |
| * p406 (A), 18 | 1 (6) | 0.39 (0.05-2.98) | 0.36 | 0.28 (0.04-2.16) | 0.22 |
| p454 (A), 167 | 35 (21) | 1.88 (1.13-3.14) | 0.02 | 1.74 (1.01-3.01) | 0.05 |
| p639 (C), 123 | 29 (24) | 2.20 (1.30-3.74) | 0.004 | 1.93 (1.07-3.48) | 0.03 |
| Combination3A, 114 | 28 (25) | 2.35 (1.37-4.01) | 0.002 | 2.02 (1.12-3.66) | 0.02 |
| Combination4A, 147 | 33 (22) | 2.10 (1.25-3.53) | 0.005 | 2.01 (1.15-3.51) | 0.01 |

Φ* mutations are part of the same model with three levels (one baseline and two mutated alleles), of combination 3A.