Investigation of QRDR-independent fluoroquinolone resistance in Irish *Clostridium difficile* isolates

Micheál Mac Aogáin, Shauna Kilkenny, Claire Walsh and Thomas Rogers

Department of Clinical Microbiology, Sir Patrick Dun Research Laboratory, Trinity College Dublin, St James's Hospital, Dublin, Ireland.

Email: m.macaogain@tcd.ie

BACKGROUND

Few antibiotic resistance mechanisms have been characterised in *Clostridium difficile*. In the case of fluoroquinolones, which are strongly implicated in precipitating *C. difficile*-associated disease (CDAD), the only known resistance mechanisms is mutational alteration of topoisomerases GyrA and GyrB at a specific region termed the quinolone resistance determining region (QRDR).

Several reports suggest the presence of a QRDR-independent fluoroquinolone resistance mechanisms with preferential activity toward ciprofloxacin in *C. difficile*. We investigated this phenomenon in Irish *C. difficile* isolates. Sixty-six isolates, collected from positive cases of CDAD in 2010 and 2011 were subjected to susceptibility testing. The majority of these strains exhibited fluoroquinolone resistance in contrast to broad susceptibility to other tested antimicrobial agents. We investigated the mechanism of fluoroquinolone resistance in resistant strains through sequencing of the QRDR as well as the entire *gyrA* and *gyrB* coding region. This confirmed the presence of extra-QRDR polymorphisms as well as the absence of mutation across the entire *gyrA* and *gyrB* regions in a number of highly ciprofloxacin-resistant isolates.

Ciprofloxacin resistance is not QRDR-dependent in *C. difficile*

Antibiotic susceptibility testing

	MIC (µg/ml)										
	≤0.05	0.06-0.13	0.13-0.25	0.25-0.5	0.5-1	1-2	2-4	4-8	8-16	16-32	≥32
Ciprofloxacin	0	0	0	0	0	0	0	7	19	6	34
Moxifloxacin	0	0	0	0	34	16	2	2	0	0	12
Vancomycin	0	0	0	26	36	4	0	0	0	0	0
Metronidazole	19	30	13	4	0	0	0	0	0	0	0
Tigecycline	66	0	0	0	0	0	0	0	0	0	0

66 isolates

↓

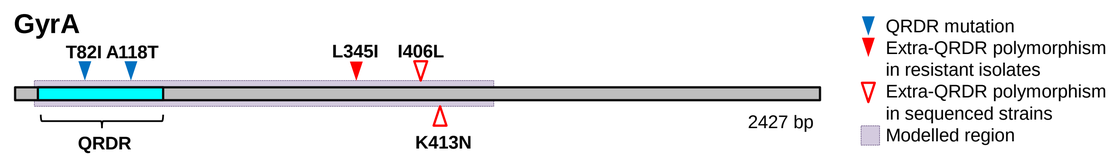
34 highly ciprofloxacin resistant (>32µg/ml)

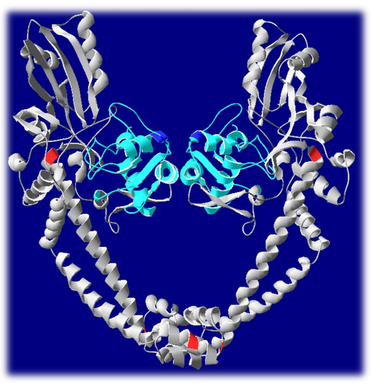
- 12 highly moxifloxacin resistant (>32µg/ml) **CIP^R MOX^R**
- 22 moxifloxacin susceptible **CIP^R MOX^S**

Sequence analysis of QRDR region

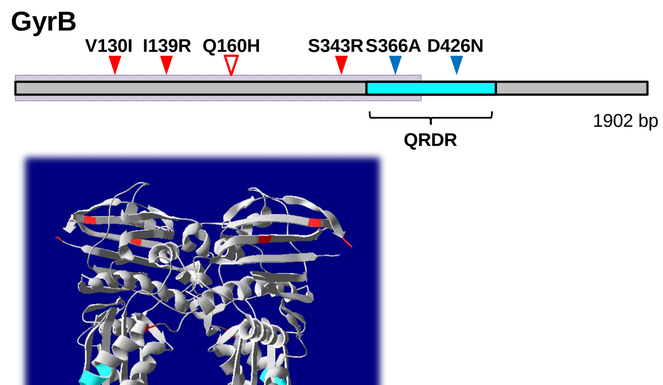
- 100% QRDR *gyrA;* T82I
- 22% QRDR *gyrA*; A118T; QRDR *gyrB*; D426N; QRDR *gyrB*; S366A
- **78% No QRDR mutation**

Sequence analysis of *gyrA/gyrB* coding region





Homology model - GyrA of *C. difficile* based on template strudcture 2xcq of *Staphylococcus aureus* (62% sequence identity) illustrating positions of polymorphisms

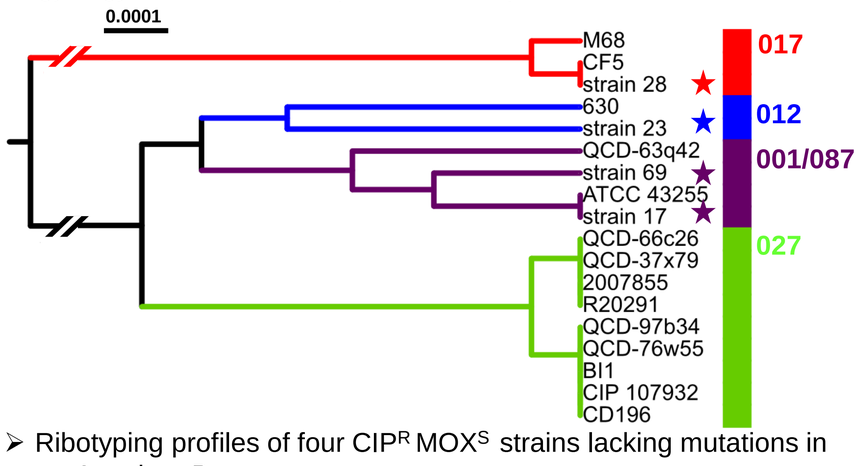


Homology model - GyrB of *C. difficile* based on template strudcture 1kij of *Thermus thermophilus (*46% sequence identity) illustrating positions of polymorphisms

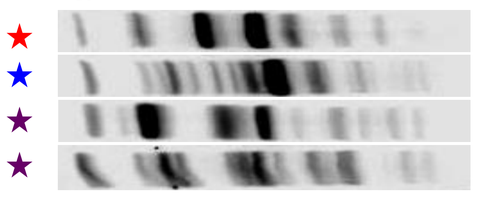
- 11/22 ciprofloxacin –resistant isolates harboured either the **V130I** or **I139R** polymorphism
- 4 ciprofloxacin-resistant isolates had no change in GyrA or GyrB at the protein level

CIP^R MOX^S strains lacking mutations in *gyrA/gyrB* are clonally diverse

- UPGMA tree comparing the *gyrA/gyrB* nucleotide sequences from four CIP^R MOX^S isolates lacking mutations in *gyrA/gyrB* with *gyrA/gyrB* nucleotide sequences of sequenced strains

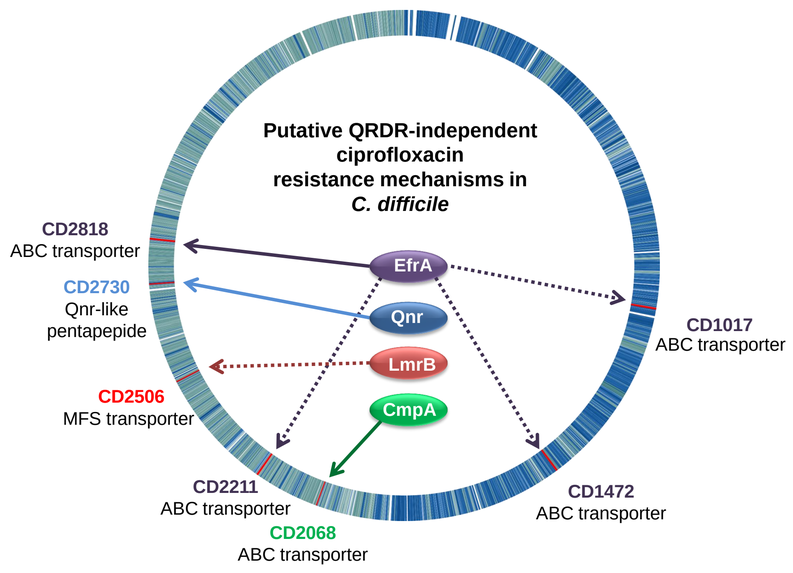


- Ribotyping profiles of four CIP^R MOX^S strains lacking mutations in *gyrA* and *gyrB*



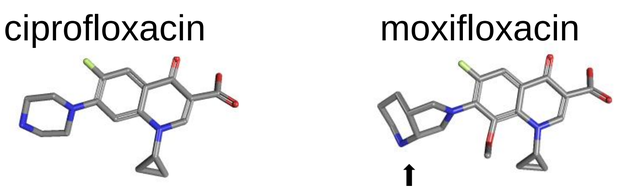
- Diverse strains exhibit *gyrAB*-independent ciprofloxacin resistance

Putative ciprofloxacin resistance mechanisms in *C. difficile*



- Several efflux systems with homology to known ciprofloxacin transporters are present in the *C. difficile* 630 genome

Investigating the impact of efflux inhibitors on CIP^R MOX^S strains



Efflux pump inhibitor	Known target	MIC	Abrogation
Sodium orthovanadate	ABC	>800	No
Verapamil	ABC	400-800	No
Probenecid	ABC	800	No
Reserpine	ABC/MFS	>800	No
Piperine	MFS	>800	No
CCCP	MFS	10-20	No
4-Phenylpiperidine	MFS	>800	No

- Abrogation of ciprofloxacin resistance in the presence of several efflux inhibitors could not be demonstrated

Summary

Surveillance of antimicrobial susceptibility among *C. difficile* isolates at St James's Hospital revealed a high prevalence of fluoroquinolone resistance. Ciprofloxacin-resistant isolates fell into two groups; those resistant to moxifloxacin (CIP^R MOX^R) and those susceptible to this agent (CIP^R MOX^S). All CIP^R MOX^R isolates harboured the previously described T82I mutation in *gyrA* whereas CIP^R MOX^S isolates comprised isolates with QRDR mutations, extra-QRDR polymorphisms and those with no mutations in either *gyrA* or *gyrB.* This further supports the presence of an alternative fluoroquinolone resistance mechanism in *C. difficile.* The fact that QRDR-independent resistance was observed in diverse strains suggests that this mechanism is widespread in *C. difficile* and is an important contributing factor to the emergence of resistance in this pathogen. Several putative QRDR-independent ciprofloxacin resistance mechanisms are present in *C. difficile* including several efflux pumps. However, the role of efflux in *C. difficile* resistance to fluoroquinolones awaits formal validation.

Acknowledgements

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