Molecular epidemiology and clinical outcomes of Clostridium difficile infections from North West London hospitals: is RT220 an under-appreciated severe ribotype?

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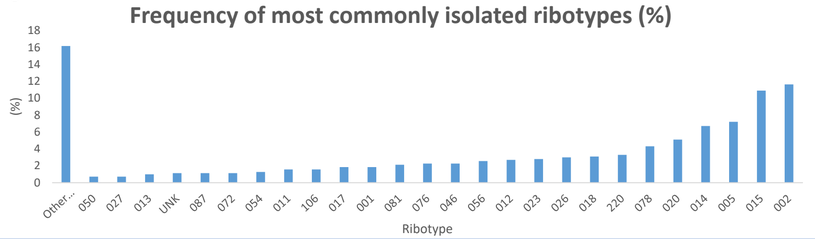
Background: Despite year on year reductions in the number of *Clostridium difficile* infections (CDI), management of CDI remains a challenge in the UK and internationally. Certain *C. difficile* ribotypes, for example 027, have been associated with complex disease phenotypes including recurrence and/or increased severity however data related to currently prevalent ribotypes are limited.

Methods: Samples for *C. difficile* testing were submitted from 5 major West London acute hospitals and from primary care to a single diagnostic laboratory. From August 2011 to June 2013 all samples from symptomatic patients >2yrs that were positive for GDH (glutamate dehydrogenase) and *C. difficile* toxin A gene (Cepheid C difficile Xpert PCR) were cultured, linked to routinely collected demographic and clinical data (where available), and biobanked. PCR ribotyping was performed on all isolates.

Figure 1



Results: A total of 758 GDH/PCR toxin A positive *C. difficile* episodes occurred in 715 unique patients aged 2 and older. *C difficile* isolates from 705/758 episodes were available for further testing including ribotyping, while 53 (6.95%) of isolates failed to grow on culture. From the viable isolates, 86 different ribotypes were identified (Figure 1).
Mortality data were available in 483 episodes (67.6%). The 30 day survival for episodes associated with RT220 was significantly less than survival associated with the two dominant ribotypes (RT002 and RT015) and with all other ribotypes (p=0.02), Figure 2. Demographic and clinical features of cases caused by RT220 were compared with RT 002/015 and other RTs (Table 1 and Figure 3). RT220 was associated with an elevated median CRP (p=0.008) and trend to reduced albumin (p=0.07) compared with RT002/RT015 and all other ribotypes at the time of stool sampling although differences in creatinine (p=0.36) and leukocyte count (p=0.23) were not apparent (Table 1). Cases associated with RT220 did not differ in age, comorbidity or clinical origin, however Toxin EIA was positive in over 60% of cases.

Ribotype	RT220	RT002 and RT015	All other ribotypes	
Demographic data				
No. of *C. difficile* PCR positive episodes (N=705), n (%)	23 (3.3)	159 (22.6)	523 (74.2)	
No. of unique patients (N=715), n (%)	21 (91.3%)	153 (96.2%)	498 (95.2%)	p=0.56
Median age/ episode, yrs (range) (N=705), n (%)	73 (35-95)	77 (4-99)	74 (3-99)	p=0.48
<65	4 (17.4)	45 (28)	165 (31.5)	
65-79	13 (56.5)	54 (33.9)	168 (32.1)	p=0.17
>80	6 (26.1)	59 (37.1)	190 (36.3)	
Sex/episode (Female) n (%)	17 (73.9)	80 (50.3)	288 (55.1)	p=0.096
Modified Elixhauser score (% with data available)	6.70 (56.5)	6.75 (57.2)	Data not available	p=0.95
Descriptives				
Hospital assoc. episodes; (n/total with data available (%)	11/22 (50)	73/141 (51.8)	244/446 (52.3)	p=0.95
Toxin EIA positive* n (%) *only after April 2012	14/21 (63.6)	43/98 (43.9)	132/319 (41.3)	**p=0.08**
Speciality (N= episodes, n (%)				
Medicine	10 (43.5)	83 (52.2)	259 (49.5)	
Surgery	5 (21.7)	23 (14.5)	95 (18.2)	p=0.69
General practice	4 (17.3)	28 (17.6)	70 (13.4)	
Other	4 (17.3)	25 (15.6)	99 (18.9)	
Outcomes				
Biomarkers, median (range)				
CRP	173 (36-319)	44 (1-358)	99 (1-364)	**p=0.008**
Leucocyte count	15.0 (6.5-22)	11.2 (2.5-30.3)	11.0 (0.3-51.4)	p=0.23
Albumin	17.5 (11-24)	23 (12-36)	23 (11-48)	P=0.07
Creatinine (exc renal)	98.5 (39-229)	80 (38-225)	68 (30-660)	P=0.36
Mean (±SD) Length of Stay (days) in 30d survivors	15.8 (±20.8)	20.2 (±21.9)	22.7 (±29.1)	p=0.74
Death within 30 days of sampling, n/total with data (%)	7/17 (41.2)	23/105 (21.9)	60/341 (17.5)	**p=0.02**

Table 1

Implications

RT220 remains one of the commoner ribotypes in this Trust and has persisted as a cause of symptomatic disease despite improvements in infection control practice. Whether there are genomic elements conferring enhanced pathogenicity or toxicity are unclear and this is the subject on ongoing research.

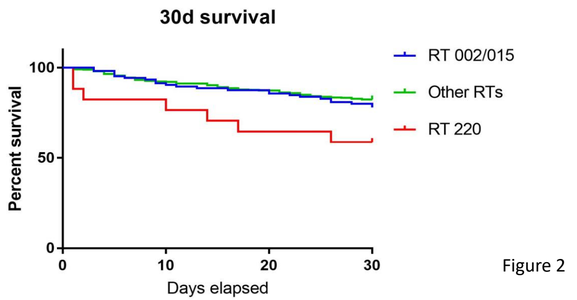


Figure 2

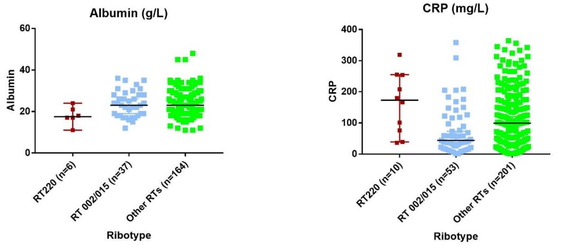


Figure 3

Discussion: This is the first large study to identify *C. difficile* RT220 as a lineage associated with increased 30d mortality and enhanced acute phase response compared with other dominant ribotypes. One strength is the inclusion of all clinically-diagnosed cases in the study period and ascertainment of ribotypes; this allowed a more refined comparison of episodes than many previous studies. Notably RT027 was infrequently found.
Limitations: As a retrospective study, not all clinical data were available limiting the analysis that was possible. We were able to examine routinely collected data relating to disease severity but could not examine individual case notes for treatment or recent antibiotic exposure that may have affected mortality or severity. Routine antimicrobial resistance testing on *C.difficile* isolates was not performed although the one RT220 isolate tested was sensitive to metronidazole and vancomycin.

Acknowledgements: Myriam Gharbi and Oliver Blandy, NIHR HPRU in HCAI and AMR; Russell Hope, Public Health England , ICHT Diagnostic laboratory.