Characteristics of *Clostridium difficile* PCR-ribotype 027 isolates and association with disease activity in a large hospital setting in Liverpool

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BACKGROUND

•The incidence and severity of *Clostridium difficile* infection (CDI) has considerably increased in the last decade.

•This has coincided with the emergence of epidemic strains, particularly PCR-ribotype 027 that has caused hospital outbreaks in North America and Europe.

•PCR-ribotype 027 has recently become the predominant strain in the United Kingdom. There is growing evidence that this strain also accounts for higher morbidity and mortality.

•Several authors have warned that *C. difficile* isolates are becoming increasingly more resistant to metronidazole and vancomycin, mainstay antimicrobials used for CDI therapy.

OBJECTIVES

•To investigate the prevalence of PCR-ribotype 027 (NAP1/BI/gc-8) among patients consecutively recruited into a prospective study and then compare with the historical strain distribution observed in the previous decades in our hospital.

•To assess its association with several clinical parameters used to evaluate the course of the disease.

•To determine the MIC of metronidazole and vancomycin for a collection of isolates based on their temporal distribution in our hospital.

METHODS

•Faecal samples were tested for *C. difficile* toxin using a TOX A/B II ELISA kit (Techlab, Blacksburg, USA) and patients with toxin-positive results were approached to participate in a prospective study on CDI.

•Ethical approval was obtained from the Liverpool Research Ethics Committee and patient information was extracted using the hospital's medical records system.

•One hundred seventy seven (177) CDI inpatients were recruited and followed-up in a large hospital setting in Liverpool between July 2008 and June 2010.

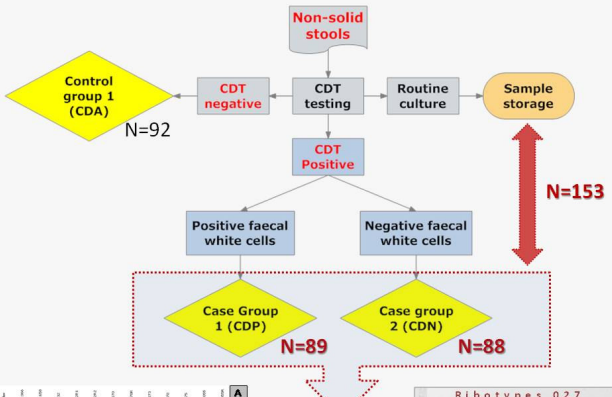
•Toxin positive samples were screened for faecal leukocytes (an indicator of intestinal inflammation) by microscopy of a wet preparation of faecal sample.

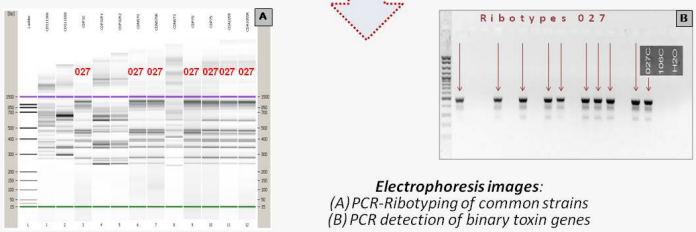
•Faecal samples were cultured for the isolation of strains and to confirm toxigenicity and determine ribotype groups.

•The MIC of metronidazole and vancomycin was determined by the E-test method.

•Clinical outcomes included determination of peak of disease severity (based on the HPA guidelines) over the 30-day follow-up period.

STUDY DESIGN





***Electrophoresis images**:*
(A) PCR-Ribotyping of common strains
(B) PCR detection of binary toxin genes

INITIAL RESULTS

- *Clostridium difficile* PCR-ribotype 027 was the commonest strain isolated from our cohort (45%), followed by types 106 (18%) and 078 (8%). Surveillance assessment also confirmed that type 027 was the main circulating strain in our hospital.
- Comparative analysis of current strains with a collection of historical strains suggests a shift towards PCR-ribotype 027 and a decrease in the diversity of strains (Figure 1).
- The distribution of PCR-ribotype 027 was significantly greater among individuals who had tested positive for the presence of faecal leukocytes (54% vs. 35%, p=0.04) (Figure 2 and Table 1).
- PCR-ribotype 027 also predicted higher levels of toxins A/B as measured by OD values (c=+0.94 , p=0.014) (Table 1).
- Symptomatic patients carrying this strain were significantly more likely to present severe disease within the follow-up period (OR=2.57, p=0.005) (Table 1).
- We found no evidence of resistance to vancomycin and metronidazole in our *C. difficile* isolates (Figures 3 and 4).

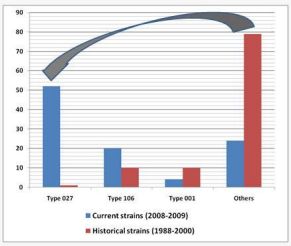


***Figure 1** – Distribution of common ribotypes in the Royal Liverpool University Hospital.*

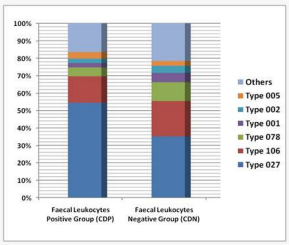


***Figure 2** – Current distribution of Clostridium difficile PCR-ribotypes based on the presence or absence of faecal leukocytes*

***Table 1** – Results of the univariate analysis*

Association results with PCR-ribotype 027		
Parameters	**P-value**	**Values (C.I. 95%)**
Presence of Faecal Leukocytes vs. Absence	0.019	OR=2.15 (1.13, 4.09) ↑
Level of toxins A/B (OD quantile regression)	0.014	C=+0.94 (0.19, 1.69) ↑
Peak of severe disease (4-week follow-up)	0.005	OR=2.57 (1.32, 5.01) ↑
Total number of observations: n=153		

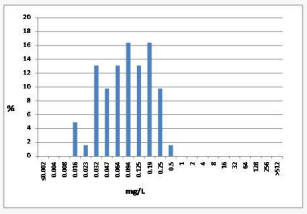


***Figure 3** – Minimum Inhibitory Concentration distribution for Metronidazole (n=61)*

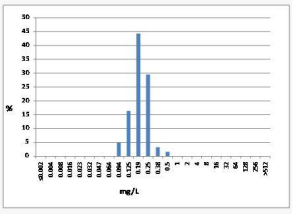


***Figure 4** – Minimum Inhibitory Concentration distribution for Vancomycin (n=61)*

CONCLUSIONS

•Our results suggest that there are important clinical features associated with symptomatic colonisation with PCR-ribotype 027, such as severity of the disease and higher levels of intestinal inflammation as denoted by the presence of faecal leukocytes.

•Although our results are interesting, further investigation is needed to validate our findings. This will be performed by using a replication set of samples.

•Additional work includes the determination of MIC per PCR-ribotype group for the entire collection of isolates.

•Further molecular screening using more discriminatory typing techniques would aid in the detection of sub-types of PCR-ribotypes allowing to track dynamic changes in the pathogen.