Ribotype 027 is Associated with Increased Lactoferrin and Toxin in Patients with Clinically Defined *C. difficile* Disease.

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ABSTRACT

Background: *C. difficile* infection (CDI) has continued to increase with hypervirulent strains causing outbreaks worldwide. The predominant outbreak strain in North America, BI/NAP1/027 (027) has been associated with an increased incidence and greater severity. 027 is fluoroquinolone resistant and produces more spores than nonhypervirulent strains contributing to its ability to spread. Currently, there is conflicting data for whether 027 actually produces more toxin than nonhypervirulent strains and if increased toxin is potentially the cause of more severe disease.

Aim: In this study, we identified ribotypes and evaluated lactoferrin and toxin levels in clinically defined patients with CDI.

Methods: Patients with CDI presenting with a spectrum of severity were recruited. Disease activity was defined by physician's assessment based on symptoms, WBC count (x10^9/L) and co-morbidities. Fecal lactoferrin (µg/mL±std error) and *C. difficile* toxins A and B were detected in stool using enzyme-linked immunoassay (EIA). Toxigenic culture was done using spore enrichment and both isolates and stool specimens were tested by tissue culture assay for cytotoxicity. Control CD strains (ARL ribotypes 001, 002, 003, 012, 014, 017, 027, 033, 036, 046, 053, 054, 078, 106, 110, 126 and 154) were used for ribotyping standards.

Results: 39 inpatients with clinically confirmed CDI (15 severe, 21 moderate and 3 mild) were tested during a 6-month period. Age ranged from 32 to 89 yrs and 50% were female. The predominant co-morbidities were diabetes (31%), cancer (23%) and renal failure (23%). WBC counts were significantly higher in pts with 027 (13) as compared to non027 (10;p<0.05). Ribotype 027 was isolated in 10/15 of severe, 10/20 of moderate and 1/3 of mild CDI. Mean lactoferrin levels for 027 vs. non027 were 941 and 56, respectively (p<0.02). Toxin level in stool expressed by mean EIA absorbance was higher (p<0.02) for pts infected with 027 (2.501) than non027 (1.491).

Conclusion: Our results demonstrate that 027 is associated with more fecal toxin, increased lactoferrin levels, and higher WBC counts, all indicators of more severe CDI.

INTRODUCTION

Clostridium difficile is the leading cause of hospital-acquired antibiotic-associated diarrhea (AAD) and colitis (Wilkins and Lyerly, 2003). Incidence of this disease has increased as more community infections are being identified. An epidemic strain (BI/NAP1/027) shows resistance to fluoroquinolones and higher levels of toxin produced as well as spores, causing numerous outbreaks. Clinical testing presents significant challenges because of the increased number of cases, the additional patient populations being affected, and the types of diagnostic methods now available to the clinician. Most cases are diagnosed based on clinical evaluations, history of antibiotic use, and the presence of toxin in the stool (toxin A/B). Biomarkers such as lactoferrin may provide additional diagnostic insight. Lactoferrin is a stable protein produced by neutrophils during an immune response, and is an indicator of inflammation (Gisbert et al., 2009). In healthy individuals, lactoferrin levels in stool exist at approximately 1-7.25 µg/mL.The assessment of lactoferrin levels along with toxin may allow clinicians to accurately predict severity of CDI and determine proper treatment.

References:

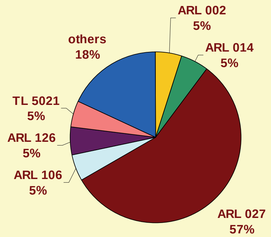
Wilkins,TD., Lyerly, DM., (2003). *Clostridium difficile* Testing: after 20 years, Still Challenging. J. Clin. Microbiol. 41(2): 531-4.

Gisbert, Javier P., McNicholl, Adrian G., Gomollon, Fernando,. (2009). Questions and Answers on the Role of Fecal Lactoferrin as a Marker of Inflammatory Bowel Disease. Inflamm Bowel Dis.(16)7:1091-2.

RESULTS

Patient Characteristics		Percent of Total N=39	Percent of Severe N=15	Percent of Moderate N=21	Percent of Mild N=3
Gender	Male	41	60	29	33
	Female	59	40	71	67
Age	< 64 yr	44	40	48	33
	≥65 yr	56	60	52	67
Co-morbidities	Diabetes	31	13	29	33
	Cancer	23	13	29	33
	Renal failure	23	20	29	0
	Pneumonia	18	27	10	33
	Other	5	27	3	1
Clinical Assessment	Severe	38	100	0	0
	Moderate	54	0	100	0
	Mild	8	0	0	100

Prevalence of Ribotypes isolated from patients in the Summa Health System



Summary:
027 was isolated in 10/15 patients with severe, 10/20 of moderate and 1/3 of mild CDI (n=38). One isolate was unable to be ribotyped and 3 isolates were nontoxigenic.

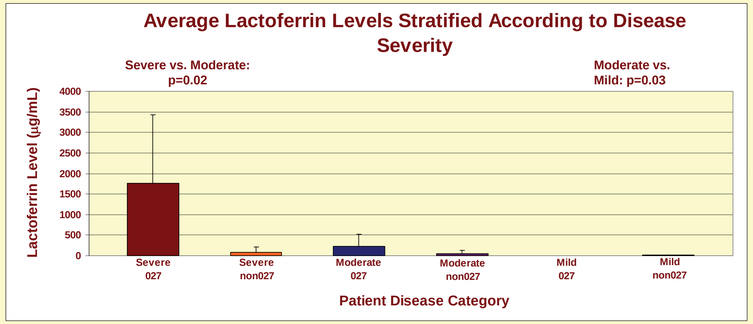
Percentage of *C. difficile* isolates by Severity and Ribotype

Patient Group	Stool GDH + (%)	Tissue Culture + (%)	Toxigenic Culture + (%)
Severe	93	87	93
Moderate	100	71	86
Mild	100	33	100
ARL 027	100	91	100
Non 027	94	53	82

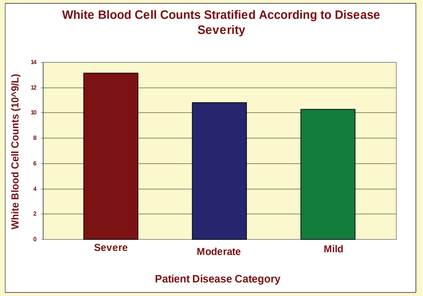
Summary:

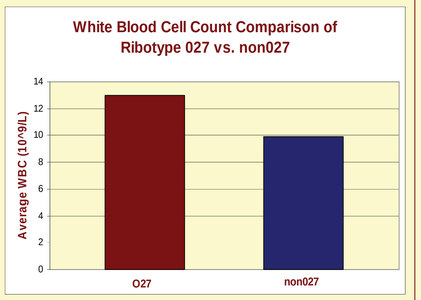
There was higher detection of GDH and toxin in patients with ribotype 027 vs. patients with non027.

RESULTS



Summary: Lactoferrin levels for infections with ribotype 027 were significantly higher than non027 infections.





Summary: WBC counts were significantly higher in severe patients compared to moderate and mild patients (p=0.08).

WBC counts were significantly higher in patients with ribotype 027 than patients with non027(p=0.02).

CONCLUSIONS

Our results demonstrate that ribotype 027 is associated with increased lactoferrin, toxin, and higher WBC counts, all indicators of severe CDI.

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