

Prevalence of *Clostridium difficile* in Fecal Samples from Inpatient and Outpatient Populations

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ABSTRACT

Background: The incidence of *C. difficile* infections (CDI) has continued to increase not only in hospitalized patients but also in outpatients as a community acquired infection. To date few studies have examined rates of CDI in stratified patient populations.

Aim: We evaluated inpatients (IP) and outpatients (OP) for the presence of *C. difficile*. We determined toxigenic vs. nontoxigenic isolation rates, and detected the organism and its toxins using a panel of *in vitro* diagnostic tests.

Methods: A prospective study was done using 435 consecutive fecal specimens submitted over 6-month period from patients suspected of CDI. Tissue culture assay (TC) was done with MRC5 cells, with neutralization noted after 48 h. Bacterial culture was done using spore enrichment, followed by plating on selective cycloserine-cefoxitin fructose agar. Isolates were grown in prerduced brain heart infusion broth and tested by TC. Isolates were confirmed as *C. difficile* by PCR for the glutamate dehydrogenase gene (*gluD*) and toxins A and B genes (*tcdA* and *tcdB*, respectively). Detection of glutamate dehydrogenase (GDH) and toxins A and B in fecal specimens was done by commercial enzyme immunoassay (EIA).

Results: The patient population was 32% (139) OP and 68% (296) IP with ages from <1 years to >94 years, and a total of 55% female patients. The isolation rate for *C. difficile* (toxigenic and nontoxigenic) trended higher in OP (28%) than in IP (20%), and both populations had similar rates (15 to 19%) for nontoxigenic isolates of total isolates. A single OP had both toxigenic and nontoxigenic *C. difficile*. GDH detection showed a correlation of 93% compared to bacterial culture. Toxigenic *C. difficile* was confirmed in 24% of OP and 16% of IP (P<0.05), with the TC positivity rate for specimens being significantly higher (p<0.05) in OP (18%) vs. IP (9%). Toxin detection by EIA, which had a correlation of 96% with TC, supported the higher stool toxin rates in OP.

Conclusion: Our results show that in this healthcare facility, the rates were higher in OP than in IP. These findings support the need for increased surveillance of CDI in previously unsuspected populations.

METHODS

- All data collection and specimen testing were done with de-identified patient information and in accordance with facility IRB requirements. Significance was determined using two-tailed z-ratio test.
- 435 fecal specimens that were submitted for routine clinical testing for CDI between February and July 2009 were included in this study. Stool consistency was defined using the Bristol Stool Chart; liquid – Type 7, Semi-solid – Types 4 – 6 and solid – Types 1 -3.
- Tissue culture – MRC-5 cell monolayers and toxin B neutralizing sera were used for specific neutralization with feces and cultures.
- Microwell ELISA - *C. DIFFICILE TOX A/B II™* test and the *C. DIFF CHEK™ -60* test.
- Bacterial and toxigenic culture – Ethanol spore enrichment with CCFA was used to identify culture-positive specimens. Isolates were subcultured to BHI and grown for 72h then tested by tissue culture for the presence of toxin B.
- PCR analysis – DNA was extracted from broth cultures using the QIAamp Mini Kit (Qiagen, Valencia, CA). Direct PCR was done for GDH, toxin A and B genes (*gluD*, *tcdA* and *tcdB*).
- Statistical analysis – Vassar Stats and Microsoft Excel.

RESULTS

Figure 1. Patient Demographics

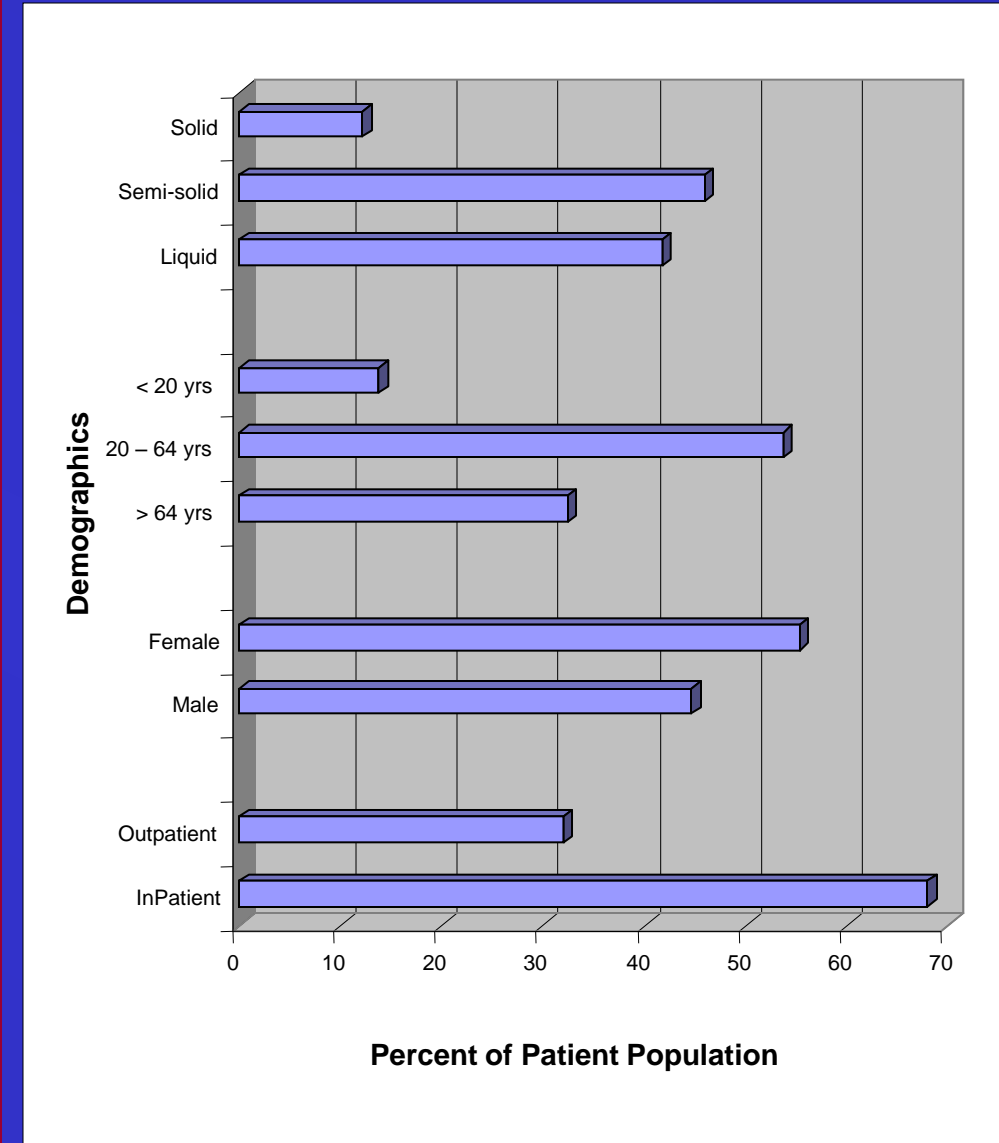


Figure 2. Total Detection Rates

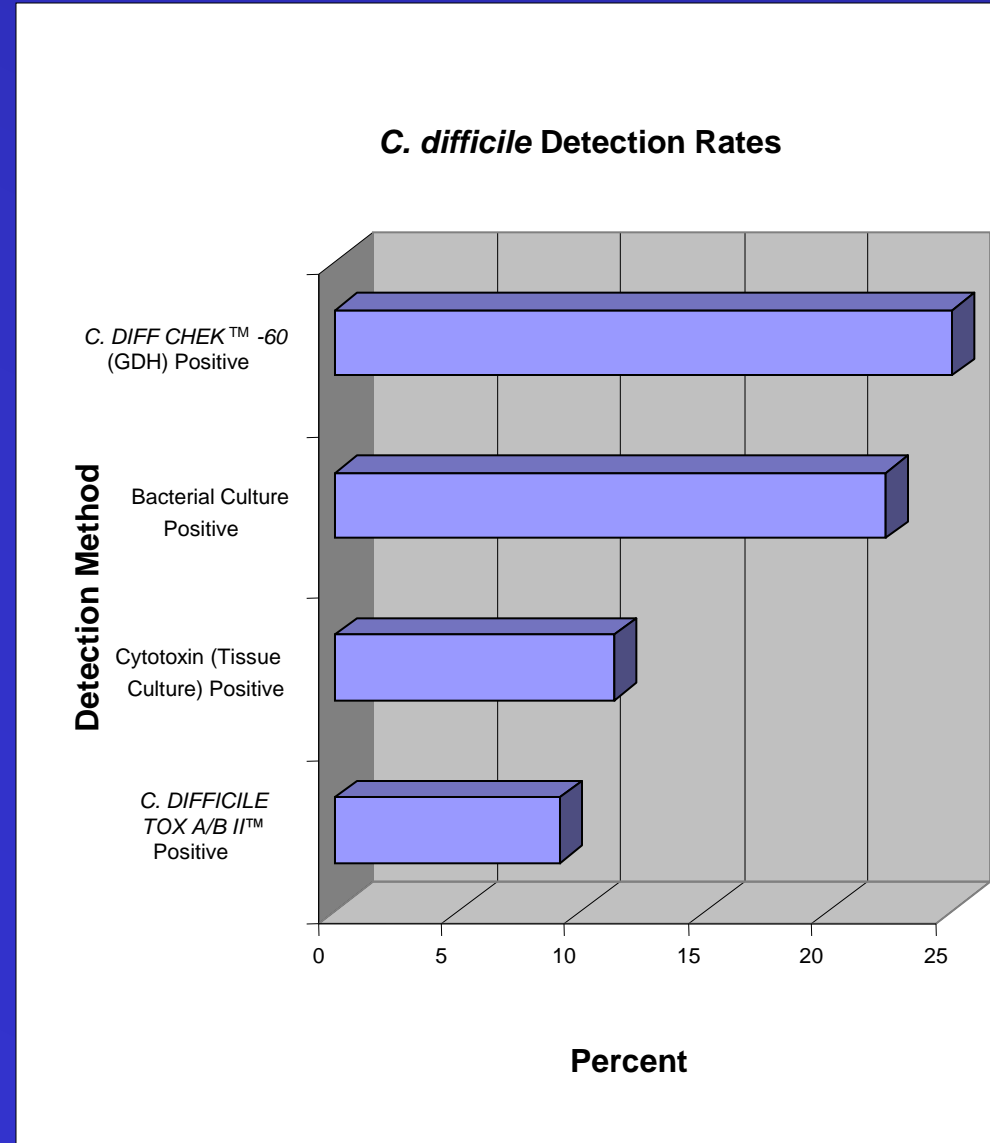
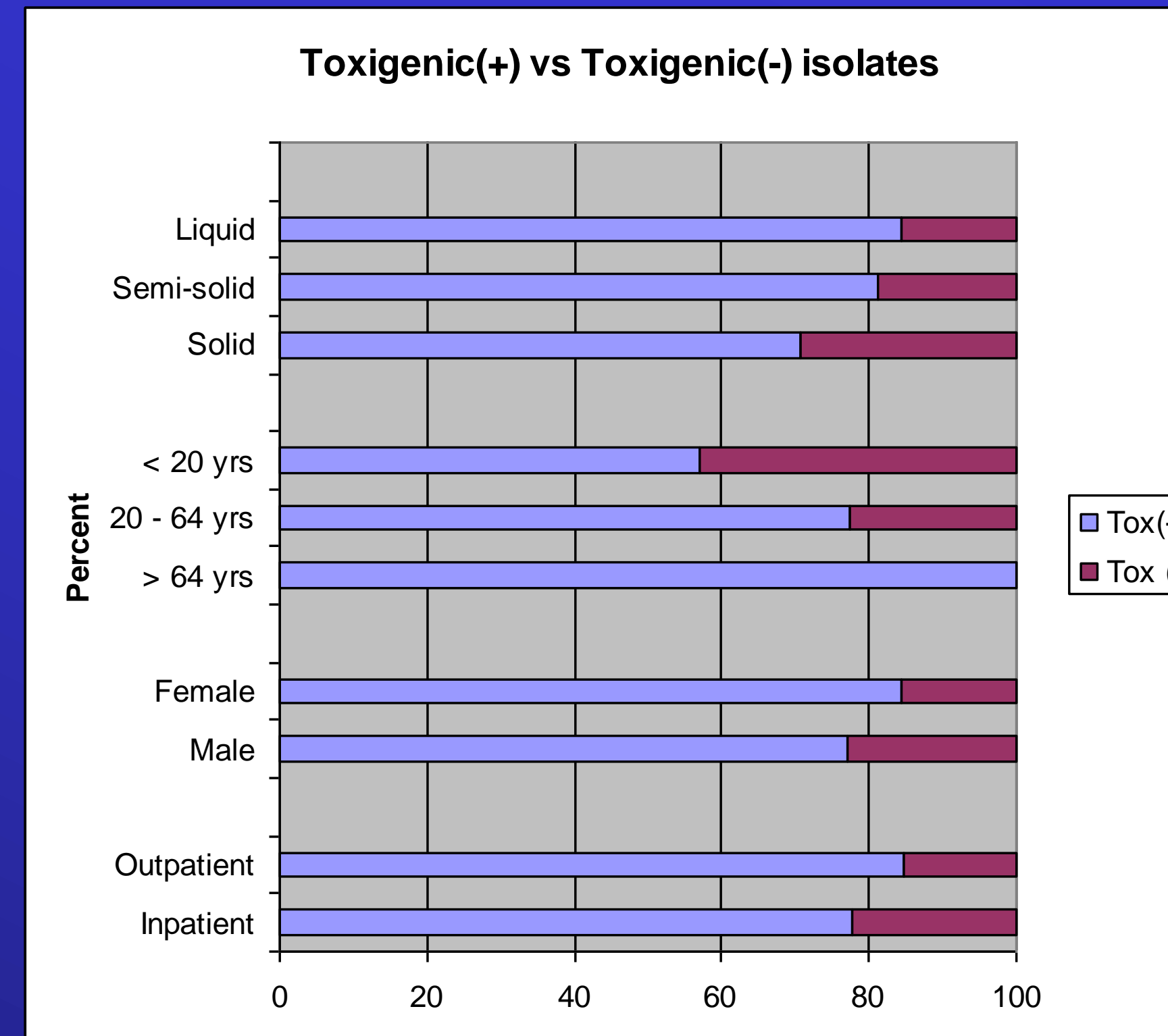


Figure 3. Toxigenic *C. difficile* Rates for Stratified Patient Populations



IP Patient Age	N	Percent culture +	Percent CHEK-60 +	Percent TC +
>64	95	18%	25%	13%
20 - 64	165	18%	21%	6%
<20	36	33%	36%	11%
OP Patient Age	N	Percent culture +	Percent CHEK-60 +	Percent TC +
>64	46	28%	28%	20%
20 - 64	69	25%	27%	17%
<20	24	38%	29%	17%

Summary: Bacterial culture and Stool GDH rates trended higher in patients less than 20 yrs for IP and OP.

IP Stool Consistency	N	Percent culture +	Percent CHEK-60 +	Percent TC +
L	153	17%	21%	9%
SS	126	20%	25%	10%
S	17	41%	41%	6%
OP Stool Consistency	N	Percent culture +	Percent CHEK-60 +	Percent TC +
L	29	21%	17%	17%
SS	74	31%	29%	19%
S	36	28%	33%	17%

Summary: Bacterial culture and stool GDH rates were highest in solid specimens for IP.

CONCLUSIONS

- Tissue culture positive rates for stool toxin are significantly higher for OP compared to IP (p<0.05) independent of age and stool consistency for this healthcare facility.
- Detection of GDH in stool by EIA compared similarly to bacterial culture (93% correlation).
- The nontoxigenic rate trended high to low with increasing age (<20 yr to >64yr).
- Bacterial culture and GDH positive rates trended higher for solid stools compared to liquid.
- When evaluating prevalence rates for *C. difficile*, the patient demographics should be considered.