JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2011, p. 3994–3996 Vol. 49, No. 11
0095-1137/11/$12.00 doi:10.1128/JCM.05349-11


# Molecular Epidemiology of *Clostridium difficile* Strains in Children Compared with That of Strains Circulating in Adults with *Clostridium difficile*-Associated Infection▽

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Received 3 August 2011/Returned for modification 2 September 2011/Accepted 12 September 2011

**Molecular analysis of *Clostridium difficile* (28 isolates) from children ($n = 128$) in Oxfordshire, United Kingdom, identified eight toxigenic genotypes. Six of these were isolated from 27% of concurrent adult *C. difficile*-associated infections studied ($n = 83$). No children carried hypervirulent PCR ribotype 027. Children could participate in the transmission of some adult disease-causing genotypes.**

*Clostridium difficile* is a major cause of adult nosocomial diarrhea, with a wide spectrum of disease severity (1). The symptoms of *Clostridium difficile*-associated infection (CDI) are attributed to the production of toxin A and/or toxin B encoded by the genes *tcdA* and *tcdB* within the pathogenicity locus (PaLoc) (15). However, both toxigenic and nontoxigenic strains may be carried asymptomatically (2, 13). Epidemic outbreaks reported in Canada, the United States, and Europe (9, 12, 22) have been particularly associated with PCR ribotype 027, which is described as hypervirulent (12).

Most studies of pediatric *C. difficile* (4, 7, 17) precede the adult 027-associated epidemic (12). Asymptomatic carriage rates range from 18 to 68%, with toxigenic isolates more likely to be recovered from hospitalized (18 to 29%) (4, 20) or symptomatic (46 to 100%) (8, 16) children. More recently, *C. difficile* has become one of the most commonly identified pathogens in pediatric nosocomial diarrhea (10), with 10 to 24% of *C. difficile* strains from symptomatic children in North America being PCR ribotype 027 (19, 21). Similar contemporary United Kingdom studies are lacking, and the potential overlap between pediatric *C. difficile* genotypes and those causing adult CDI in the same locality has yet to be investigated. This pilot study used an established multilocus sequence typing (MLST) scheme (6) and partial sequencing of the PaLoc-associated *tcdB* and *tcdC* loci to characterize and compare pediatric *C. difficile* isolates with contemporaneous adult CDI isolates.

One hundred twenty-eight anonymized fecal samples from children aged <30 months were included (1 November 2008 to 1 February 2009). Samples comprised (i) nondiarrheal samples from discarded diapers from healthy children attending a breastfeeding clinic, Oxford, United Kingdom, or living in the Oxford area ($n = 80$) and (ii) diarrheal samples from symptomatic children ($n = 48$), submitted to the John Radcliffe Hospital microbiology laboratory for routine enteropathogen testing. All contemporaneous adult (≥18 years) *C. difficile* enzyme immunoassay (EIA)-positive (Premier Toxins A&B enzyme immunoassay; Meridian Bioscience, Italy) diarrheal samples from Oxfordshire processed routinely by the same laboratory were cultured ($n = 83$; 68 from inpatients, 15 from outpatients). Samples from the same individual with the same sequence type (ST) within a 14-day period were excluded from analysis.

As no patient-identifiable data were collected and anonymized samples were discards from routine testing or the breastfeeding clinic or were voluntarily donated, this study was considered a pilot falling under local generic approval for anonymized microbiological surveillance studies. Approval for using adult and pediatric diarrheal samples without individual consent was also obtained within a larger Oxfordshire *C. difficile* study (Berkshire Research Ethics Committee; 10/H0505/83).

Samples underwent spore-selective "alcohol shock" and anaerobic culture on modified Brazier's agar (6). *C. difficile* DNA was obtained from clarified boiled cell suspensions (6), and MLST was performed (6). The presence of the PaLoc (3), *tcdA*, *tcdB*, *tcdC*, and binary toxin genes (*cdtA* and *cdtB*) was confirmed by PCR (5, 11, 18). Part of the *tcdB*

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▽ Published ahead of print on 21 September 2011.

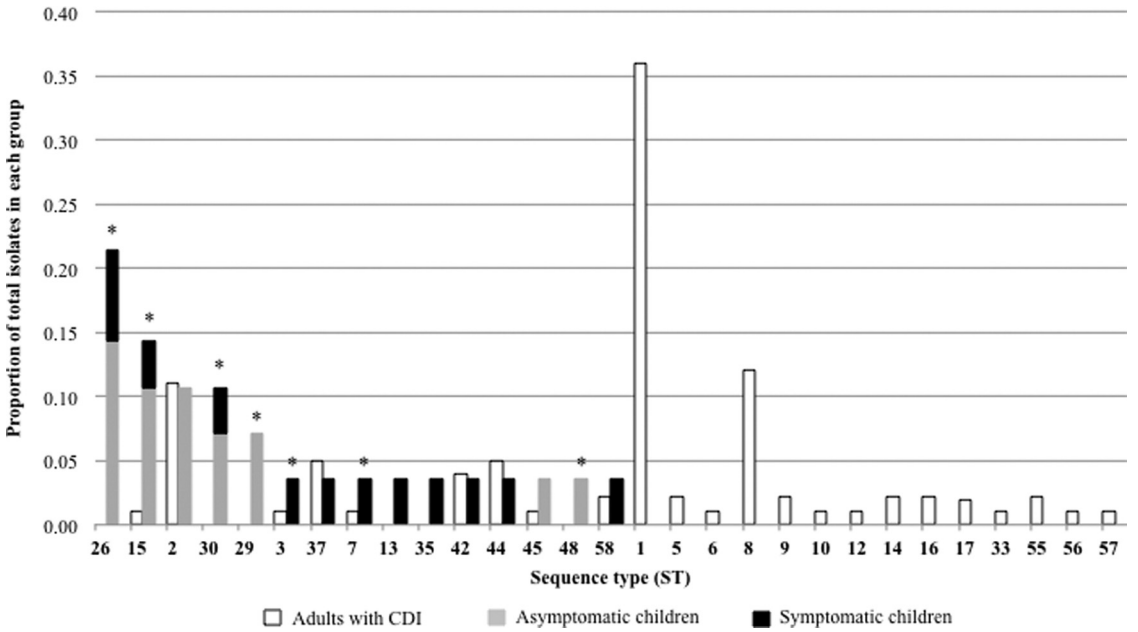


FIG. 1. Distribution of STs in children and adults with CDI as a proportion of the total number of isolates in each group (1 November 2008 to 1 February 2009). Asymptomatic or symptomatic refers to the absence or presence of diarrhea, respectively. Asterisks denote STs identified in children in this study that were nontoxigenic in this group. $A^- B^+$ isolates were exclusively ST-37.

receptor-binding domain (*tcdB*-RBD) and the *tcdC* gene were sequenced (5).

Statistical analysis was performed using Fisher's exact test (FET) with Stata 11.1.

*C. difficile* was cultured from 28/128 (22%) children, 16/80 (20%) of whom were asymptomatic and 12/48 (25%) of whom were symptomatic (FET, $P = 0.52$). For the adults, 84 isolates from 83 individuals were studied, since one culture yielded colonies of different morphologies and both of these were analyzed.

Fifteen STs were identified among 28 pediatric isolates, and 23 STs were identified among 84 adult isolates. Six STs were unique to children and 14 were unique to adults, whereas nine were shared (Fig. 1). Nontoxigenic ST-26 and ST-15 were the most common genotypes in children ($n = 10/28$; 36%). In contrast, 36% ($n = 30/84$) of adult isolates were toxigenic ST-1 (PCR ribotype 027), which was not found in children. The frequency distribution of specific STs in children compared to that in adults with CDI was significantly different (FET, $P < 0.0001$ for all isolates and $P = 0.03$ for toxigenic isolates only; Fig. 1).

Ten of 28 (32%) pediatric isolates were toxigenic (nine $A^+ B^+$, one $A^- B^+$). A negative lok1/lok3 PCR (3) indicated that these contained the PaLoc in the expected genomic position. Four asymptomatic ($n = 4/80$; 5%) and six symptomatic ($n = 6/48$; 13%) children carried toxigenic strains (FET, $P = 0.19$). Two isolates from adults with CDI were nontoxigenic (ST-7 and ST-15), indicating an inaccurate EIA result or coinfection with an uncultured toxigenic strain; four isolates were $A^- B^+$.

All pediatric isolates were negative for binary toxin genes. Thirty-two isolates from adults with CDI were binary toxin gene positive (ST-1 and ST-5; FET, $P < 0.0001$ for all isolates and $P = 0.03$ for toxigenic strains only).

Six of the eight toxigenic pediatric STs accounted for 23/84 (27%) of the adult isolates. The remaining two (ST-13 and ST-35) were not identified in adults in this study but have been found in a larger study of adult CDIs in Oxfordshire (5). There was no difference in *tcdB*-RBD and *tcdC* alleles of equivalent STs isolated from children and adults with CDI, and all pediatric isolates lacked the *tcdC* truncations described in hypervirulent strains (5).

Identifying reservoirs colonized by disease-causing *C. difficile* strains is essential for understanding transmission networks and developing effective infection control. Given that asymptomatic children are carriers of toxigenic *C. difficile* strains, they represent a potential reservoir of strains causing disease in adults. Although we did not demonstrate direct transmission events, our data confirmed that a significant proportion of our pediatric isolates shared the same STs and PaLoc variants as those isolates cocirculating in adults with CDI ($n = 8/28$; 28%), confirming that the potential for transmission exists. Notably, however, the two most common genotypes in adults with CDI (ST-1 and ST-8 [PCR ribotype 002]; $n = 40$ [48%]) were absent in children, suggesting that other possible reservoirs remain to be identified. Both observations are supported by a recent study identifying close contact with children <2 years as a risk factor for community-associated adult CDI (23) and by a study of French infants, which found no evidence of hypervirulent *C. difficile* strains in this group (14).

A limitation of this pilot study is the small sample size. In addition, larger studies are required to confirm whether toxigenic strains are causally associated with pediatric diarrhea or whether their isolation is coincidental. Extending sampling to hospitalized, symptomatic children could identify whether hypervirulent STs are present, as reported in the United States (21).

Comparative studies contemporaneously sampling multiple potential *C. difficile* reservoirs are best placed to accurately characterize chains of transmission. Longitudinal studies employing high-resolution genotyping of isolates from the food chain and animal and human subpopulations will enhance our understanding of the transmission networks of different genotypes.

This work was supported by the United Kingdom Clinical Research Collaboration (UKCRC) and the National Institute for Health Research (NIHR) Biomedical Research Centre, Oxford (OxBRC).

We acknowledge the help of Sally Inch, Infant Feeding Specialist, and staff at the Breastfeeding Clinic and all the staff in the Gastroenterology, Colorectal and Endoscopy Departments, John Radcliffe Hospital, Oxford, United Kingdom.

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