

Emergence of an Epidemic Strain of *Clostridium difficile* in the United States, 2001-4:
Potential Role for Virulence Factors and Antimicrobial Resistance Traits

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Background: Recent reports suggest increasing rates and severity of *Clostridium difficile*-associated disease (CDAD) in the United States. This may reflect the emergence of a new strain of CD with increased virulence or resistance.

Methods: A total of 151 isolates were collected from 7 hospitals (median 13 per hospital, range 3-46) located in 6 states (GA, IL, ME, NJ, OR and PA); all 7 hospitals had outbreaks of CDAD in 2001-4. Strain typing was performed using a combination of restriction endonuclease analysis (REA), pulsed-field gel electrophoresis (PFGE), and toxinotyping. REA types were compared with those in a database of >6,000 historic (before 12/31/2000) isolates. Potential virulence factors were analyzed using PCR to detect genes encoding binary toxin and a deletion in the negative regulatory gene *tcdC* that could increase toxin A and B production. Antimicrobial susceptibility testing was performed using E-tests with appropriate controls.

Results: One REA profile was common among isolates from 4 hospitals tested; isolates from one of these hospitals were identical to isolates in 2 additional hospitals by PFGE. The REA profile, first reported in 1984, was uncommon (14 cases) in the historic database. Both historic and current isolates were toxinotype III, binary toxin positive, and contained the *tcdC* deletion. Isolates with the same toxinotype and virulence properties were identified in the remaining hospital. The epidemic strain was predominant in 6/7 hospital samples. Antimicrobial susceptibility testing was performed on 19 isolates of the epidemic strain (14 current; 5 historic). Clindamycin non-susceptibility was similar in current (7/14) vs. historic (1/5; $P=0.3$) isolates. In contrast, moxifloxacin/gatifloxacin resistance was more common in current (14/14) vs. historic (0/5; $P<0.01$) isolates.

Conclusion: A previously uncommon strain of CD has emerged as a cause of geographically dispersed outbreaks. The development of fluoroquinolone resistance in a strain with pre-existent virulence factors may have provided a selective advantage that promoted widespread dissemination.