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Clindamycin Resistant clone of C. difficile PCR ribotype 027 in Europe

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Clindamycin-Resistant Clone of *Clostridium difficile* PCR Ribotype 027, Europe

To the Editor: Since 2003, outbreaks of *Clostridium difficile*–associated disease (CDAD) associated with the emergence of a hypervirulent strain have been reported worldwide (*1*,*2*; www.eurosurveillance.org/em/v12n06/1206-221.asp). This strain has been associated with increased disease severity and attributable mortality. Patients infected with *C. difficile* 027 fail to respond to metronidazole therapy (*1*). Several typing methods have been applied to further characterize *C. difficile* PCR ribotype-027, including pulsed-field gel electrophoresis (PFGE) (North American pulsed field type 1) and restriction enzyme analysis (REA) (BI). PFGE and REA are widely used in the United States; PCR ribotyping is more commonly used throughout Europe. More recently, 2 multiple-locus variable-number tandem-repeat analysis (MLVA) protocols have been applied to type *C. difficile*, and these proved more discriminatory compared to other methods (*3*,*4*). Furthermore, MLVA can subgroup geographically diverse 027 isolates (G. Killgore et al., unpub data) as well as 027 isolates that are common to 1 institution (*5*).

We reported a case of *C. difficile* PCR 027 in Ireland, where the isolate had an identical antibiogram profile compared with those strains reported across Europe (*6*,*7*) (i.e., resistant to fluoroquinolones and erythromycin, susceptible to clindamycin). We have subsequently identified *C. difficile* 027 in 6 more healthcare settings. To date >100 Irish *C. difficile* 027 isolates have been characterized by analysis of their antibiogram profiles, toxinotyping, and 16S–23S rDNA PCR ribotyping. All *C. difficile* 027 isolates were resistant to moxifloxaxin, gatifloxacin,

ciprofloxacin (MIC >32 mg/L), and erythromycin (MIC >256 mg/L) but susceptible to metronidazole (MIC 0.25 mg/L) and vancomycin (MIC >0.5 mg/L). Clindamycin susceptibility varied between isolates from unrelated institutions. Isolates from 2 healthcare settings were susceptible to clindamycin (n = 11: MIC_{90} 4 mg/L). However, clindamycin-resistant PCR 027 isolates (n = 96: MIC_{90} >256 mg/L) were identified in the other 5 healthcare institutions. All clindamycin-resistant PCR 027 isolates were positive for the *erm*B gene, encoding the macrolide-lincosamide-streptogramin-B genotype.

A subset of clindamycin-sensitive and -resistant Irish 027 strains isolated throughout 2006 (n = 22) were further characterized by using a recently described MLVA protocol (*3*). Six clindamycin-susceptible isolates were selected from 2 healthcare settings. One hospital conducted active routine laboratory surveillance and molecular genotyping (n = 3). The second hospital submitted only random isolates (n = 3) for typing during a *C. difficile* outbreak. Sixteen clindamycin-resistant PCR 027 isolates were also included in the MLVA. Resistant isolates were selected from 5 healthcare settings. These included isolates from 2 *C. difficile* outbreaks with ongoing laboratory surveillance (n = 5, n = 6, respectively); a third hospital with ongoing laboratory surveillance (n = 3) and 2 hospitals that each submitted fecal samples from patients with severe cases of *C. difficile* disease (n = 1). The Stoke-Mandeville control strain R20291 was included for comparison.

MLVA determined that all strains within the clindamycin-resistant cluster were closely related and were single- or double-locus variants with a maximum 5 summed tandem-repeat difference (STRD). In contrast, the closest relationship between the clindamycin-resistant and the clindamycin-sensitive clusters was a triple-locus variant with an STRD of 17. The nonrelated reference strain of the Stoke-Mandeville outbreak (R20291) differed considerably from all Irish isolates but was more related to the clindamycin-sensitive cluster than to the clindamycin-resistant cluster (Figure). We thus linked a defined genetic marker with the clindamycin-resistant phenotype in *C. difficile* PCR-027. MLVA could clearly differentiate clindamycin-resistant and -susceptible isolates from the same geographic region and subgrouped them into 2 distinct clusters (Figure).

Although high-level resistance to fluoroquinolone antimicrobial agents has been well documented in PCR 027 (*1*,6), resistance to clindamycin is rare. Subsequently, clindamycin has been considered as a "protective" antimicrobial agent for the development of CDAD in an epidemiologic survey in the Netherlands (*8*). Currently, resistance to this agent in NAP 1/PCR 027 has been restricted to the United States. McDonald and colleagues reported that 19 (79%) of 24 NAP 1 isolates were classified as less susceptible (MIC 4 mg/L) or resistant (MIC 8 mg/L) to clindamycin when Clinical and Laboratory Standards Institute criteria were used (*2*). Unfortunately, MIC values were not reported, and the corresponding resistance genes were not investigated. In contrast, Canadian studies to date have not reported clindamycin resistance in this strain type. The MIC_{90} of Canadian NAP 1 isolates for clindamycin was 4 mg/L (*9*,*10*). Although outbreaks and sporadic cases of PCR 027 have been identified in several European countries, to date no clindamycin-resistant clone has been reported.

Detection of clindamcyin-resistant *C. difficile* PCR 027 strains is an important and worrying development. Resistance to this antimicrobal agent increases the risk for CDAD in patients, and its use may be an important factor contributing to the persistence and spread of PCR 027. A similar feature has already been observed when fluoroquinolones and cephalosporins are prescribed. Clindamcyin-resistant PCR

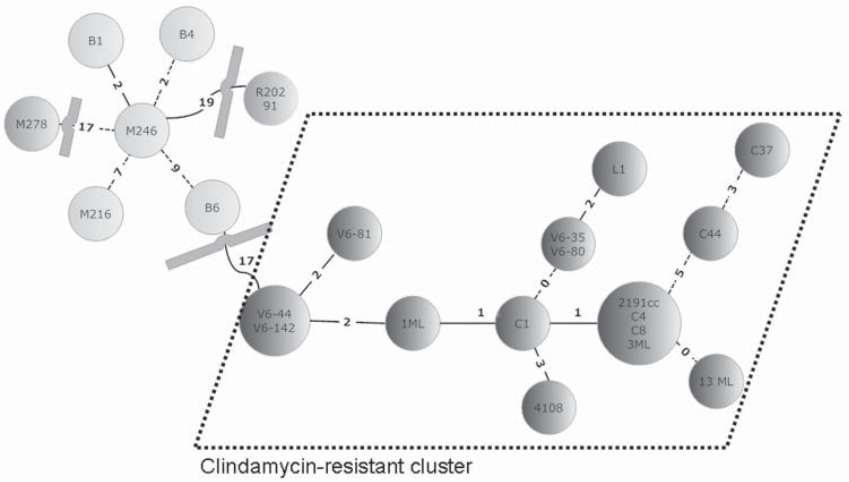

Figure. Minimal spanning tree of 23 *Clostridium difficile* isolates. In the circles, the individual isolates are mentioned. The numbers between the circles represent the summed tandem repeat differences (STRDs) between multiple-locus variable-number tandem-repeat analysis types. Straight lines represent single-locus variants, dashed lines double-locus variants. Curved lines represent triple-locus variants. Two related clusters can be discriminated: the light gray cluster (isolates B1, B4, M246, B6, and M216) and the cluster within dotted lines (isolates V6–44, V6–142, V6–81, 1ML, C1, 4108, V6–35, V6–80, L1, 2191cc, C4, C8, 3ML, C44, C37, and 13ML) The isolates in the light gray cluster are sensitive to clindamycin; isolates in the cluster surrounded by dotted lines are resistant. Two isolates (M278 and R20291) did not belong to a cluster but were more related to the sensitive cluster than to the resistant cluster. Genetically related clusters were defined by an STRD ≤10.

027 probably reflects the emergence of a new clone because MLVA clearly differentiates between clindamycin-susceptible and -resistant isolates.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have one Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Increasing Incidence of *Clostridium difficile*–associated Disease, Singapore

To the Editor: *Clostridium difficile*–associated disease (CDAD) has increased in incidence across North America and Europe (*1*). Recent reports document the emergence of an epidemic strain of *C. difficile*, NAP1/BI/027, associated with increased virulence (*2*,*3*). However, less information is available regarding CDAD epidemiology in Asia. We examined the incidence of *C. difficile* among hospitalized patients in Singapore from 2001 through 2006 and conducted a case–control study to evaluate risk factors for testing positive for *C. difficile* toxin (CDT) in our population.

Tan Tock Seng Hospital (TTSH) is a 1,200-bed, acute-care general hospital in Singapore that serves an urban population of 4 million. We calculated CDAD incidence using the number of patients testing positive for CDT per 10,000 patient days from 2001 through 2006. We used this calculation because CDT testing would have been ordered for clinical indications. CDT testing was performed by using the same ELISA (Premier Toxins A&B; Meridian Bioscience, Inc., Cincinnati, OH, USA) throughout the entire period of investigation.

Case-patients and controls were selected from patients hospitalized at TTSH from January 1 through December 31, 2004. Microbiology laboratory records were used to define 3 groups. Case-patients were defined as CDT-positive inpatients (group 1). Two sets of negative controls were defined: the first (group 2) consisted of patients who tested negative for CDT. However, because false-negatives could nullify differences between groups 1 and 2, we defined a second set of negative controls (group 3) from among 18,000 inpatients not tested for CDT.