

Letters to the Editor

Genotyping of *Clostridium difficile* Isolates

With regard to the recent report (2) on typing of *Clostridium difficile* by arbitrarily primed PCR (AP-PCR), we would like to make readers aware of another typing approach (1). This approach uses PCR to amplify the 16S-23S rRNA gene (rDNA) spacer region, which is situated in the evolutionarily stable rRNA operon (5). It was demonstrated that in *C. difficile* there were 16 variable-length 16S-23S rDNA spacer alleles: different strains contained different combinations of variable-length alleles (1).

By AP-PCR, 41 isolates were separated into nine groups, with 66% falling into one group (2). In a separate study, AP-PCR was used to separate 20 isolates into four groups (3). By amplifying the 16S-23S rDNA spacer region (1), 24 isolates were divided into 14 ribotypes. Although the total number of genotypes is not known, the 16S-23S rDNA spacer typing is more discriminatory than AP-PCR typing.

The 16S-23S spacer region typing is based on PCR, and so the laboratory time taken to type organisms is comparable to that taken with AP-PCR: about 1.5 working days from extraction to gel reading.

AP-PCR is based on the partial homology of short primers (5 to 10 nucleotides) to genomic DNA, resulting in primers randomly annealing (4): the method produces results which vary from run to run and from operator to operator and are highly dependent on PCR conditions. On the other hand, 16S-23S spacer typing is based on the presence or absence of long regions of DNA (150 to 500 bp) in an operon that is stable in evolutionary terms (1, 5). Taking this and the improved discriminatory power together, 16S-23S rDNA spacer region typing has advantages over AP-PCR.

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Author's Reply

As pointed out in the references of both our paper and the letter of Drs. Gürtler and Mayall, several methods of typing *Clostridium difficile* isolates have been tried and the merits and pitfalls of each presented. Their method of using PCR to detect variation in the spacer region between the genes for 16S and 23S rRNA appears to be another promising molecular technique. However, before we can determine the comparative usefulness of these methods with regard to complexity, cost, typeability, reproducibility, and discriminatory power, they must be performed in several laboratories using a common set of *C. difficile* isolates. Because the 41 isolates we examined came from a hospital outbreak, it should not be surprising that a great number of them fell into the same group.

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Bordetella bronchiseptica in an AIDS Patient Cross-Reacts with *Legionella* Antisera

Previous reports (1, 4) have identified false-positive test results with *Legionella* polyclonal antisera for direct fluorescent-antibody (DFA) staining, due to cross-reactivity of the sera with microorganisms such as *Pseudomonas fluorescens* and *Bordetella pertussis*. We would like to add *Bordetella bronchiseptica* to the list of cross-reacting organisms.

A 41-year-old black male diagnosed with human immunodeficiency virus infection in 1988 was admitted to the Northport Veterans Affairs Medical Center for evaluation of fever, mild cough, and sputum production for approximately 2 weeks. He had a previous history of drug use and herpes zoster but had no other opportunistic infections. The CD4 count was 11. Because of prior sulfonamide intolerance, he was being treated with aerosolized pentamidine as prophylaxis for *Pneumocystis carinii* pneumonia. He received no antiviral medications. His

physical examination showed fine crackles at both lung bases. A chest roentgenogram showed bilateral interstitial infiltrates. Analysis of his sputum showed numerous polymorphs with gram-negative rods, and *B. bronchiseptica* grew on culture. Examination of bronchioalveolar lavage showed both *B. bronchiseptica* and *P. carinii*. *Legionella* DFA staining of the bronchial washings was strongly reactive. No attempt was made to grow *Legionella* spp. in charcoal plates. The patient's clinical condition improved after treatment with both pentamidine and erythromycin.

Because *B. pertussis* has been reported to cross-react with *Legionella* DFA antisera (1, 4), we decided to directly test the patient's *B. bronchiseptica* isolate. A suspension of the culture of *B. bronchiseptica* was prepared to a turbidity of 0.5 McFarland. Several dilutions of the suspension were prepared in