JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1995, p. 3169–3173 Vol. 33, No. 12
0095-1137/95/$04.00+0

Comparison of Arbitrarily Primed PCR with Restriction Endonuclease and Immunoblot Analyses for Typing *Clostridium difficile* Isolates

YAJARAYMA J. TANG,[1] SHERARD T. HOUSTON,[1] PAUL H. GUMERLOCK,[1] MAURY E. MULLIGAN,[2] DALE N. GERDING,[3] STUART JOHNSON,[3] F. ROBERT FEKETY,[4] AND JOSEPH SILVA, JR.[1]*

Cancer and Molecular Research Laboratory, Department of Internal Medicine, University of California, Davis Medical Center, Sacramento,[1] and Veterans Administration Medical Center, Long Beach,[2] California; VA Lakeside Medical Center and Northwestern University Medical School, Chicago, Illinois[3]; and University of Michigan, Ann Arbor, Michigan[4]

Received 20 April 1995/Returned for modification 8 June 1995/Accepted 15 September 1995

Arbitrarily primed PCR (AP-PCR) was used to genotype 26 clinical isolates of *Clostridium difficile* previously analyzed by immunoblotting (IB) and 20 isolates typed by restriction endonuclease analysis (REA) with *Hin*dIII. Two levels of differentiation were achieved with the AP-PCR approach by use of two different arbitrary primers. With the 19-mer arbitrary primer T-7 (first level of differentiation), a good correlation was found between IB and AP-PCR typing. Twenty isolates grouped into six IB types were separated into seven major AP-PCR types. These seven AP-PCR groups were further discriminated into 12 subtypes after genotyping with the arbitrary primer PG-05 (second level of differentiation). The remaining six isolates, all of different IB types, showed a unique and distinct DNA banding pattern with both of the arbitrary primers, T-7 and PG-05. Twenty isolates representing 20 REA types from 15 REA groups were resolved into 13 AP-PCR DNA profiles with the arbitrary primer T-7. A good correlation was found at this level of differentiation between the major REA groups, Y and M, and AP-PCR typing. While AP-PCR with this primer failed to differentiate isolates in REA groups J, G, R, and B, AP-PCR with PG-05 resolved these four isolates into four distinct AP-PCR types. In addition, one of three M strains and one of four Y strains displayed a slightly different DNA banding pattern by AP-PCR (with PG-05) from that of the other strains in the group. We conclude that AP-PCR is a rapid and sensitive method which not only complements other typing schemes but also may be a substitute and prove to be especially suited for immediate epidemiological tracking of nosocomial infections due to *C. difficile.*

Toxigenic *Clostridium difficile* is the leading cause of nosocomial diarrhea. Cross-infection between patients and contamination from environmental surfaces and hospital personnel are considered important factors in the nosocomial spread of this enteropathogen (2, 6, 27). A number of typing schemes have been developed to identify strains of *C. difficile* and to better understand its transmission in hospital settings. Some of these techniques are based on phenotypic characteristics, including bacteriocin and bacteriophage susceptibility, electrophoretic protein patterns, and immunologic markers (8, 21, 26, 29). While these methods are useful, they are often more labor-intensive and less discriminatory than methods targeting genetic markers. Among the latter are restriction endonuclease analysis (REA), ribotyping, pulse-field gel electrophoresis (PFGE), and, recently, arbitrarily primed PCR (AP-PCR). Initial use of these techniques has proven useful in epidemiological studies of *C. difficile* infections (5, 18, 20, 22, 28).

These methods have allowed the characterization of a number of strains of *C. difficile*, and in some cases, epidemiological correlations to outbreaks have been made (1, 24). Recently, Kristjansson et al. (20) found REA and PFGE to be comparable in discriminatory power to and more sensitive than ribotyping for differentiating strains of *C. difficile*. Killgore and Kato (19), comparing AP-PCR with immunoblot (IB) typing for *C. difficile* isolates, found AP-PCR to be more sensitive than IB typing. In a previous study, we reported the use of AP-PCR to genotype serogrouped strains of *C. difficile* as well as patient and environmental isolates from our institution (28). In the latter study, four major AP-PCR groups, some of which contained subgroups, were identified among 20 isolates with the single 15-mer arbitrary primer PG-05. We have since refined our typing system by using a two-level AP-PCR approach with a 19-mer arbitrary primer, T-7, which groups strains into major types (first level of differentiation). In addition, a second arbitrary primer (PG-05) further discriminates strains within a type into subtypes (second level of differentiation). A similar system has been used by Barbut et al. to genotype a limited number of *C. difficile* strains (1). This study described a two-level AP-PCR approach to genotype isolates previously typed by IB or analyzed by their restriction enzyme digestion pattern. The discriminatory power and reproducibility of AP-PCR is compared with that of IB and REA typing.

* Corresponding author. Mailing address: Department of Internal Medicine, University of California, Davis Medical Center, 2315 Stockton Blvd., Sacramento, CA 95817. Phone: (916) 734-5356. Fax: (916) 734-7766.

MATERIALS AND METHODS

***C. difficile* strains.** Forty-six isolates of *C. difficile* were studied. Twenty-six of these isolates had been typed previously by IB (21); the other 20 isolates had been analyzed by REA (5). We included in this analysis a diverse group of isolates, most of which were from hospital epidemiological studies or from hospital surveillance of *C. difficile* diarrhea cases at the Minneapolis Veterans Administration Medical Center. The isolates represented both asymptomatic carriers and diarrhea patients. Two of the strains in REA group B were outbreak strains. Table 1 lists a number of these isolates and their characteristics.

Oligonucleotides used as AP-PCR primers. The arbitrary primers used in this study were T7, a 19-mer (5′GTAATACGACTCACTATAG3′) which grouped strains into major types, and PG-05, a 15-mer (5′AGCCCAGCTATGAAC3′) used to discriminate isolates in major types into subtypes (15). These primers

TABLE 1. Molecular profiles of selected *C. difficile* isolates genotyped by AP-PCR with the arbitrary primer T-7

Isolate	Toxigenicity	IB type	REA group	AP-PCR (T-7) type
Y (1, 2, 4, 6)	+	ND	Y	1
WA 197, KY 17	+	1	ND[a]	1
WA 182, WA 148				
WA 171, WA 105				
WA 40, WA 37	−	10B	ND	2
WA 150				
KY 28, WA 114	+	7B	ND	4
WA 146, WA 50	−	7C	ND	5
WA 84, WA 74	+	18	ND	6
PC 406, PC 997	+	10C	ND	7
L1	+	ND	L	8
M (1, 3, 4)	−	ND	M	9
J1, G1	+	ND	J, G	10
E1	+	ND	E	11
N1	+	ND	N	12
Z1	+	ND	Z	13
BM1	+	ND	BM	14
T1	−	ND	T	15
WA 114	+	4	ND	16
B1, R1	+	ND	R, B	17
AL1	+	ND	AL	18
BD1	+	ND	BD	19

[a] ND, not done.

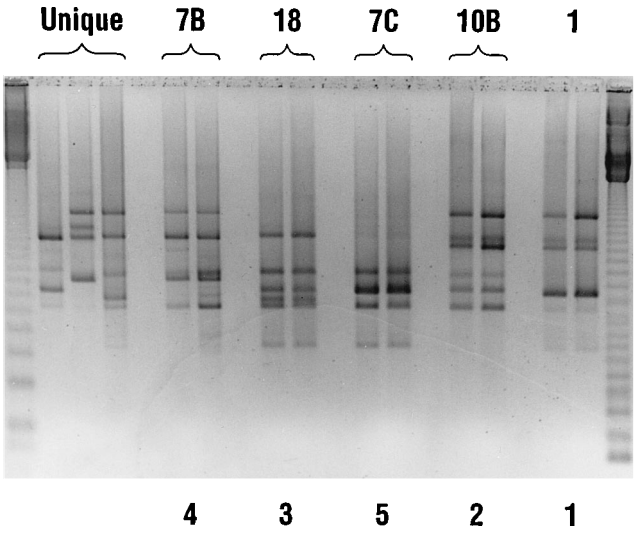

FIG. 1. Genotyping of IB-typed *C. difficile* isolates by AP-PCR. A negative print of an ethidium bromide-stained 1.5% agarose gel of DNA patterns generated by AP-PCR with the arbitrary primer T-7 is shown. Outer lanes, 123-bp DNA marker ladder. IB types are indicated by the numbers given at the top of the lanes. AP-PCR types are given by the numbers at the bottom of the lanes. Unique types had unique and different DNA banding patterns by AP-PCR (T-7) from those of all other isolates analyzed.

were obtained from a commercial source (Integrated DNA Technologies, Coraville, Iowa).

IB analysis. IB was based on the method for enzyme-linked immunoelectrotransfer blotting described by Tsang et al. (31). Bacterial cell proteins from various strains and isolates were extracted with EDTA and used as the recognition antigens following gel electrophoresis and blotting. Pooled human sera (from 100 patients) were used as the primary antibodies. Goat anti-human immunoglobulin G (γ chain) conjugated with horseradish peroxidase was used as the secondary antibody. The blotting and detection procedure used was described previously (23).

Restriction endonuclease digestion. Whole-cell DNA was prepared as described previously (5). Briefly, a single *C. difficile* colony from a 48-h anaerobic blood agar plate was inoculated into 10 ml of reduced brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and grown anaerobically for 18 h at 35°C. A modification of the method of Cleary et al. was used for extracting DNA (7). Briefly, approximately 3 to 5 μg of DNA obtained by the GES method was incubated with 20 U of *Hin*dIII restriction endonuclease and digested as described in the manufacturer's instructions (Promega, Madison, Wis.) with the addition of 1 μl of a 50-mg/ml concentration of bovine serum albumin (Gibco-BRL, Gaithersburg, Md.) and 3 μl of a 100-μg/μl concentration of spermidine (Sigma Chemical Co., St. Louis, Mo.) to each reaction mixture. The restricted DNA fragments were separated in an 0.7% agarose gel (FMC Bioproducts) by electrophoresis in Tris-acetate buffer (40 mM Tris, 20 mM acetate, 2 mM EDTA [pH 7.9], with glacial acetic acid) at 50 V for 16 h. Gels were stained for 30 min in 2 μg of ethidium bromide per ml, visualized by UV transillumination, and photographed. The restricted DNA pattern for each isolate was compared with that of all known types. Isolates were designated as distinct types if the DNA band patterns were unique (number designation) and grouped (letter designation) if the band pattern displayed a ≥90% similarity (5). For example, types M1, M3, and M4 each display unique band patterns but are all designated as belonging to the M group because of ≥90% similarity of band patterns.

AP-PCR methodology. AP-PCR was performed as described previously (28). DNA was extracted from the cultures by scraping a single colony from each isolate with an inoculating loop into a 0.6-ml microcentrifuge tube containing 100 μl of sterile water. After boiling for 10 min, the sample was centrifuged at 5,000 × *g* for 3 min to remove cell debris, and the DNA in the supernatant was quantitated as described previously (28). PCRs were prepared in PCR buffer (50 mM KCl, 20 mM Tris-HCl, 2.5 mM $MgCl_2$, 100 μg of bovine serum albumin per ml [pH 8.4]) containing 40 pmol of the respective arbitrary primer, 1 mM each deoxynucleoside triphosphate, 2 U of recombinant thermostable DNA polymerase (r*Taq*; Perkin-Elmer Cetus, Norwalk, Conn.), and 1 μg of DNA. The reaction mixtures were covered with 150 μl of light mineral oil (Sigma). The PCR profile was initiated with two cycles of low stringency, which included a denaturing step at 95°C for 1 min, followed by annealing of the primer at 26°C for 1 min, with an extension for 2 min at 72°C. After the initial 2 cycles, annealing of the arbitrary primer was done at 50°C for the subsequent 55 cycles. To ensure reproducibility, all amplifications were done in duplicate and on DNA extracted on different days. All strains were analyzed with both of the primers, T-7 and PG-05. Amplification products were visualized by running 12 μl of the reaction mixture in a 1.5% agarose gel (Life Technologies, GIBCO BRL, Grand Island, N.Y.) in Tris-borate-EDTA buffer for 70 min at 110 V. A 123-bp DNA ladder was included as a size marker in all gels. Gels were stained in a 0.5-μg/ml ethidium bromide solution for 30 min, destained for 30 min, and photographed under UV light with a Polaroid Land camera.

Analysis of DNA banding patterns. The DNA profile of each isolate was compared with the DNA profile of each other previously typed isolate by running the amplified products simultaneously on the same gel. Scanning densitometry was then used, after visualization of the DNA banding patterns, to confirm the assignation of the strains to AP-PCR types. For this purpose, a negative of each gel was scanned through a laser densitometer (LKB, Pharmacia) with the computer program GSXL. The peaks produced by the DNA banding patterns of each isolate were compared with the scanning pattern of each other isolate. In addition, for those isolates which differed by more than one band, the Dice similarity coefficient analysis was used to determine relatedness, arbitrarily setting 85% similarity as the cutoff point (9) for AP-PCR types. This similarity coefficient has frequently been used by others as the cutoff point for determining relatedness of isolates (17) and thus was selected in this study.

Amplification of toxin A and B genes. The primers and conditions for amplification of the toxin A and B genes have been described elsewhere (16, 30).

RESULTS

AP-PCR and IB analysis. AP-PCR with the arbitrary primer T-7 separated 26 isolates assigned to six different IB types into seven major AP-PCR types as shown in Table 1. A near-complete correlation in typing between IB and AP-PCR was found at this level. In general, strains in the same IB type showed the same DNA banding pattern by AP-PCR, with the exception of isolates in IB type 1, which were separated by AP-PCR into two groups. Strains genotyped with the primer T-7 were assigned to major types (Table 1). Strains within these types were discriminated at the second level with the arbitrary primer PG-05. Twelve subtypes were identified with this primer. The DNA banding patterns generated with this primer showed some unique bands in addition to displaying the major common bands of the group. Figure 1 shows the AP-PCR profiles of selected IB typed isolates with the primer T-7. Strains in unique IB types were also unique by AP-PCR (Table 1).

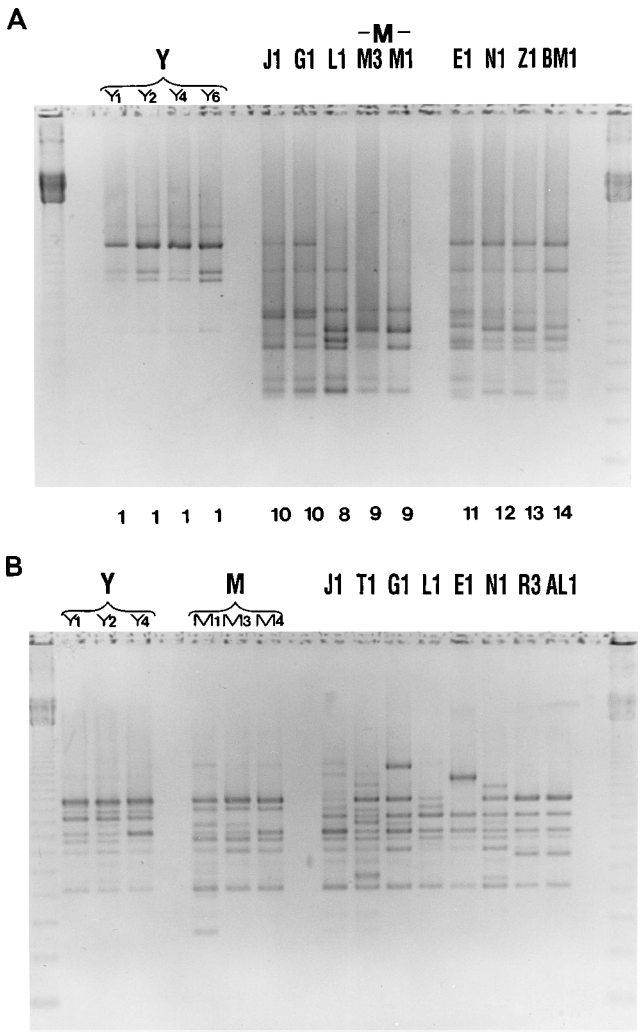

FIG. 2. Genotyping of REA-typed *C. difficile* isolates by AP-PCR with the arbitrary primers T-7 (A) and PG-05 (B). Negative prints of ethidium bromide-stained 1.5% agarose gels of DNA patterns are shown. Outer lanes, 123-bp DNA marker ladder. REA groups and types are indicated by the letters at the top of the lanes. (A) AP-PCR types are indicated by the numbers at bottom of lanes (see Table 1). (B) AP-PCR types were unique for all isolates of a particular REA group. Isolates Y4 and M4 displayed a DNA banding pattern slightly different from that of the other isolates in these groups.

AP-PCR and REA. Genotyping *C. difficile* by AP-PCR with the primer T-7 had a slightly less discriminatory power than REA typing. Fifteen REA groups were resolved into 13 AP-PCR types. A good correlation was found between the major REA groups (Y and M) and our AP-PCR typing with T-7 (Fig. 2A); however, AP-PCR failed to discriminate the J and G groups and the B and R REA groups and did not differentiate M and Y types within these groups. A second level of typing with PG-05 yielded a much higher level of discrimination. Isolates G1, J1, R1, and B1 were all resolved into four different AP-PCR types; in addition, one isolate in the Y group and one isolate in the M group displayed a DNA banding pattern slightly different from that of the other isolates in these groups (Fig. 2B). However, by REA, each isolate within the Y and M REA groups displayed minor but reproducible band differences representing unique REA types. All strains in unique REA groups displayed unique DNA banding patterns by AP-PCR with the PG-05 primer. In the analysis of these isolates, we did not find any case in which the T-7 primer further discriminated isolates grouped in the same AP-PCR type with PG-05.

As indicated in Materials and Methods, the Dice coefficient was used to group strains by percentage of similarity. In this case, the isolates compared shared all the major bands but differed in that they either lack or had one or more minor (light) bands.

DISCUSSION

AP-PCR has been used to type a number of bacterial species (3, 12, 32). McMillin and Muldrow first reported the use of AP-PCR to generate DNA banding patterns from *C. difficile* (22). This technique has also been used by Barbut et al. (1) to study an outbreak of *C. difficile* diarrhea in AIDS patients. A number of studies have correlated AP-PCR to other typing systems. AP-PCR has been compared with ribotyping in typing strains of *Legionella pneumophila* (14) and with PFGE in typing strains of *Staphylococcus aureus* (25) and *Stenotrophomonas maltophilia* (33). With regards to *C. difficile* strain typing, Killgore and Kato (19) applying AP-PCR analyzed 41 strains typed by IB and which were isolated from patients during a *C. difficile* colitis outbreak. These investigators found AP-PCR to be comparable in discriminative power to IB, and in addition, AP-PCR discriminated seven individual isolates which were untypeable by IB.

In our study, a two-level AP-PCR technique was used to type strains analyzed by IB and REA. This two-primer AP-PCR approach provided a relative increase in discriminatory power when compared with IB and AP-PCR typing with a single arbitrary primer. At the first level of differentiation, AP-PCR and IB typing correlated closely. These results are consistent with the study of Killgore and Kato (19) comparing AP-PCR with IB analysis. However, AP-PCR typing at this first level was less discriminatory than REA. At the second level of differentiation, the REA types that were grouped in the same AP-PCR type with the first (T-7) arbitrary primer (J1, G1, R1, and B1) resolved into separate types with the PG-05 primer. It is interesting that REA types G1 and J1, which were identified as the same type by AP-PCR with the primer T-7, have the same ribotyping pattern (I) (5). At the second level of differentiation, in some cases, strains in the same REA group were further resolved into subtypes. Whether these AP-PCR subtype strains are different strains or represent strain heterogeneity within a single type is not known; however, it is known that the REA type is stable and highly reproducible with repeated typing. Because of the good correlation between T-7 AP-PCR types and the major groups as defined by REA and IB, this primer has value for typing by itself. However, the clinical significance of typing with either oligonucleotide (T-7 or PG-05) remains unknown. It is possible that virulence may correlate better to the major groups as identified with the T-7 primer. Typing with PG-05 leads to unique DNA fingerprinting in most cases. These minor differences in DNA banding patterns may not be significant enough to consider these strains as different types; however, further clinical correlations may reveal their importance.

Not all of the IB-typed strains analyzed in this study have been typed by REA. Although strains in IB type 1 and REA group Y were grouped in AP-PCR type 1 with the T-7 primer, the DNA banding pattern obtained with the arbitrary primer PG-05 was different for IB type 1 and REA group Y.

As was observed with REA typing, genotyping with both oligonucleotides correlated with the toxigenicity of isolates. All types within an REA group are either toxin positive or toxin

negative, and the same observation was found with AP-PCR typing.

Kristjansson et al. (20) found the discriminatory power of REA and PFGE to be comparable for typing strains of *C. difficile*; however, REA was more sensitive than ribotyping in differentiating strains. In a recent study, Chachaty et al. (4) compared ribotyping with PFGE and AP-PCR in differentiating between 30 unrelated strains of *C. difficile*. The three methods showed a good correlation for clustering of strains; however, PFGE was slightly more discriminatory than AP-PCR, with three different primers used in their study. PFGE, however, is labor-intensive and requires specialized equipment not available to all clinical laboratories (20).

The selection of the two oligonucleotide primers used here was based on the reproducibility of the DNA banding patterns, the ability to type all of the strains, and the number of bands obtained (in general, with the T-7 primer, the DNA profile contained 6 to 10 bands, while with the primer PG-05, the number of bands observed was usually 7 to 16). We also tested three other arbitrary primers (10- to 12-mers), which resulted in either no reproducible banding pattern, a small number of bands (which we consider insufficient for an accurate typing system), or no bands at all. Because of the inconclusive results and poor reproducibility obtained with these three primers, they were not used in this study. Although we made no attempt to use primers with a GC content matching that of the *C. difficile* genome, both primers used in this study (T-7 and PG-05) provided a DNA banding pattern which was reproducible and adequate for analysis. As has been reported by others (11), some of the drawbacks of AP-PCR are its sensitivities to changes in primer concentration, DNA concentration, brand of thermal cycler used, and *Taq* polymerase. The DNA banding pattern obtained from all 46 strains was reproducible from day to day with both of the primers, T-7 and PG-05. The assignation of isolates to AP-PCR types not only was based on visual inspections of the DNA banding patterns in the gels but was determined by laser scanning densitometry. This allowed us to objectively discriminate true bands from background bands. The number of AP-PCR types obtained by visualization of the gels and by scanning densitometry showed 100% correlation (data not shown). Scanning densitometry has been used extensively to analyze protein patterns of polyacrylamide gel electrophoresis analysis and has proved to be useful in analyzing complex banding patterns (10). The use of similarity coefficient analysis for strains in which the DNA banding patterns differed slightly allowed us to separate the strains into distinct types when the isolates showed less than 85% similarity.

An important issue to be considered in the interpretation of DNA banding patterns is the criterion used by the investigators to score the DNA banding patterns. As has been the experience of others (1, 13, 19), we observed the presence of faint amplification products in some of the reactions when either primer was used. Even though these bands were reproducible in most cases, we chose not to include them when scoring the DNA banding pattern. Inclusion of these bands would have resulted in a larger number of subtypes being identified, making the system unnecessarily complex.

In summary, AP-PCR typing is a reproducible, rapid, and simple technique. Like the other typing methods (REA and IB), it results in a sensitive and accurate identification of *C. difficile* strains. The applicability of these typing methods to a clinical laboratory is dependent on their economic feasibility. AP-PCR can be performed in 36 to 48 h compared with the 3 to 4 days it takes to carry out some of the other typing methods; thus, it can be considered a cost-effective method for determining the source of hospital outbreaks. (The approximate cost of duplicate reactions with both primers would likely be in the range of $150 to $200 per specimen.) Thus, the primarily practical advantage of AP-PCR is the greater rapidity of this procedure compared with that of other molecular typing methods.

ACKNOWLEDGMENT

We thank Gloria Marquez for excellent typing of the manuscript.

REFERENCES

1. **Barbut, F., N. Mario, J. Frottier, and J. C. Petit.** 1993. Use of the arbitrarily-primed polymerase chain reaction for investigating an outbreak of *Clostridium difficile*-associated diarrhea in AIDS patients. Eur. J. Clin. Microbiol. Infect. Dis. **12:**794–795.
2. **Barlett, J. G.** 1990. *Clostridium difficile*: clinical considerations. Rev. Infect. Dis. **12**(Suppl. 2)**:**S192–S199.
3. **Bingen, E., C. Boissinot, P. Desjardins, H. Cave, N. Brahinai, N. Lambert-Zechovsky, E. Denamur, P. Blot, and J. Elion.** 1993. Arbitrarily primed polymerase chain reaction provides rapid differentiation of *Proteus mirabilis* isolates from a pediatric hospital. J. Clin. Microbiol. **31:**1055–1059.
4. **Chachaty, E., P. Saulnier, A. Martin, N. Mario, and A. Andremont.** 1994. Comparison of ribotyping, pulsed field-gel electrophoresis and random amplified polymorphic DNA for typing *Clostridium difficile* strains. FEMS Microbiol. Lett. **122:**61–68.
5. **Clabots, C. R., S. Johnson, K. M. Bettin, P. A. Mathie, M. E. Mulligan, D. R. Schaberg, L. R. Peterson, and D. N. Gerding.** 1993. Development of a rapid and efficient restriction endonuclease analysis typing system for *Clostridium difficile* and correlation with other typing systems. J. Clin. Microbiol. **31:** 1870–1875.
6. **Clabots, C. R., S. Johnson, M. M. Olson, L. R. Peterson, and D. N. Gerding.** 1992. Acquisition of *Clostridium difficile* by hospitalized patients: evidence for colonized new admissions as a source of infection. J. Infect. Dis. **166:** 561–567.
7. **Cleary, P. P., E. L. Kaplan, C. Livdahl, and S. Skjold.** 1988. DNA fingerprints of *Streptococcus pyogenes* are M type specific. J. Infect. Dis. **158:**1317–1323.
8. **Delmee, M., Y. Laroche, B. Ayesani, and G. Cornelis.** 1986. Comparison of serogrouping and polyacrylamide gel electrophoresis for typing *Clostridium difficile*. J. Clin. Microbiol. **24:**991–994.
9. **Dice, L. R.** 1945. Measures of the amount of ecological association between species. Ecology **26:**297–302.
10. **Ehret, W., M. Turba, P. Pfaller, W. Heizmann, and G. Ruckdeschel.** 1988. Computer-aided densitometric analysis of protein patterns of *Clostridium difficile*. Eur. J. Microbiol. Infect. Dis. **7:**285–290.
11. **Ellsworth, D. L., K. D. Rittenhouse, and R. L. Honeycutt.** 1993. Artifactual variation in randomly amplified polymorphic DNA banding patterns. Biotechniques **14:**214–217.
12. **Fang, F. C., M. McClelland, D. G. Guiney, et al.** 1993. Values of molecular epidemiologic analysis in a nosocomial methicillin-resistant *Staphylococcus aureus* outbreak. JAMA **270:**1323–1328.
13. **Fekete, A., J. A. Bantle, S. M. Halling, and R. W. Stich.** 1992. Amplification fragment length polymorphism in *Brucella* strains by use of polymerase chain reaction with arbitrary primers. J. Bacteriol. **174:**7778–7783.
14. **Gomez-Lus, P., B. S. Fields, R. F. Benson, W. T. Martin, S. P. O'Connor, and C. M. Black.** 1993. Comparison of arbitrarily primed polymerase chain reaction, ribotyping, and monoclonal antibody analysis for subtyping *Legionella pneumophila* serogroup 1. J. Clin. Microbiol. **31:**1940–1942.
15. **Gumerlock, P. H., Y. J. Tang, M. E. Mulligan, and J. Silva, Jr.** 1994. *Clostridium difficile*: correlation of genotyping and immunoblot analysis. (Fourth Annual Meeting for the Society of Hospital Epidemiology of America.) Infect. Control Hosp. Epidemiol. **15**(Suppl.)**:**16.
16. **Gumerlock, P. H., Y. J. Tang, J. B. Weiss, and J. Silva, Jr.** 1993. Specific detection of toxigenic strains of *Clostridium difficile* in stool specimens. J. Clin. Microbiol. **31:**507–511.
17. **Herwaldt, L. A., J. Pottinger, S. Coffman, M. J. Jaqua-Stewart, and J. Tjaden.** 1994. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage. (Fourth Annual Meeting for the Society of Hospital Epidemiology of America.) Infect. Control Hosp. Epidemiol. **15**(Suppl.)**:**S20.
18. **Kato, H., N. Kato, K. Watanabe, K. Ueno, H. Ushijima, S. Hashira, and T. Abe.** 1994. Application of typing by pulsed-field gel electrophoresis to the study of *Clostridium difficile* in a neonatal intensive care unit. J. Clin. Microbiol. **32:**2067–2070.
19. **Killgore, G. E., and H. Kato.** 1994. Use of arbitrary primer PCR to type *Clostridium difficile* and comparison of results with those by immunoblot typing. J. Clin. Microbiol. **32:**1591–1593.
20. **Kristjansson, M., M. H. Samore, D. N. Gerding, P. C. DeGirolami, K. M. Bettin, A. W. Karchmer, and R. D. Arbeit.** 1994. Comparison of restriction endonuclease analysis, ribotyping, and pulsed-field gel electrophoresis for

molecular differentiation of *Clostridium difficile* strains. J. Clin. Microbiol. **32:**1963–1969.
21. **McFarland, L. V., G. W. Elmer, W. E. Stamm, and M. E. Mulligan.** 1991. Correlation of immunoblot type, enterotoxin production, and cytotoxin production with clinical manifestations of *Clostridium difficile* infection in a cohort of hospitalized patients. Infect. Immun. **59:**2456–2462.
22. **McMillin, D. E., and L. L. Muldrow.** 1992. Typing of toxigenic strains of *Clostridium difficile* using DNA fingerprints generated with arbitrary polymerase chain reaction primers. FEMS Microbiol. Lett. **79:**5–10.
23. **Mulligan, M. E., L. R. Peterson, R. Y. Kwok, C. R. Clabots, and D. N. Gerding.** 1988. Immunoblots and plasmid fingerprints compared with serotyping and polyacrylamide gel electrophoresis for typing *Clostridium difficile*. J. Clin. Microbiol. **26:**41–46.
24. **Samore, M. H., K. M. Bettin, P. C. DeGirolami, C. R. Clabots, D. N. Gerding, and A. W. Karchmer.** 1994. Wide diversity of *Clostridium difficile* types at a tertiary referral hospital. J. Infect. Dis. **170:**615–621.
25. **Saulnier, P., C. Bourneik, G. Prevost, and A. Andremont.** 1993. Random amplified polymorphic DNA assay is less discriminant than pulsed-field gel electrophoresis for typing strains of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **31:**982–985.
26. **Sell, T. L., D. R. Schaberg, and R. Fekety.** 1983. Bacteriophage and bacteriocin typing scheme for *Clostridium difficile*. J. Clin. Microbiol. **17:**148–152.
27. **Silva, J., Jr., and C. Iezzi.** 1988. *Clostridium difficile* as a nosocomial pathogen. J. Hosp. Infect. **11**(Suppl. A)**:**378–385.
28. **Silva, J., Jr., Y. J. Tang, and P. H. Gumerlock.** 1994. Genotyping of *Clostridium difficile* isolates. J. Infect. Dis. **169:**661–664.
29. **Tabaqchali, S.** 1990. Epidemiological markers of *Clostridium difficile*. Rev. Infect. Dis. **12**(Suppl.) **2:** S192–S199.
30. **Tang, Y. J., P. H. Gumerlock, J. B. Weiss, and J. Silva, Jr.** 1994. Specific detection of *Clostridium difficile* toxin A gene sequences in clinical isolates. Mol. Cell. Probes **8:**463–467.
31. **Tsang, V. C., J. M. Peratta, and A. R. Simons.** 1983. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificity of antigens and antibodies separated by gel electrophoresis. Methods Enzymol. **92:**377–391.
32. **vanBelkum, A., M. Struelens, and W. Quint.** 1993. Typing of *Legionella pneumophila* strains by polymerase chain reaction-mediated DNA fingerprinting. J. Clin. Microbiol. **31:**2198–2200.
33. **VanCouwenberghe, C. J., S. H. Cohen, Y. J. Tang, P. H. Gumerlock, and J. Silva, Jr.** 1995. Genomic fingerprinting of epidemic and endemic strains of *Stenotrophomonas maltophilia* (formerly *Xanthomonas maltophilia*) by arbitrarily primed PCR. J. Clin. Microbiol. **33:**1289–1291.