JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1995, p. 3083–3086 Vol. 33, No. 12
0095-1137/95/$04.00+0

Analysis of *Bordetella pertussis* Isolates from an Epidemic by Pulsed-Field Gel Electrophoresis

BERNARD BEALL,* PAMELA K. CASSIDAY, AND GARY N. SANDEN

Childhood and Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333

Received 12 April 1995/Returned for modification 15 June 1995/Accepted 29 August 1995

We examined genetic variation among 78 clinical isolates of *Bordetella pertussis*, including 54 strains recovered during a 1986 pertussis epidemic. A total of 16 pulsed-field gel electrophoresis (PFGE) profiles, generated with each of three different enzymes (*Xba*I, *Spe*I, and *Dra*I), were obtained from the epidemic and sporadic isolates included in the study. Indistinguishable profiles were seen among strains unrelated temporally or geographically, as well as among strains isolated sporadically from the same geographic areas. All isolates from the epidemic had indistinguishable PFGE profiles. The PFGE pattern of the epidemic strains was shared with only 1 of 25 strains isolated independently of the outbreak. This isolate was cultured from a specimen from a laboratory scientist who had been working with the epidemic strains, further implicating the usefulness of PFGE for the epidemiologic study of clinical strains of *B. pertussis*. Differences in PFGE profiles for single epidemic strains occurred occasionally upon repeated passage on agar medium, suggesting that subculturing of initial isolates should be minimized before pulsed-field analysis.

Pulsed-field gel electrophoresis (PFGE) has been used to construct a restriction map of the *Bordetella pertussis* chromosome (8), as it has for many other bacterial species. Previous studies also demonstrated that PFGE of chromosomal DNA digested with *Xba*I was useful in subtyping clinical isolates of *B. pertussis* (2–4). DNA fingerprints of individual *B. pertussis* strains were shown to be reproducible and stable with repeated subculture (4). In contrast, serology-based methods and multilocus enzyme electrophoresis failed to adequately discriminate strains for epidemiologic study (3). It has also been reported that *B. pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* were not differentiated by electrophoresis of chromosomal DNA fragments digested with the frequent cutter *Eco*RI (3). Using the same approach, we have observed that the frequent cutters *Pst*I, *Sal*I, and *Eco*RV distinguished between *Bordetella* species but not between *B. pertussis* strains. Similarly, we have found that random amplification with arbitrary primers distinguished between *Bordetella* species but failed to differentiate between epidemiologically unassociated *B. pertussis* strains distinguishable by PFGE (7).

In the study described here we examined 54 strains isolated from individual patients during a whooping cough outbreak that occurred in 1986 (1, 6) and 24 additional isolates from temporally or geographically independent patients. We have extended the use of PFGE as a useful epidemiologic tool for studying *B. pertussis* by using two rare-cutting enzymes in addition to *Xba*I: *Dra*I and *Spe*I.

MATERIALS AND METHODS

Bacterial strains. *B. pertussis* strains were acquired during a pertussis outbreak of 270 reported cases in Kent County, Delaware, during March through May of 1986 among members of a partially vaccinated rural Amish community of about 2,000 people (1). Fifty-eight of the reported cases (21%) were confirmed by culture (1). We used 54 of the strains from patients with confirmed cases of pertussis for the present study. Also, strains were isolated from patients with sporadic cases of pertussis and were sent to the Centers for Disease Control and Prevention for additional evaluations or were derived from independent collections. All strains isolated at the Centers for Disease Control and Prevention were cultured from nasopharyngeal swabs on Regan Lowe agar at 37°C (6) and were passed on this medium no more than four times after primary isolation.

Restriction endonuclease digestion and PFGE of chromosomal DNA. Unless indicated otherwise, plugs were prepared from stocks after 3 days of growth on Regan Lowe agar. The restriction enzymes *Xba*I, *Dra*I, and *Spe*I were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. DNA plugs of about 70 μl in volume were prepared in low-melting-point agarose as described previously (5). Plugs were suspended in 100 μl containing the supplied restriction enzyme buffer and 20 U of restriction enzyme. The restriction digests were incubated overnight at 37°C before loading the plugs onto a 1% pulsed-field agarose gel for PFGE with a CHEF-DRII (Bio-Rad, Melville, N.Y.) apparatus. Gels were run at 200 V, with the switch time ramped from 5 to 45 s over a 27-h period at 14°C, and photographed. The bacteriophage lambda ladder size standard was a preparation of phage lambda concatemers of 48.5 kb (New England Biolabs, Beverly, Mass.). For the purpose of the present study, any PFGE profile indicating a unique restriction enzyme cleavage pattern constituted a PFGE type.

RESULTS

The PFGE types of the study strains are given in Table 1. A total of 16 PFGE patterns were identified among the 78 clinical isolates examined. The *Xba*I PFGE types of all 54 of the epidemic strains were indistinguishable, and many are shown in Fig. 1A to C. The respective *Dra*I (Fig. 1A to C and Fig. 2A, lanes a to j) and *Spe*I (Fig. 2B, lanes m and n) profiles of these strains were also indistinguishable. The profiles of the epidemic strains with each of the three restriction enzymes were different from the respective patterns of 23 of the 24 strains isolated from diverse geographic areas (Fig. 2A and 2B). Although strain 73 (Fig. 2B, lanes j, s, and 3) was recovered from a patient who was geographically and temporally segregated from the outbreak, it produced PFGE profiles indistinguishable from the twice-passed epidemic isolates, regardless of the enzyme used (Fig. 2B, lanes d and e, m and n, and w and x, show the *Dra*I, *Spe*I, and *Xba*I patterns, respectively; the *Xba*I profile of strain 73 is also shown in Fig. 2A, lane q).

Fifteen PFGE types were obtained with the 24 sporadic isolates included in the study (Table 1). Seven of the 10 sporadic isolates shown in Fig. 2A and B had unique profiles with each of the three restriction enzymes used. Strains 74 and 75 showed indistinguishable profiles with each of the three en-

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Mailstop C02, Atlanta, GA 30333. Fax: (404) 639-3123. Electronic mail address: beb0@CIDDBD2.CDC.GOV.

TABLE 1. Culture site and PFGE type of test strains

Strain no.	PFGE type	Culture site	Mo/yr of isolation
12	1	Wisconsin	11/85
14-67	2	Delaware	3-5/86
68	3	ATCC type strain[a]	
73	2	Georgia (laboratory scientist)	10/86
74	4	Derivative of strain from Kendrick-Eldering collection	ca. 9/49
75	4	Hawaii	7/87
80	5	Arizona	8/87
82	6	Alaska	5/88
83	7	Maryland	6/88
84	8	Senegal	4/88
85	8	Senegal	4/88
108	9	Missouri	9/89
115	9	Missouri	5/90
122	10	Missouri	9/89
130	11	Missouri	6/89
139	12	Missouri	7/89
IH28	13	New York	3/94
143, 146	9	Senegal	8/90
154, 156	9	Senegal	4-5/92
163, 165	14	Massachusetts	12/92
059	15	New Hampshire	1/94
200	16	New York	5/90

[a] ATCC, American Type Culture Collection.

zymes (Fig. 2B, lanes h and i, q and r, and 1 and 2, show the results obtained with *Dra*I, *Spe*I, and *Xba*I, respectively), even though these strains were separated geographically.

We examined the effects of repeated subculture and extended incubation periods on the reproducibilities of the PFGE patterns of several epidemic strains. The respective profiles generated by each of three restriction enzymes were identical for each epidemic strain subcultured twice over a period of 6 days (Fig. 2B, lanes d, e, m, n, w, and x). No differences in PFGE patterns were observed whether DNA from each of four epidemic isolates was prepared after a single 2-week incubation (Fig. 3B, lanes g, m, s, and y) or after approximately six subcultures over 12 days (Fig. 3B, lanes a to f, h to o, and n to r; faint nonrepeatable differences in banding patterns are seen in lanes n and o and are disregarded). However, we found that the PFGE patterns determined with DNA from three of four epidemic strains after subculturing every 3 to 4 days for 1.5 months (Fig. 3A, lanes g, m, y, y′, and y″) differed from the respective patterns generated from the same isolates similarly subcultured five times over 10 days (Fig. 3A, lanes a to e, n to r, and t to x). A subsequent experiment showed that the PFGE pattern of one (strain 17) of the four strains lost a 290-kb *Xba*I fragment and gained a 415-kb fragment after seven subcultures within 1 month (Fig. 3B, compare lanes t, u, and v with lanes w, x, and y). This observation was reinforced by variations observed among *Spe*I restriction fragments of 290 to 485 kb generated from DNA prepared from strain 17 after two subcultures, approximately 15 passages over 1.5 months, or 9 passages over 25 days (Fig. 2A, lanes w, x, and y, respectively). Similar variations were observed with *Dra*I and *Xba*I (data not shown).

The benefit of comparing the profiles determined by each of the three restriction enzymes was evident with some strains. For example, the *Spe*I profiles shared by strains 74 and 75 (Fig. 2B lanes q and r, respectively) were very similar to the pattern for isolate 82 (Fig. 2B, lane p). The two PFGE types were differentiated only by a slightly smaller fragment in the pattern for strains 74 and 75 corresponding to the 440-kb cleavage product observed in the profile for isolate 82. However, the respective *Xba*I (Fig. 2B, lanes 1, 2, and z) and *Dra*I (Fig. 2B, lanes g, h, and i) profiles readily differentiated isolate 82 from isolates 74 and 75. In another instance, the *Xba*I pattern of one isolate from Missouri (Fig. 4, lane g) differed from the profile shared by four West African strains (Fig. 4, lanes d, e, and f) only in having a doublet rather than a single fragment of about 125 kb. In contrast, the *Dra*I profile of the Missouri strain (Fig. 4, lane c) lacked a 280-kb fragment present in the West African PFGE type (Fig. 4, lanes a and b).

Additional enzyme profiles were also useful for substantiating slight differences between profiles associated with the subculture routine, as in the case of the *Dra*I profiles of strain 17.

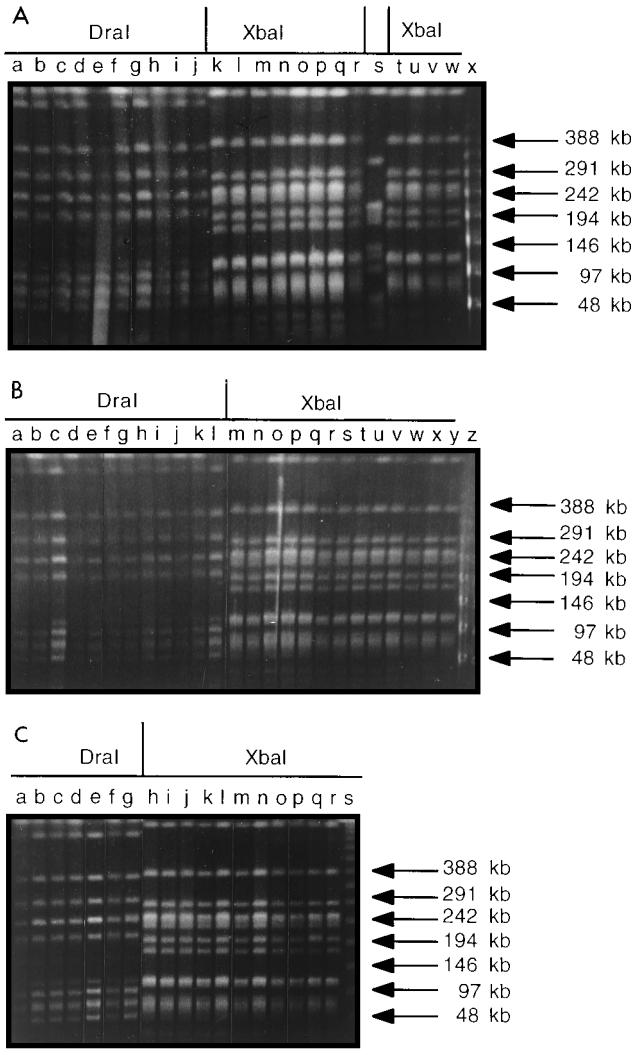

FIG. 1. PFGE profiles of DNAs from isolates involved in the Delaware pertussis epidemic digested with two different restriction enzymes. (A) Lanes a to j, *Dra*I PFGE profiles of strains 42, 40, 38, 37, 36, 35, 32, 31, 30, and 29, respectively; lane s, *Xba*I PFGE profile of a *Corynebacterium* isolate originally misidentified as a *B. pertussis* strain; lanes k to r, *Xba*I profiles of strains 43, 42, 39, 38, 37, 36, 35, and 34 respectively; lanes t to w, *Xba*I profiles of 32, 31, 30, and 29, respectively; lane x, size markers. (B) Lanes a to l, *Dra*I PFGE profiles of strains 56, 55, 54, 53, 52, 50, 48, 47, 46, 45, 44, and 49, respectively; lanes m to y, *Xba*I profiles of strains 49, 56, 55, 54, 53, 52, 51, 50, 48, 47, 46, 45, and 44, respectively; lane z, size markers. (C) Lanes a to g, *Dra*I PFGE profiles of strains 66, 65, 64, 63, 60, 57, and 16, respectively; lanes h to r, *Xba*I profiles of strains 67, 66, 65, 64, 63, 61, 60, 59, 57, 16, and 14 respectively; lane s, size markers.

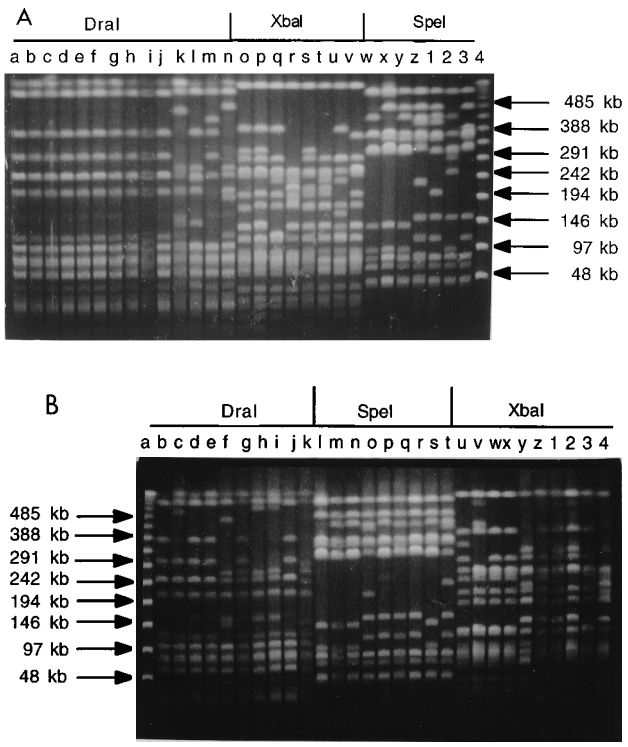

FIG. 2. Comparison of PFGE profiles of Delaware isolates with the same isolates passed multiple times and with 10 isolates from patients with sporadic cases of pertussis. (A) Lanes a to j, *Dra*I profiles of Delaware epidemic strains 56, 55, 51, 24, 23, 22, 21, 19, 18, and 17, respectively; lanes k to n, *Dra*I profiles of sporadic case strains 200, 122, 059, and IH28, respectively; lanes o to v, *Xba*I profiles of sporadic case strains 75, 74, 73, 12, 200, 122, 059, and IH28, respectively; lane w, *Spe*I profile of epidemic strain 17 after multiple passages over 1.5 months; lanes x and y, *Spe*I profiles of strain 17 after the 10th and 2nd passages, respectively, on Regan Lowe medium; lanes 1 to 3, *Spe*I profiles of strains 200, 122, and 059, respectively; lane 4, DNA size standards. (B) Lane a, size standards; lanes b, c, and d, *Dra*I profiles of strain 17 after 15 passages over 1.5 months, 10 passages over 1 month, and 2 passages, respectively; lanes e to k, *Dra*I profiles of strains 26, 83, 82, 75, 74, 73, and 12, respectively; lanes l and m, *Spe*I profiles of strain 17 after 10 and 2 passages, respectively; lanes n to t, *Spe*I profiles of strains 26, 83, 82, 75, 74, 73, and 12, respectively; lanes u to w, *Xba*I profiles of strain 17 after approximately 15 passages over 1.5 months, 10 passages over 20 days, and 2 passages over 6 days, respectively; lanes x to z, *Xba*I profiles of strains 26, 83, and 82, respectively; lanes 1 to 4, *Xba*I profiles of strains 75, 74, 73, and 12, respectively.

The PFGE types determined after two passages (Fig. 2B, lane d) and after repeated subculture for 1.5 months (Fig. 2B, lane b) differed only by a single band of 120 kb instead of a barely discernible doublet of 120 kb. The discriminatory power of this subtle variation was subsequently supported with the *Xba*I patterns of the same two chromosomal preparations, which clearly showed two differences within 220 to 390 kb (Fig. 2B, lanes u and w).

DISCUSSION

Previous studies (2–4) reported that PFGE of *Xba*I-digested DNA sensitively distinguished among epidemiologically distinct strains of *B. pertussis* isolates. Similarly, our PFGE results with each of three enzymes suggest that only 1 (strain 73) of 24 isolates from patients with nonoutbreak-related cases of pertussis shared a profile common to 54 isolates cultured during an investigation of an epidemic (6). Strain 73 was recovered from a laboratory worker in Georgia with a cough illness lasting >6 weeks who was working with the epidemic isolates at the time of, and prior to, the onset of the cough. This, in the absence of additional risk factors, suggests that this particular isolate was a laboratory-acquired epidemic strain. Together, epidemiologic data and PFGE results clearly justify an association between a single PFGE type and epidemic pertussis.

Although the results described here associated a single *B. pertussis* strain with epidemic illness, a previous report related multiple strains with a province-wide epidemic in Canada (2). However, this difference may be explained by the epidemiology of the respective outbreaks. The province-wide Canadian outbreak involved at least 19 municipalities over 18 months. In contrast, the 1986 Delaware epidemic associated with a single PFGE type occurred within a more compact geographic area, involved a smaller and an ethnically and socially less heterogeneous population, and progressed over a much shorter time period (1, 2). These characteristics better describe the pertussis outbreak occurring over 3 months in Fort Smith, Alberta, Canada, rather than the province-wide epidemic of 1989 to 1991 (2). Interestingly, laboratory results linked a single PFGE type with pertussis in Fort Smith (2). Thus, our results associating a single strain of *B. pertussis* with epidemic disease are consistent with previous reports and also demonstrate the utility of PFGE profiling for investigations of epidemic pertussis.

As shown here, all three test enzymes were equally suitable for the analysis of the PFGE profiles of *B. pertussis* genomic DNA encompassing fragments of approximately 50 to 490 kb.

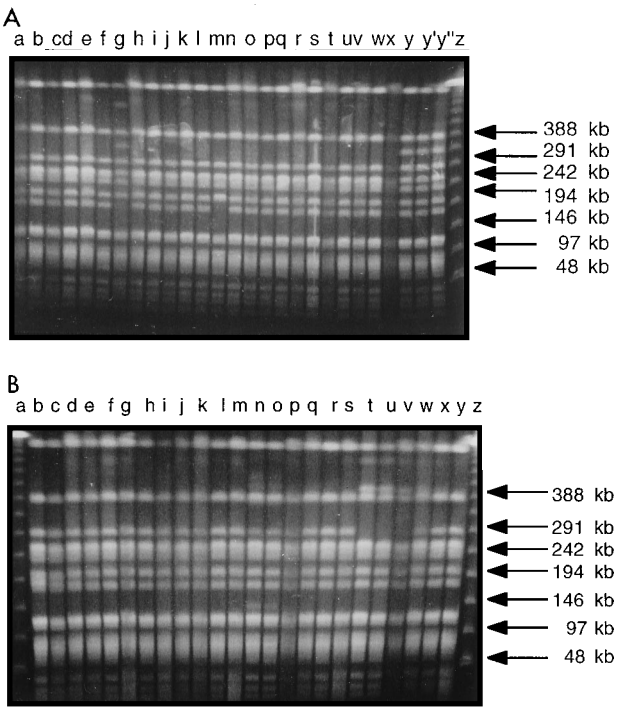

FIG. 3. Effect of multiple passages of strains on *Xba*I PFGE profiles. (A) Lanes a to e, h to l, n to r, and t to x, passages one through five of strains 49, 42, 26, and 17, respectively; lanes f and g, digests prepared from two different cultures of strain 49 that were subcultured 15 times over a period of 1.5 months; lanes m and s, digests from strains 42 and 26, respectively, passed as described for strain 49 for 1.5 months; lanes y, y′, and y″, three digests from three separate DNA samples prepared from the same culture of strain 17 passed 1.5 months, as described for the other three strains. (B) Lanes b to g, h to m, n to s, and t to y, *Xba*I digests from strains 49, 42, 26, and 17, respectively; lanes g, m, s, and y, strains from a culture allowed to incubate for 2 weeks without subculture. The other lanes contain digests from passages 6 through 10 as described in the text. Note that only lanes t to v, which contain passages 8 through 10 of strain 17, show a clearly different profile.

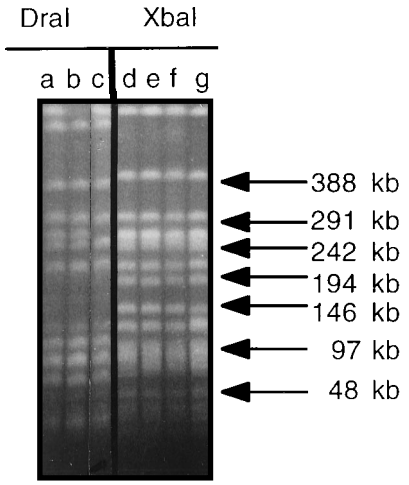

FIG. 4. Verification of similar PFGE types with two restriction enzymes (*Dra*I and *Xba*I). Lanes c and g, digests from Missouri strain 139; lanes b and e, digests from Senegal strain 146; lanes a and d, digests from Senegal strain 154; lane f, a digest from Senegal strain 143.

Although several of the fragments are not well resolved and in fact comigrate (8), there are many resolvable bands in this size range. *Spe*I and *Dra*I were particularly useful when *Xba*I profiles showed minor differences such as a doublet in place of a single band or small but reproducible variations in cleavage fragment size. In such instances, a PFGE profile generated with a second or third enzyme successfully discriminated epidemiologically unrelated strains. Consequently, additional enzyme profiles may be useful for distinguishing between strains showing equivocal patterns with the primary enzyme.

Our results also demonstrated detectable genetic variation after repeated subculture of some strains. In all of these cases the profiles generated by all three restriction enzymes changed. These results are more likely the result of DNA rearrangement than of multiple point mutations, since DNA rearrangement could occur by a single recombination event. This was not recognized previously, perhaps because of differences in the frequency of subculture or variations in incubation periods between the present and other studies. Alternatively, the cumulative number of strains examined before our study may not have been sufficient to observe this relatively infrequent variation. In any case, this result suggests that subculturing and incubation periods should be minimized prior to PFGE analysis.

In conclusion, the profile identities of DNAs prepared from 55 isolates associated with an epidemic in Delaware, one of which was later cultured from a patient with pertussis in Georgia, establishes the utility of PFGE for typing epidemic *B. pertussis* strains. Other data indicate that two additional restriction enzymes, *Dra*I and *Spe*I, are as effective as *Xba*I for PFGE typing and that additional enzyme patterns can differentiate strains showing equivocal profiles with a primary endonuclease. However, the reliability of PFGE could be compromised by the variation that the present study associated with extended subculturing. Prospective PFGE of restriction digests of *B. pertussis* DNA would facilitate epidemiologic and pathobiologic studies focused on the characterization of endemic and epidemic patterns, secondary attack rates, vaccine efficacy, and the role of atypical illness in transmission.

REFERENCES

1. **Biellik, R. J., P. A. Patriarca, W. Paul, G. Sanden, E. W. Brink, and P. Silverman.** 1989. Pertussis in an Amish community in Delaware, abstr. 286, p. 145. *In* Program and abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
2. **de Moissac, Y. R., S. L. Ronald, and M. S. Peppler.** 1994. Use of pulsed-field gel electrophoresis for epidemiological study of *Bordetella pertussis* in a whooping cough outbreak. J. Clin. Microbiol. **32:**308–402.
3. **Khattak, M., and R. C. Matthews.** 1993. Genetic relatedness of *Bordetella* species as determined by macrorestriction digests resolved by pulsed field electrophoresis. Int. J. Syst. Bacteriol. **43:**659–664.
4. **Khattak, M., R. C. Matthews, and J. P. Burnie.** 1992. Is *Bordetella pertussis* clonal? Br. Med. J. **304:**813–815.
5. **Maslow, J. N., A. M. Slutsky, and R. D. Arbeit.** 1993. Application of pulsed field gel electrophoresis to molecular epidemiology, p. 523–572. *In* D. H. Persing, T. F. Smith, F. C. Turner, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology Washington, D.C.
6. **Patriarca, P. A., R. J. Biellik, G. Sanden, D. G. Burstynn, P. D. Mitchell, P. R. Silverman, J. P. Davis, and C. R. Manclark.** 1988. Sensitivity and specificity of clinical case definitions for pertussis. Am. J. Public Health **78:**833–836.
7. **Sanden, G. N., P. K. Cassiday, and B. Beall.** 1994. Typing of *Bordetella* isolates by multiple arbitrary amplicon profiling, abstr. C-27, p. 495. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
8. **Stibitz, S., and T. L. Garletts.** 1992. Derivation of a physical map of the *Bordetella pertussis* chromosome. J. Bacteriol. **174:**7770–7777.