Journal of Clinical Microbiology, Aug. 1997, p. 2111–2114 Vol. 35, No. 8
0095-1137/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Molecular Epidemiology of *Streptococcus pyogenes* in an Area Where Acute Pharyngotonsillitis Is Endemic

LAURENCE NGUYEN,[1] DELPHINE LEVY,[1] AGNÈS FERRONI,[1] PIERRE GEHANNO,[2] AND PATRICK BERCHE[1]*

Service de Microbiologie, Hôpital Necker-Enfants Malades, 75743 Paris Cedex 15,[1] and Service d'Oto-Rhino-Laryngologie, Hôpital Bichat-Claude Bernard, 75018 Paris,[2] France

Received 24 February 1997/Returned for modification 11 April 1997/Accepted 15 May 1997

During an open clinical trial in an area where streptococcal infections are hyperendemic, we studied the genetic polymorphism of *Streptococcus pyogenes* isolates collected from patients and from healthy carriers living in close contact with them. The clonal diversity of isolates was analyzed by pulsed-field gel electrophoresis with three restriction enzymes (*Sma*I, *Apa*I, and *Sac*II). The pharynx of each patient and healthy carrier was colonized by a single clone, suggesting the clonal nature of streptococcal colonization in individuals. Among 52 isolates obtained from patients with acute pharyngotonsillitis, we found 14 genetically unrelated clones, showing the genetic diversity of *S. pyogenes*. However, two clones belonging to the M1 and M12 serotypes represented about 70% of isolates in carriers and patients. Pharyngeal colonization in cured patients was monitored for 3 to 4 months. After the initial elimination of *S. pyogenes* following antibiotic therapy, the rate of recolonization was high by day 30 (about 20%) and was also at that level between days 90 and 120; this was similar to the carriage rate in family contacts. Thus, cured patients can be recontaminated by unrelated clones, suggesting that colonization of healthy carriers might be a potential source of spread and redistribution of *S. pyogenes* isolates.

Streptococcus pyogenes (group A *Streptococcus*) is a specific human pathogen responsible for very common infections, such as acute pharyngotonsillitis, that are particularly frequent in children (4). The pharyngeal carriage of *S. pyogenes* in adult populations is usually estimated to be under 5 to 10% in industrialized countries (3). These infections can be complicated by severe nonsuppurative sequelae, including acute rheumatic fever and glomerulonephritis (22). The incidence of these complications has rapidly declined during the last decades in industrialized countries (9, 15) due to improved living conditions and to systematic antibiotic therapy with penicillin, both of which limit the spread of bacterial strains in the population (1, 13). However, the rate of recurrence of streptococcal pharyngitis following penicillin therapy can be as high as 20 to 25% in children, especially in semiclosed communities (7, 8). Unexpected outbreaks of rheumatic fever due to the reemergence of certain virulent M clones has been recently described in industrialized countries where penicillin therapy is widely used (1, 4, 22, 23). This and the high incidence of poststreptococcal nonsuppurative sequelae in the developing countries illustrate how streptococcal infections remain a major problem of public health.

Most reports on the epidemiology of streptococcal pharyngitis are based on M and T and opacity factor serotyping (10, 12). These methods might not adequately reflect the clonal diversity of bacterial strains, as suggested by the finding that isolates expressing the same M serotypes can be distinguished by genetic methods, including restriction endonuclease polymorphism analysis (2, 14), ribotyping (6, 19), multilocus enzyme electrophoresis (10, 16), and pulsed-field gel electrophoresis (PFGE) (20). In this work, we used PFGE to study the clonal diversity of *S. pyogenes* isolates collected from patients with acute streptococcal pharyngitis monitored for 3 to 4 months and from healthy carriers living in contact with these patients.

* Corresponding author. Mailing address: Service de Microbiologie, Hôpital Necker-Enfants Malades, 149, rue de Sèvres, 75743 Paris Cedex 15, France. Phone: 33 1 44 49 49 61. Fax: 33 1 44 49 49 60. E-mail: berche@necker.fr.

MATERIALS AND METHODS

Patients. *S. pyogenes* isolates were collected during a randomized clinical trial from patients with acute pharyngotonsillitis treated by a 10-day course of penicillin V (10^6 IU of penicillin) or by a 5-day course of josamycin (1 g twice a day). Acute pharyngotonsillitis was diagnosed on the basis of fever over 38°C, sore throat, pharyngeal exudate, acute inflammatory tonsillitis, and a positive result in a rapid diagnostic test (Test Pack StreptA; Abbott, Chicago, Ill.). Fifty-two patients living in the Ile de la Réunion, age 12 to 65 years, were monitored by 13 general practitioners for 3 to 4 months. Samples were also collected from 92 healthy carriers living in close family contact with 39 patients (one to four persons per patient) and from a group of 25 adult patients with acute streptococcal pharyngotonsillitis in the Paris region (Ile-de-France). As control strains, we used *S. pyogenes* BM 105, *Streptococcus agalactiae* BM 106, *S. agalactiae* 3766 and 109191 from the collection at the hospital Necker-Enfants Malades (Paris, France), and *Streptococcus pneumoniae* NEM 01 and NEM 02 isolates obtained from nasopharyngeal flora from children living in France.

Bacteriological methods. A swab applicator was applied over both tonsils and the posterior pharynx and then was shaken carefully in 500 μl of Lablemco broth (Oxoid, Unipath, Dardilly, France), which was immediately stored at −20°C in the medical office for 40 days (maximum). Swabs were collected from patients before antibiotic therapy, on day 30, and between days 90 and 120, when possible. All specimens were placed in dry ice and sent by air within 24 h to the Laboratory of Microbiology of the Hôpital Necker-Enfants Malades where they were stored at −80°C until examination. Specimens were rapidly thawed and cultured on 5% sheep blood agar (bioMérieux, Marcy-l'Etoile, France). Beta-hemolytic colonies were identified, and the serogroup A, as defined by Lancefield, was determined by using the Slidex Strepto Kit (bioMérieux). Biotyping was performed using biochemical tests of identification on API-Strept 50 (bioMérieux) as previously described (5). Strains were then examined for M and T serotypes at the Centre de Reference des Streptocoques (H. de Montclos, Institut Pasteur de Lyon). T-protein types were identified by slide agglutination with rabbit antisera (24). Strains that did not agglutinate with the anti-T serogroup were considered nontypeable. Strains found to be positive were tested for M-protein serotypes by microdiffusion with acid extracts and rabbit antisera (18).

PFGE. Bacteria grown overnight on 5% sheep blood agar plates at 37°C in 10% CO_2 were suspended in 2 ml of phosphate-buffered saline, pH 7.2, to an optical density of 1.4 at 600 nm. Then, 300 μl of the suspension was mixed with an equal volume of liquid 2% low-melting-point agarose (Sigma Chemical Co., St. Louis, Mo.) and cooled to 50°C. The mixture was poured into plug molds (BioRad, Richmond, Calif.). Bacterial DNA was released by treatment in Tris-EDTA (TE) buffer containing 10 mg of lysozyme per ml for 1 h at 37°C followed

by a second treatment with lysis buffer containing 0.5 M EDTA (pH 8.1; Sigma), 10% (wt/vol) sodium dodecyl sulfate, and 0.5 mg of proteinase K (Sigma) per ml. The mixture was incubated for 24 h at 50°C. Proteinase K was inactivated by adding 40 μl of 0.25 M phenylmethylsulfonyl fluoride (Sigma) for 1 h at room temperature, followed by washing in 0.5× TE buffer (10^{-2} M Trismabase [Sigma], 10^{-3} M EDTA [pH 7.5]), in 0.5× TE buffer diluted 1 in 2 with distilled water, and finally in distilled water. DNA was then digested with the restriction enzymes *Sac*II (5 IU), *Sma*I (20 IU), or *Apa*I (20 IU) in the appropriate buffer overnight at 25°C (*Sma*I) or 37°C (*Sac*II and *Apa*I). Fragments of DNA were separated on 1% agarose gel (Gibco BRL, Paisley, Scotland) in 0.5× TBE buffer (0.5 M Tris-borate-EDTA) in a Chef Mapper DR II apparatus (BioRad), under appropriate conditions: for 24 h with an 8- to 30-s switch time ramp at a 120° angle and 7 V/cm. Gels were stained with ethidium bromide (30 min), rinsed in distilled water, and examined and photographed (Polaroid 667 film) under UV light. DNA markers from Boehringer (Mannheim, Germany) were included in each gel.

Data analysis. Restriction fragment length polymorphism patterns were analyzed by calculating the Dice coefficient (*D*) of similarity with appropriate software (Vilber Lourmat; Biogène 6.21a, Marne la Vallée, France). The equation $D = 2n_{xy}/n_1 + n_2$, where n_1 is the total number of DNA fragments from strain X, n_2 is the total number of fragments from strain Y, and n_{xy} is the number of identical fragments, was used. A Dice value for two PFGE patterns of ≥0.80 represents closely related strains. A dendrogram was then constructed from the Dice coefficients.

RESULTS

Clonality of *S. pyogenes* infection in patients with acute pharyngotonsillitis. We isolated *S. pyogenes* from a group of 52 patients at the onset of acute pharyngotonsillitis. Each patient gave positive rapid diagnostic test results and was included in a randomized clinical trial. All patients were living in the Ile de la Réunion. Isolates were analyzed by PFGE after digestion of the chromosomal DNA by *Sma*I, *Apa*I, or *Sac*II. Each of these enzymes produced about 10 to 14 fragments for each isolate, allowing various pulsotypes to be defined. The results obtained with each of the three enzymes were quite similar. We present the results with *Sma*I restriction, which gave well-defined profiles that were easy to interpret. We first tested whether patients were infected by a single clone by analyzing five separate colonies randomly chosen from primary cultures on sheep blood agar. For each of the 52 patients, the five separate colonies displayed the same pulsotype (Fig. 1). This strongly suggests the clonal nature of acute streptococcal pharyngotonsillitis. The same approach was used in asymptomatic patients (day 30), as described below. A single clone of *S. pyogenes* was also detected in the pharyngeal flora of each of these colonized persons.

Genetic diversity of *S. pyogenes* isolates. The genetic diversity of *S. pyogenes* isolates collected from the 52 patients was further studied by PFGE, M and T serotyping, and biotyping. The results obtained by *Sma*I digestion are illustrated in Fig. 2A and B for the 52 isolates obtained at day 0. Fourteen different genetic patterns, designated A to N, were detected (Fig. 2A and B). As controls, we studied PFGE isolates collected from a group of 25 adult patients with acute streptococcal pharyngotonsillitis living in the Paris region. Eighteen pulsotypes were detected, no one being more prevalent than the others, suggesting a heterologous distribution of bacterial clones in this area of low endemicity of streptococcal infections (Fig. 2C). The dendrogram for all the 52 isolates illustrates the genetic polymorphism of *S. pyogenes* isolates from the Ile de la Réunion (Fig. 3). Thirty-five isolates (67.3%) belonged to two pulsotypes: pulsotypes A (17 isolates) and C (18 isolates), indicating that there were two epidemic strains in the population. These two strains belonged to two different biotypes and serotypes: clone A, serotype T12M12 and biotype 3; clone C, serotype T1M1 and biotype 1 (Fig. 3). Pulsotypes F and G and I and K were genetically closely related (Dice indexes >80%) (Fig. 3). There was a correlation between pulsotype, serotype, and biotype (Fig. 3). As previously reported, PFGE could discriminate isolates of the same serotype and the same biotype (Fig. 3).

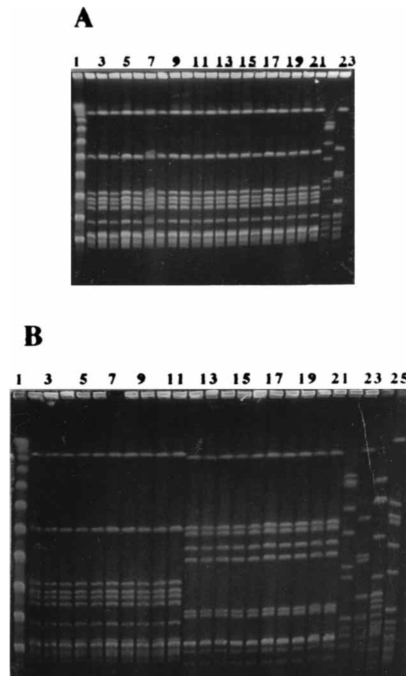

FIG. 1. PFGE restriction profiles of five separate colonies of *S. pyogenes* chosen randomly from patients on day 0 and day 30. Chromosomal DNA was digested with *Sma*I. (A) Lane 1, phage λ DNA ladder; lanes 2 to 6, isolates from patient 1R (day 0); lanes 7 to 11, isolates from patient 1R (day 30); lanes 12 to 16, isolates from patient 2R (day 0); lanes 17 to 21, isolates from patient 2R (day 30); lane 22, strain *S. agalactiae* BM 106; lane 23, strain of *S. pyogenes* BM 105. (B) Lane 1, phage λ DNA ladder; lanes 2 to 6, isolates from patient 16R (day 0); lanes 7 to 11, isolates from patient 16R (day 30); lanes 12 to 16, isolates from patient 17R (day 0); lanes 17 to 21, isolates from patient 17R (day 30); lane 22, strain of *S. agalactiae* BM 106; lane 23, strain of *S. pyogenes* BM 105; lanes 24 and 25, *S. pneumoniae* NEM 01 and NEM 02, respectively. Isolates were obtained from four patients living in the Ile de la Réunion.

Follow-up of streptococcal colonization in asymptomatic patients and contact healthy carriers. All 52 patients were rapidly cured by antibiotics, with complete regression of clinical symptoms within 3 to 4 days. *S. pyogenes* was undetectable in oropharyngeal flora two days after the end of antibiotic therapy. No clinical recurrences were observed during the following 3 to 4 months. Pharyngeal streptococcal colonization of asymptomatic patients was monitored for a similar period. Among 47 cured patients tested on day 30, 11 (21.2%) were colonized by *S. pyogenes*. The same clones as those isolated initially were found in 10 patients, as assessed by pulsotyping, biotyping, and M and T serotyping (Table 1). Only one patient (30R) was colonized on day 30 by a genetically unrelated isolate (pulsotype C instead of pulsotype A) (Table 1). By days 90 to 120, 8 of 39 tested patients (20%) were colonized by *S. pyogenes*. Among these eight asymptomatic patients, only four patients (2R, 5R, 13R, and 26R) had also been positive on day 30, one having been colonized by an isolate different from that found on day 0 and day 30 (patient 5R). Of the four other patients who were negative on day 30, two were colonized by an isolate different from that initially found (patients 19R and 27R). At least three patients (5R, 19R, and 27R) positive on days 90 to 120 had therefore been colonized by a new clone during the follow-up period (Table 1). Finally, spontaneous elimination of *S. pyogenes* by days 90 to 120 was observed in four cases (1R, 16R, 17R, and 30R).

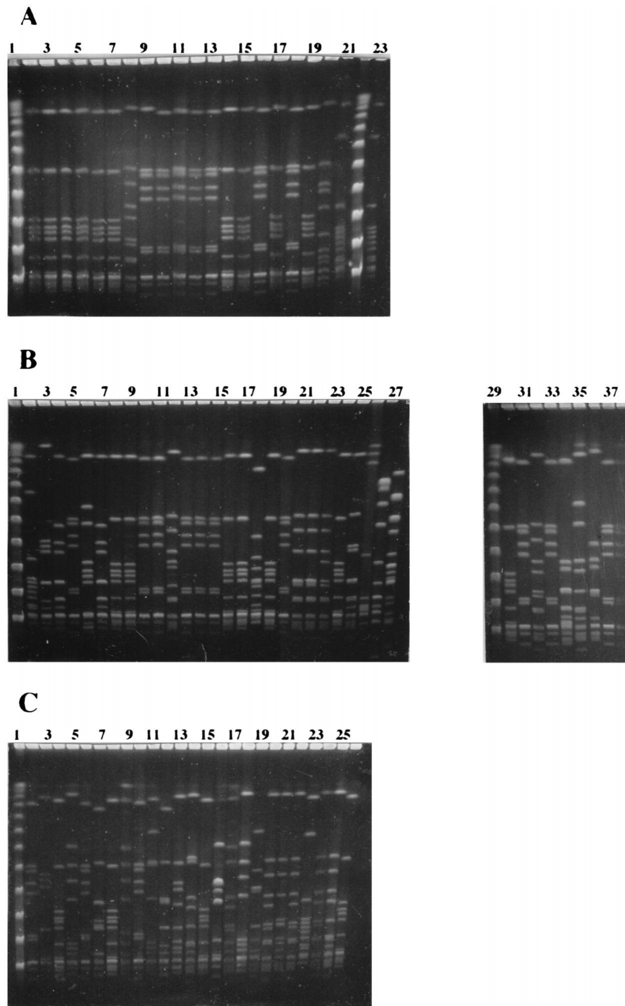

FIG. 2. PFGE restriction profiles of clinical isolates of *S. pyogenes* from patients with pharyngotonsillitis. Chromosomal DNA was digested with *Sma*I. (A and B) 52 isolates from patients (1R to 52R) living in the Ile de la Réunion. (A) Lanes 1 and 22, phage λ DNA ladder; lane 2, 1R; lane 3, 2R; lane 4, 3R; lane 5, 4R; lane 6, 5R, lane 7, 6R; lane 8, 7R; lane 9, 8R; lane 10, 9R; lane 11, 10R; lane 12, 11R; lane 13, 12R; lane 14, 13R; lane 15, 14R; lane 16, 15R; lane 17, 16R; lane 18, 17R; lane 19, 18R; lane 20, 19R; lane 21, 20R; lane 23, 20R. (B) Lanes 1 and 29, phage λ DNA ladder; lane 2, 21R; lane 3, 22R; lane 4, 23R; lane 5, 24R; lane 6, 25R; lane 7, 26R; lane 8, 27R; lane 9, 28R; lane 10, 29R; lane 11, 30R; lane 12, 31R; lane 13, 32R; lane 14, 33R; lane 15, 34R; lane 16, 35R; lane 17, 36R; lane 18, 37R; lane 19, 38R; lane 20, 39R; lane 21, 40R; lane 22, 41R; lane 23, 42R; lane 24, 43R; lane 25, strain BM 105; lane 26, *S. agalactiae* BM 106; lane 27, *S. agalactiae* NEM 3766; lane 28, *S. agalactiae* NEM 109191; lane 30, 44R; lane 31, 45R; lane 32, 46R; lane 33, 47R; lane 34, 48R; lane 35, 49R; lane 36, 50R; lane 37, 51R; lane 38, 52R. (C) 25 isolates from patients (1F to 25F) living in the Paris region. Lane 1, phage λ DNA ladder; lane 2, 1F; lane 3, 2F; lane 4, 3F; lane 5, 4F; lane 6, 5F; lane 7, 6F; lane 8, 7F; lane 9, 8F; lane 10, 9F; lane 11, 10F; lane 12, 11F; lane 13, 12F; lane 14, 13F; lane 15, 14F; lane 16, 15F; lane 17, 16F; lane 18, 17F; lane 19, 18F; lane 20, 19F; lane 21, 20F; lane 22, 21F; lane 23, 22F; lane 24, 23F; lane 25, 24F; lane 26, 25F.

Ninety-two persons living in close family contact with the 39 patients included in the study were examined by days 90 to 120 for the presence of *S. pyogenes* in their oropharyngeal flora. We found 18 healthy carriers of *S. pyogenes* (19.6%). This percentage of carriage is very similar to that found in asymptomatic patients. A genetic analysis of isolates by PFGE showed two strains to be most prevalent in the patients: pulsotype A (33.3%) and pulsotype C (50%). Except in two cases (patients 30R and 31R), healthy carriers harbored the same isolates as those found in the corresponding patients. Moreover, subjects in contact with two patients (2R and 19R) were colonized with two different isolates (Table 1). These results indicate that healthy carriers living in close contact with patients are often colonized by the same isolates as those found in patients.

DISCUSSION

We used PGFE to study *S. pyogenes* isolates collected from a group of 52 patients with acute pharyngotonsillitis living in a semiclosed area hyperendemic to streptococcal infections (Ile de la Réunion). The group was monitored for 3 to 4 months. The pharynx of each patient and healthy carrier was colonized by a single clone, suggesting the clonal nature of streptococcal infections. At least 14 different clones, as defined by their pulsotypes, were present in this group of patients. However, two genetically unrelated, dominant clones were isolated from 35 of 52 patients (67.3%). These clones, designated pulsotypes A and C, corresponded to serotypes T12M12 and T1M1, respectively, which are frequently associated with poststreptococcal glomerulonephritis and rheumatic fever (10, 11, 21). In contrast, at least 18 unrelated clones, without a dominant type, were found in a group of 25 patients with pharyngotonsillitis living in an urban area of low endemicity (Paris) where poststreptococcal sequelae are exceptional. These results demonstrate the genetic polymorphism of *S. pyogenes* isolates in cases of acute streptococcal pharyngotonsillitis. Such genetic diversity among *S. pyogenes* isolates associated with invasive dis-

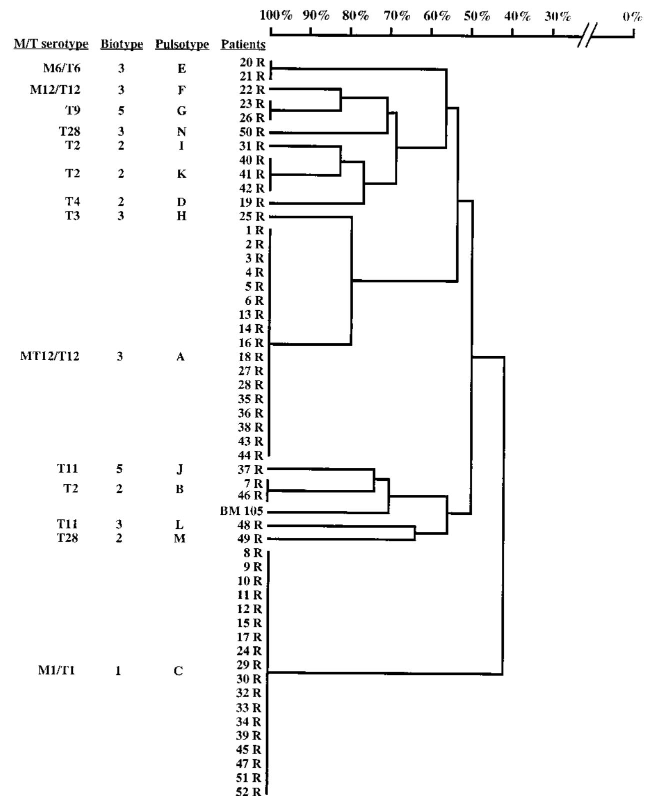

FIG. 3. Dendrogram illustrating the genetic diversity of *S. pyogenes* isolates from patients living in the Ile de la Réunion. The dendrogram was generated from *Sma*I restriction patterns of *S. pyogenes* isolates obtained by PFGE. The correspondences with the M and T serotypes and the biotypes are also reported. Clones A (M12T12) and C (M1T1) were predominant among the 52 isolates. The percent scale at the top is the Dice index.

TABLE 1. Carriage of *S. pyogenes* from patients and family contacts monitored by PFGE

Patient	Pulsotype of:			
	Patients at day(s):			Family contacts at days 90–120
	0	30	90–120	
1R	A	A	–[a]	–
2R	A	A	A	A[b]/C[b]
3R	A	A	ND[c]	ND
5R	A	A	C	C[d]
6R	A	–	–	A[b]
11R	C	–	C	C[b]
13R	A	A	A	–
14R	A	–	A	–
16R	A	A	–	–
17R	C	C	–	C[b]
18R	A	–	–	A[d]
19R	D	–	A	A[d]/D[b]
23R	G	G	ND	ND
26R	G	G	G	–
27R	A	–	C	C[b]
29R	C	–	–	C[b]
30R	C	A	–	B[b]
31R	I	–	–	K[b]
33R	C	C	ND	ND
36R	A	–	–	C[b]

[a] –, negative culture.
[b] One family contact.
[c] ND, not determined.
[d] Two family contacts.

eases and toxic shock-like syndrome has been described in a study using multilocus enzyme electrophoresis (17). This highly discriminatory method could distinguish 33 electrophoretic types among 108 isolates, although two dominant clones were detected (16).

During the 3 to 4 months of follow-up of the 52 patients with acute streptococcal pharyngotonsillitis, the rate of *S. pyogenes* recolonization was about 20%. By genetic analysis of isolates by PFGE, we found that some cured patients were colonized by new clones. *S. pyogenes* can spontaneously disappear from asymptomatic patients without antibiotic therapy, suggesting that active immunization may eliminate bacteria in the pharyngeal flora. The source of recontamination might be the family contacts living with patients. The rate of colonization in contact subjects was similar, about 20%, to that in asymptomatic patients. Most healthy carriers harbored the same clones as those found in the corresponding patient, with the two dominant clones being A and C. Interestingly, we detected in two members of one family two clones which were sequentially isolated in the corresponding patient, evidence of supporting cross-contamination within families (Table 1). It is likely that the rate of cross-contamination between asymptomatic patients and healthy carriers was underestimated since most patients were colonized by one of two dominant clones (Table 1). Our results suggest a rapid spreading and redistribution of *S. pyogenes* isolates in persons living in close contact. This has also been previously documented in confined areas, for example, day care centers, where up to 50% of the children are colonized by *S. pyogenes* (7). Our results also provide direct evidence for the early recontamination of asymptomatic patients recovering from acute pharyngotonsillitis by new clones, presumably originating from healthy carriers. This raises questions as to the significance of the so-called bacteriological failure to evaluate the efficacy of antibiotic therapy and emphasizes the importance of molecular typing to analyze the results.

ACKNOWLEDGMENTS

We thank O. Lescale, A. Scheimberg, M. Farges, A. Kosowsk, C. Coppin, A. Duport, F. Garnier, E. Manche, D. Passalacqua, G. Sebatigita, S. Arguillère, F. Nobileau, J. L. Carmeille, J. P. Degui, M. J. Fournier, F. Kabagema, C. Ottenwalder, S. Stoïanoff, and P. Tamburini for their help in collecting isolates and A. Scheimberg and O. Lescale for helpful discussion and help in designing the trial.

This work was supported by INSERM 411 and the University Paris V.

REFERENCES

1. **Bach, J. F., S. Chalons, E. Forier, G. Elana, and J. Jouanelle.** 1996. 10-year educational programme aimed at rheumatic fever in two French Caribbean islands. Lancet **347:**644–648.
2. **Bingen, E., E. Denamur, N. Lambert-Zechovsky, N. Braimi, M. el Lakany, and J. Elion.** 1992. DNA restriction fragment length polymorphism differentiates recurrence from relapse in treatment failures of *Streptococcus pyogenes* pharyngitis. J. Med. Microbiol. **37:**162–164.
3. **Bisno, A. L.** 1994. *Streptococcus pyogenes*, p. 1786–1799. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. Churchill-Livingstone, New York, N.Y.
4. **Bisno, A. L.** 1991. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. **325:**783–793.
5. **Bouvet, A., P. Geslin, P. Kriz-Kuzemenska, V. Blanc, C. Devime, and F. Grimont.** 1994. Restricted association between biotypes and serotypes within group A streptococci. J. Clin. Microbiol. **32:**1312–1317.
6. **Bruneau, S., H. De Montclos, E. Drouet, and G. A. Denoyel.** 1994. rRNA gene restriction patterns of *Streptococcus pyogenes*: epidemiological applications and relation to serotypes. J. Clin. Microbiol. **32:**2953–2958.
7. **Feldman, D., A. L. Bisno, L. Lott, R. Dodge, and R. E. Jackson.** 1987. Efficacy of benzathine penicillin G in group A streptococcal pharyngitis: reevaluation. J. Pediatr. **110:**783–787.
8. **Gastanaduy, A. S., E. L. Kaplan, B. B. Huwe, C. McKay, and L. W. Wannamaker.** 1980. Failure of penicillin to eradicate group A streptococci during an outbreak of pharyngitis. Lancet **ii:**488–502.
9. **Gordis, L.** 1985. The virtual disappearance of rheumatic fever in the United States: lessons in the use and fall of disease. Circulation **72:**1155–1162.
10. **Johnson, D. R., D. L. Stevens, and E. L. Kaplan.** 1992. Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. J. Infect. Dis. **166:**374–382.
11. **Kaplan, E. L.** 1991. The resurgence of group A streptococcal infections and their sequelae. Eur. J. Clin. Microbiol. **10:**55–57.
12. **Lancefield, R. C.** 1962. Current knowledge of the type specific M antigens of group A streptococci. J. Immunol. **89:**307–313.
13. **Markowitz, M., A. Michael, M. A. Gerber, L. Edward, and E. L. Kaplan.** 1993. Treatment of streptococcal pharyngotonsillitis: report of penicillin's demise are premature. J. Pediatr. **123:**679–685.
14. **Martin, D. R., and L. A. Single.** 1993. Single molecular epidemiology of group A *Streptococcus* M type 1 infections. J. Infect. Dis. **167:**1112–1117.
15. **Massel, B. F., C. G. Chute, A. M. Walker, and K. S. Kurland.** 1988. Penicillin and the marked decrease in morbidity and mortality from rheumatic fever in the United States. N. Engl. J. Med. **318:**280–286.
16. **Musser, J. M., A. R. Hauser, M. H. Kim, P. M. Schlievert, K. Nelson, and R. K. Selander.** 1991. Streptococcus pyogenes causing toxic-shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. Proc. Natl. Acad. Sci. USA **88:**2668–2672.
17. **Musser, J. M., B. M. Gray, P. M. Schlievert, and M. E. Pichichero.** 1992. *Streptococcus pyogenes* pharyngitis: characterization of strains by multilocus enzyme genotype, M and T protein serotype, and pyrogenic exotoxin gene probing. J. Clin. Microbiol. **30:**600–603.
18. **Rotta, J., and R. R. Facklam.** 1980. Manual of microbiology diagnostic methods for streptococcal infections and their sequelae. Bull. W. H. O. **1:**1–50.
19. **Seppala, H., J. Vuopo-Varbila, M. Osterblad, M. Jahkoba, M. Rummukainen, S. Holm, and P. Huoviner.** 1994. Evaluation of methods for epidemiologic typing of group A streptococci. J. Infect. Dis. **169:**519–525.
20. **Single, L. A., and D. R. Martin.** 1992. Clonal differences within M types of the group A *Streptococcus* revealed by pulsed field gel electrophoresis. FEMS Microbiol. Lett. **91:**85–90.
21. **Stollerman, G. H.** 1969. Nephritogenic and rheumatogenic group A streptococci. J. Infect. Dis. **120:**258–263.
22. **Stollerman, G. H.** 1990. Rheumatogenic group A streptococci and the return of rheumatic fever. Arch. Intern. Med. **35:**1–26.
23. **Veasy, L. G., S. E. Wiedmeier, G. S. Orsmond, H. D. Ruttenberg, M. M. Boucek, S. J. Roth, and V. F. Tait.** 1987. Resurgence of acute rheumatic fever in the intermountain area of the United States. N. Engl. J. Med. **316:**421–427.
24. **Williams, R. E. O.** 1968. Laboratory diagnosis of streptococcal infection. Bull. W. H. O. **19:**153–173.