Transmission of *Streptococcus pyogenes* causing successive infections in a family

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The objective of this study was to determine the characteristics of *Streptococcus pyogenes* isolated during a 10-month period from members of a family with infections and asymptomatic carriage. T-serotyping and pulsed-field gel electrophoresis confirmed that distinct GAS clones were introduced into the family over a short period of time.

Keywords *Streptococcus pyogenes*, T-typing, PFGE, siblings

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INTRODUCTION

The spectrum of diseases caused by *Streptococcus pyogenes* group A β-hemolytic streptococcus (GAS) ranges from uncomplicated pharyngitis to severe invasive infections (necrotizing fasciitis and toxic shock-like syndrome) and the post-streptococcal non-suppurative sequelae of acute glomerulonephritis and acute rheumatic fever [1]. The decreases in the morbidity and mortality resulting from streptococcal infections in industrialized countries have been attributed to improved living conditions and the introduction of antibiotic therapy [1]. However, there is still a high incidence of post-streptococcal non-suppurative sequelae in the developing countries, and streptococcal infections still represent a major public-health problem in many parts of the world [1]. Furthermore, in recent years, rheumatic fever outbreaks have been described in the USA, and reports of outbreaks of necrotizing fasciitis or other severe invasive infections are relatively frequent [2–5]. Although the use of penicillin has limited the spread of GAS strains in the population, their high transmissibility in closed communities is very frequently documented, with the emergence of clusters of invasive infections and other streptococcal syndromes in families, hospitals, and nursing home communities [6–10]. We describe here the clinical characteristics and the molecular epidemiology of a family outbreak with successive infections during a 10-month period.

MATERIALS AND METHODS

Patients

The family studied consisted of the parents and three siblings (A, female 11 years old; B, male ten years old; and C, male four years old) as the only household contacts. This family had a good socioeconomic level. Siblings A and B went to the same school but were in different classes, and sibling C went to another school. The siblings had several infections and periods of asymptomatic carriage during a period between March and December of 2000 (Table 1). None of the three siblings had any previous relevant illnesses or immunologic disorders.

Bacterial strains

During the study, 13 isolates of *Streptococcus pyogenes* were found. Eleven of them were isolated from throat swabs, and two from perianal lesions. The clinical samples were inoculated on trypticase soy agar plates with 5% sheep blood, and incubated overnight at 36 °C in 5% CO_2. The strains were confirmed as group A by a latex agglutination method (Slidex Strepto Kit, Bio-Merieux Marcy I'Etoile, France).

Antibiotic susceptibility

Antimicrobial susceptibility testing of GAS isolates was performed by the agar dilution method according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [11]. The antibiotics studied were penicillin, erythromycin, clindamycin, and tetracycline.

T-typing

Anti-T-typing serum was obtained from Seiken (Tokyo, Japan). A loop of GAS grown from blood agar plates was placed in Todd–Hewit broth and incubated overnight at 30 °C. The agglutination conditions were as recommended by the supplier.

Pulsed-field gel electrophoresis (PFGE)

The relatedness of isolates was determined by comparing *Sma*I and *Apa*I restriction enzyme digests of chromosomal DNA separated by PFGE, according to a previously described method [12], with some modifications. Briefly, the PFGE was performed with initial and final switch times of 0.1 s and 40 s, respectively, with a linear ramping factor and a run time of 22 h at 6.0 V/cm.

RESULTS

During the ten-month period of the study, the three siblings had several streptococcal infections or periods of carriage with four different T types (4, 6, 13 and 28). The chronologies of isolates and infections are shown in Table 1 and Figure 1.

Between March and May, six GAS isolates were obtained from the three siblings. All of them were of type T28. The clinical syndromes registered were: acute pharyngotonsillitis (two episodes, siblings A and B); sore throat (one episode, A); and perianal dermatitis (one episode, C). Two isolates were from asymptomatic carriers (siblings A and B).

Sibling A, after the first episode of pharyngotonsillitis, was treated with amoxicillin (500 mg every 8 h for seven days); 16 and 45 days later, the same serotype was found in the nasopharynx of this sibling, by then asymptomatic.

Sibling B also experienced an episode of pharyngotonsillis, and received similar treatment. The same strain was isolated when he, too, became an asymptomatic carrier.

Sibling C developed perianal dermatitis at the same time as sibling A was shown to be an asymptomatic carrier. All six T28 isolates showed identical PFGE profiles with both *Sma*I and *Apa*I (5b and 11, respectively) (Table 1, Figure 2).

On 4 May, the siblings were each treated with a single injection of benzathine-penicillin (50 000 IU/kg/L) to eradicate the carrier state. However, in June, three isolates of a different strain (13T, *Sma*I-31–*Apa*I-31) were obtained from the three siblings, two of whom were asymptomatic carriers (A and B) and one of whom (C) had a new episode of perianal dermatitis.

The first episode of perianal dermatitis was treated with oral amoxicillin, 50 mg/kg per day, for seven days, and the second episode was treated with oral cloxacillin, 50 mg/kg per day, for seven days. Throat swabs from the parents were negative for GAS.

In October, sibling C developed scarlet fever; a GAS strain of the T4 type was isolated, not digested by *Sma*I, but with the *Apa*I profile 7. The same strain was isolated from the nasopharynx of the asymptomatic sibling B. The parents and sibling A had negative cultures for GAS.

On 30 November, sibling A again developed pharyngotonsillitis, and a GAS T6 strain was isolated. A T6 strain was also isolated from the asymptomatic nasopharynx of sibling C. Both isolates had the same PFGE profiles with the two enzymes (33, 33).

Eleven isolates were sensitive to erythromycin, clindamycin, tetracycline, and penicillin. Two isolates (both T4) were erythromycin resistant.

DISCUSSION

The person-to-person spread of group A streptococcal infections within families or other closed communities is well recognized, producing secondary cases of uncomplicated pharyngotonsillitis as well as invasive infections [5–10,12]. The possibility of spread is greater during acute infection, although carriers are considered to represent a low-risk source.

In a study performed on members of the households of 52 patients with pharyngotonsillitis, it was found that the GAS carriage rate was up to 20%, and most family carriers had the same strain as the patient [12]. In another survey of 114 families, the carriage rate was 33% [13]. Family outbreaks have been ascribed to 'ping-pong

Table 1 Clinical and microbiological characteristics of *Streptococcus pyogenes* isolates.

Patient	Isolate no.	Isolation date (day/month/year)	Isolation site	Syndrome	T type	PFGE (*Sma*I)	PFGE (*Apa*I)	Erythromycin
Sibling A	393	21/03/00	Throat swab	Pharyngotonsillitis	28	5b[a]	11[a]	S[d]
	394	6/04/00	Throat swab	Asymptomatic	28	5b	11	S
	395	4/05/00	Throat swab	Sore throat/fever	28	5b	11	S
	396	12/06/00	Throat swab	Asymptomatic	13	31	31	S
	–[b]	31/10/00	Throat swab	Asymptomatic	–	–	–	–
	515	30/11/00	Throat swab	Pharyngotonsillitis	6	33	33	S
	–	5/12/00	Throat swab	Asymptomatic	–	–	–	–
Sibling B	397	28/03/00	Throat swab	Pharyngotonsillitis	28	5b	11	S
	398	4/05/00	Throat swab	Asymptomatic	28	5b	11	S
	399	12/06/00	Throat swab	Asymptomatic	13	31	31	S
	481	31/10/00	Throat swab	Asymptomatic	4	ND[c]	7	R
	–	5/12/00	Throat swab	Asymptomatic	–	–	–	–
Sibling C	400	19/04/00	Skin	Perianal dermatitis	28	5b	11	S
	401	12/06/00	Skin	Perianal dermatitis	13	31	31	S
	480	31/10/00	Throat swab	Scarlet fever	4	ND	7	R
	516	5/12/00	Throat swab	Asymptomatic	6	33	33	S
Mother	–	12/06/00	Throat swab	Asymptomatic	–	–	–	–
	–	31/10/00	Throat swab	Asymptomatic	–	–	–	–
Father	–	12/06/00	Throat swab	Asymptomatic	–	–	–	–
	–	31/10/00	Throat swab	Asymptomatic	–	–	–	–

[a]In our reference laboratory, we have studied 425 GAS isolates by PFGE (*Sma*I and *Apa*I). We have found 55 *Sma*I patterns, one pattern named ND (not digested), and 62 *Apa*I patterns. The pattern numbers cited were assigned according to our laboratory scheme (unpublished).
[b]Culture negative.
[c]Not digested by *Sma*I.
[d]S, sensitive; R, resistant.

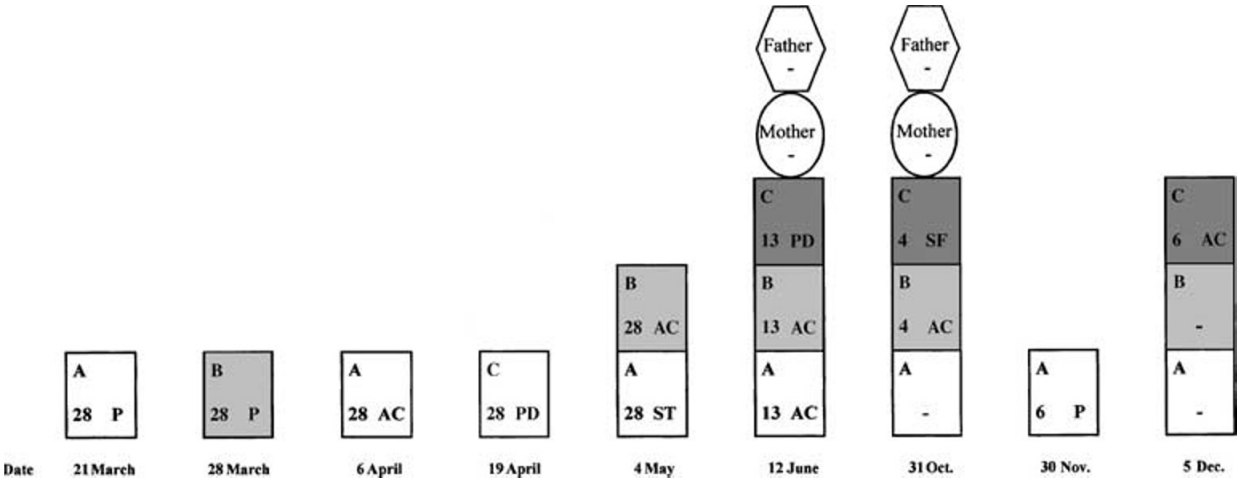

Figure 1 Chronology of *Streptococcus pyogenes* isolates from family members during a 10-month period. A, sister 11 years; B, brother 10 years; C, brother 4 years; P, pharyngotonsillitis; ST, sore throat; PD, perianal dermatitis; SF, scarlet fever; AC, asymptomatic carrier; –, culture negative. Numbers indicate the T type.

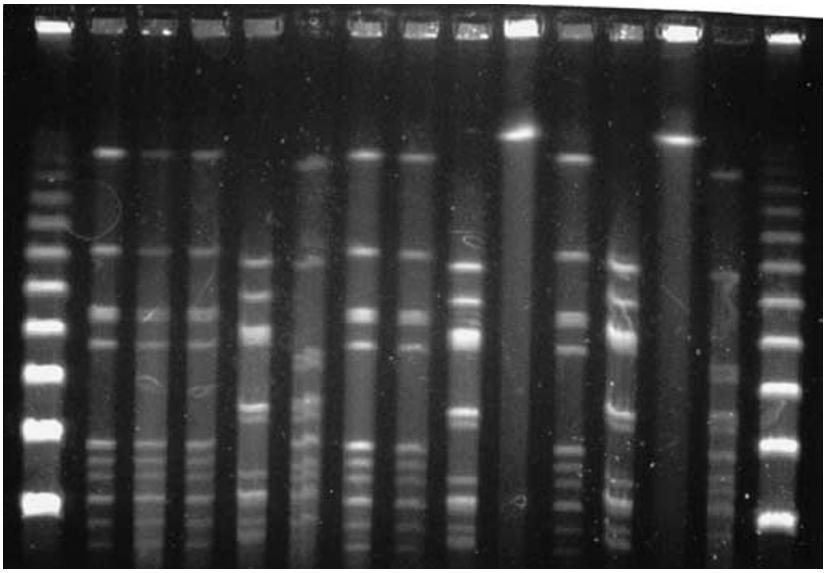

Figure 2 Pulsed-field gel electrophoresis of *Sma*I-digested chromosomal DNA from 13 *S. pyogenes* isolates from family members. Lanes 1 and 15: Molecular size standard. Lanes 2–6: sibling A (isolates 393, 394, 395, 396, and 515). Lanes 7–10: sibling B (isolates 397, 398, 399, and 481). Lanes 11–14: sibling C (isolates 400, 401, 480, and 516) (Table 1).

spread'—spread between the family members of distinct serotypes acquired from the community [13].

During the ten-month period of our study, 13 GAS isolates of four different strains were found, each of them being isolated from two or more family members. Six isolates were clearly associated with acute infections (pharyngotonsillitis, perianal dermatitis, and scarlet fever), while the seventh case, of sore throat and fever, may have been a carrier, the symptoms being due to viral infection.

Although there are some clinical and epidemiologic signs that suggest the streptococcal etiology of the pharyngotonsillitis, the clinical syndrome caused by other bacterial species or viruses may be indistinguishable from that of GAS infection. For this reason, the definite diagnosis of GAS pharyngotonsillitis must be made with a positive throat swab. However, the asymptomatic carriage rate in the general population ranges between 5% and 30%, after an acute episode of pharyngotonsillitis or because of colonization from other carriers or patients [14].

To confirm the role of GAS in a given patient, it would be necessary to detect an increase in specific antibodies during the acute phase of infection. These studies are very difficult in clinical practice. Moreover, the immune response against the M-protein is type specific, and does not confer cross-immunity against the 90 types described [15], so new infections with new types are frequent in the same individuals. Additional new serotypes have been recently described, increasing the number of serotypes to 124 [16].

In our family, sibling C had experienced two episodes of perianal dermatitis and one of scarlet fever caused by distinct clones, T28, T13 and T4, and sibling A had experienced two episodes of pharyngotonsillitis caused by two different clones, T28 and T6. The spread of the four clones (T28, T13, T4, and T6) between siblings suggests 'ping-pong' dissemination, because the isolates were found in patients and asymptomatic carriers after different episodes of infection, and because of the persistence of the carrier state principally in T28 episodes (between March and May). Such a carrier state has been described as GAS isolation from asymptomatic individuals without a detectable immune response, usually after antibiotic therapy for a pharyngotonsillitis episode. GAS persistence could be related to adherence to and penetration through the pharyngeal epithelium. Neeman et al. [14] found a high prevalence of the *prtF1* gene, which encodes fibronectin (a protein involved in adherence to mucous surfaces), in patients in whom it was possible to eradicate GAS from the nasopharynx after an infection. However, this fact has not been confirmed in a similar study [17]. As carriers are considered to represent a low-risk source of infection, and clinical sequelae after infection are not possible due to type-specific immune responses, treatment for the eradication of a possible carrier state is not recommended in patients who have had recent therapy, and it is not necessary to take eradication control GAS cultures [18].

Since the 1960s, several studies have been published on the failure to eradicate GAS with penicillin. 'Ping-pong' infection as a possible cause of therapeutic failure has been given little attention; its importance in recurrence has been well documented [13,19–21], but also denied [22–24]. Another hypothesis has been established in which there is interference from β-lactamase producers in the normal flora of the nasopharynx [25]. However, in these studies, the successive GAS isolates were not compared by means of microbiological markers to establish their similarity.

As second pharyngitis episodes with the original strain in the same patient are very rare [18], before therapeutic failure is diagnosed it is necessary to eliminate other possibilities, such as the persistence of the carrier state during a viral infection, or a new episode produced by another GAS strain acquired in the family or from the community.

In our cases, the successive acute infections cannot be ascribed to therapy failure, because they were caused by distinct strains, except in the sore throat and fever episode in sibling A, which could be attributed to simultaneous viral infection and GAS carriage of the strain previously isolated during the episode of pharyngotonsillitis (Table 1).

Unfortunately, typing methods are not used in many laboratories, and clinicians cannot know if a new isolate in a patient is the same as that previously isolated from the same patient or their household. Only the clinical and epidemiologic signs can help in determining whether further antibiotic therapy is needed.

In our study, the inclusion of the T-typing and a precise molecular marker (PFGE) permitted adequate characterization of isolates. This method has been used in several studies to characterize GAS isolates [26–28].

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