Antimicrobial susceptibility patterns and genomic diversity in strains of *Streptococcus pyogenes* isolated in 1978–1997 in different Brazilian cities

Maria Celeste Nunes De Melo, Agnes Marie Sá Figueiredo and Bernadete Teixeira Ferreira-Carvalho

Correspondence

Bernadete Teixeira Ferreira-Carvalho
carvalho@acd.ufrj.br

Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Professor Paulo de Góes, Laboratório de Biologia Molecular de Bactérias, CCS, Bloco I, Cidade Universitária, Rio de Janeiro, RJ, 21941-590, Brazil

Penicillin has been the antimicrobial of choice for the treatment of *Streptococcus pyogenes* infections for almost six decades. Although penicillin-resistant isolates have not been described to date, clinical failures have been reported after treatment with β -lactams. In this study, we analysed the antimicrobial susceptibility and genetic diversity of *S. pyogenes* isolates obtained from healthy carriers or patients in different cities in the south and south east of Brazil. The MICs were determined for penicillin and seven other antimicrobials. Penicillin tolerance was also investigated. Genetic diversity was analysed by PFGE after *Sma*I fragmentation of the genomic DNA. All 211 isolates tested were susceptible to penicillin (MIC 0.0025–0.02 mg l⁻¹). Four isolates were moderately penicillin-tolerant (MBC/MIC = 16 mg l⁻¹). Most of the other drugs tested were very active against the strains examined, except for tetracycline, to which 50 % of strains were resistant. We also found extensive genetic diversity, in that 60 different patterns were recognized in the 96 strains studied. Indeed, we found no correlation between tetracycline resistance and clonality. Despite this diversity, some PFGE patterns persisted for up to 18 years and specific clone types were spread over different geographical locations

Received 11 April 2002

Accepted 23 October 2002

INTRODUCTION

Streptococcus pyogenes (frequently referred to as group A *Streptococcus*; GAS) is an important human pathogen and causes both mild infections, such as pharyngitis and impetigo, and severe disease, such as toxic-shock-like syndrome and necrotizing fasciitis. GAS infection can also give rise to sequelae such as acute rheumatic fever and acute glomerulonephritis (Cunningham, 2000).

Despite 50 years of extensive use, penicillin remains the treatment of choice for GAS infections. However, reports of the failure of penicillin to eradicate *S. pyogenes* from the oropharynx, possibly as a result of penicillin tolerance (Orrling *et al.*, 1996; Pichichero, 1996; van Asselt *et al.*, 1996), are now causing concern. In contrast to penicillin, erythromycin resistance in GAS emerged in the late 1950s (Lowbury & Hurst, 1959), soon after the introduction of this drug into clinical practice. In the same way, tetracycline resistance has been reported in many countries since the first resistant isolate was reported in 1954 (Lowbury & Cason,

1954). In Brazil, surveillance data on the occurrence of antimicrobial resistance in GAS are scarce (Benchetrit *et al.*, 1981; Teixeira *et al.*, 2001).

Ripa *et al.* (2001), using PFGE to study genome diversity, suggested that most erythromycin-resistant GAS circulating in Italy were derived from the spread of a limited number of clones. However, the spread of tetracycline resistance among a population of Iranian GAS isolates was mostly due to multiclonal dissemination of the resistance trait rather than the epidemic spread of a few clones (Jasir *et al.*, 2000). Recently, a clonal epidemic of GAS infections associated with serotype M25 was reported among intravenous drug users (Böhlen *et al.*, 2000). Another study, involving 79 clinical isolates of GAS from hospital patients, showed that the majority of strains (71 %) displayed one of 12 clones. The largest clone (M type 1) occurred endemically and was frequently involved with severe disease (Léchoy *et al.*, 2001).

The aim of this study was to analyse the antimicrobial-susceptibility pattern and genetic diversity in a population of GAS isolates separated by clinical origin, geographical distance and time of isolation.

Abbreviations: GAS, group A *Streptococcus*; MBC, minimal bactericidal concentration.

METHODS

Strains. GAS were isolated from various sites from infected patients or

from the oropharynx of healthy carriers. The isolates were collected between 1978 and 1997 in hospitals or communities in the south or south east of Brazil (Table 1). All isolates were identified by colonial

Table 1. Genotyping characterization of 96 *S. pyogenes* human isolates obtained in different Brazilian cities

Strain*	Clinical source†	Isolation year	City of isolation	PFGE pattern‡
3-78	Oropharynx	1978	Rio de Janeiro, RJ	A
10-84*	Oropharynx	1984	São Paulo, SP	D
4-86	Oropharynx	1986	Rio de Janeiro, RJ	E
7-86	Oropharynx	1986	Rio de Janeiro, RJ	F ₁
5-87	Oropharynx	1987	Rio de Janeiro, RJ	G
1-89*	Oropharynx	1989	Rio de Janeiro, RJ	H ₁
2-89	Oropharynx	1989	Rio de Janeiro, RJ	H ₂
5-89	Oropharynx	1989	Rio de Janeiro, RJ	I ₁
8-89	Oropharynx	1989	Rio de Janeiro, RJ	H ₂
1-90*	Oropharynx	1990	Rio de Janeiro, RJ	J ₁
2-90*	Oropharynx	1990	Rio de Janeiro, RJ	L
3-90	Oropharynx	1990	Rio de Janeiro, RJ	I ₁
5-90*	Oropharynx	1990	Rio de Janeiro, RJ	K ₁
8-90	Oropharynx	1990	Rio de Janeiro, RJ	M
4-91*	Oropharynx	1991	Rio de Janeiro, RJ	N ₁
5-91	Oropharynx	1991	Rio de Janeiro, RJ	O
7-91*	Oropharynx	1991	Rio de Janeiro, RJ	B ₁
8-91*	Oropharynx	1991	Rio de Janeiro, RJ	P
9-91	Oropharynx	1991	Rio de Janeiro, RJ	Q
3-92	Oropharynx	1992	Rio de Janeiro, RJ	X
4-92*	Oropharynx	1992	Rio de Janeiro, RJ	S
5-92	Oropharynx	1992	Rio de Janeiro, RJ	T ₁
8-92	Oropharynx	1992	Rio de Janeiro, RJ	U
2-93*	Oropharynx	1993	Rio de Janeiro, RJ	W ₁
3-93	Oropharynx	1993	Rio de Janeiro, RJ	R
4-93*	Oropharynx	1993	Rio de Janeiro, RJ	Y
5-93*	Oropharynx	1993	Rio de Janeiro, RJ	L
6-93*	Oropharynx	1993	Rio de Janeiro, RJ	L
7-93*	Oropharynx	1993	Rio de Janeiro, RJ	G
9-93*	Oropharynx	1993	Rio de Janeiro, RJ	AE
10-96*	Oropharynx	1996	Rio de Janeiro, RJ	Z
11-96	Oropharynx	1996	Rio de Janeiro, RJ	AA ₁
21-96	Oropharynx	1996	Rio de Janeiro, RJ	I ₁
38-96	Oropharynx	1996	Rio de Janeiro, RJ	AB
1-97*	Oropharynx	1997	Rio de Janeiro, RJ	AC
13-97*	Oropharynx	1997	Rio de Janeiro, RJ	I ₁
15-97	Oropharynx	1997	Rio de Janeiro, RJ	AD
16-97	Oropharynx	1997	Rio de Janeiro, RJ	B ₂
37-97	Oropharynx	1997	Rio de Janeiro, RJ	AA ₁
1-78	Impetigo	1978	Rio de Janeiro, RJ	T ₂
2-78	Impetigo	1978	Rio de Janeiro, RJ	C
4-79*	Impetigo	1979	Rio de Janeiro, RJ	F ₁
6-79*	Impetigo	1979	Rio de Janeiro, RJ	AF
11-79*	Impetigo	1979	Rio de Janeiro, RJ	T ₂
1-84	Impetigo	1984	São Paulo, SP	AG ₁
3-84	Impetigo	1984	São Paulo, SP	AH
7-84	Impetigo	1984	São Paulo, SP	I ₂

Table 1. cont.

Strain*	Clinical source†	Isolation year	City of isolation	PFGE pattern‡
8-86	Impetigo	1986	São Paulo, SP	BC
12-87	Impetigo	1987	São Paulo, SP	AG ₂
17-87*	Impetigo	1987	Rio de Janeiro, RJ	T ₂
16-88*	Impetigo	1988	São Paulo, SP	AF
13-89	Impetigo	1989	Rio de Janeiro, RJ	B ₁
4-90*	Impetigo	1990	Rio de Janeiro, RJ	AI
15-90	Impetigo	1990	São Paulo, SP	BA
21-91*	Impetigo	1991	São Paulo, SP	T ₂
24-91	Impetigo	1991	São Paulo, SP	F ₂
28-96	Impetigo	1996	Niterói, RJ	BB
44-96	Impetigo	1996	Rio de Janeiro, RJ	AJ
45-96*	Impetigo	1996	Niterói, RJ	BD
49-96	Impetigo	1996	Rio de Janeiro, RJ	BK
7-97*	Impetigo	1997	Rio de Janeiro, RJ	G
21-87	Bacteraemia	1987	São Paulo, SP	AZ
11-88	Bacteraemia	1988	São Paulo, SP	AL
14-88*	Bacteraemia	1988	São Paulo, SP	AK
20-88	Bacteraemia	1988	São Paulo, SP	AN
18-90	Bacteraemia	1990	São Paulo, SP	AP
13-91*	Bacteraemia	1991	São Paulo, SP	BI
36-97	Bacteraemia	1997	Rio de Janeiro, RJ	AA ₁
12-79*	Abscess secretion	1979	Rio de Janeiro, RJ	B ₁
10-86	Abscess secretion	1986	São Paulo, SP	BA
14-87	Abscess secretion	1987	São Paulo, SP	O
22-87	Abscess secretion	1987	São Paulo, SP	AI
19-89*	Abscess secretion	1989	São Paulo, SP	AR
14-90	Abscess secretion	1990	São Paulo, SP	BJ
25-91	Abscess secretion	1991	São Paulo, SP	AS
26-91	Abscess secretion	1996	São Paulo, SP	AT
50-96	Abscess secretion	1996	Rio de Janeiro, RJ	W ₂
11-97	Abscess secretion	1997	Niterói, RJ	AV
9-92	Urethral secretion	1992	Florianópolis, SC	V
30-97*	Urethral secretion	1996	Rio de Janeiro, RJ	G
46-96*	Vaginal secretion	1996	Rio de Janeiro, RJ	AX
47-96	Vaginal secretion	1996	Niterói, RJ	AY
27-96	Tracheal secretion	1996	Rio de Janeiro, RJ	AX
19-88	Ascitic liquid	1988	São Paulo, SP	AM
51-96*	Skin wound	1996	Rio de Janeiro, RJ	AW
18-87	Surgical wound	1987	São Paulo, SP	J ₁
19-87*	Pustule	1987	São Paulo, SP	AV
52-96	Mucosal infection	1996	Niterói, RJ	T ₂
21-88	Human mastitis	1988	São Paulo, SP	H ₁
20-89*	Ganglion	1989	São Paulo, SP	BG
10-97	Urinary infection	1997	Rio de Janeiro, RJ	N ₂
26-97	Finger scrape	1997	Rio de Janeiro, RJ	BE
22-88	Unknown	1988	São Paulo, SP	F ₂
15-91	Unknown	1991	São Paulo, SP	BF
18-91	Unknown	1991	São Paulo, SP	BJ
22-91*	Unknown	1991	São Paulo, SP	BH

*Strains showing tetracycline resistance.

†Swabs of oropharynx were obtained from carriers or from patients presenting with pharyngitis.

‡PFGE following genomic digestion of the isolates with the endonuclease *Sma*I.

morphology, haemolysis on blood agar, bacitracin susceptibility (0.04 UI; Cecon, São Paulo, Brazil) and commercial latex agglutination (Streptococcal Grouping Kit; Oxoid). The strains were maintained in glycerol broth at -70°C .

Susceptibility tests. The MIC of penicillin (Wyeth-Whitehall) was determined for 211 strains of GAS by agar dilution, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). MICs were also determined for seven other antimicrobials, namely erythromycin (Sigma), clarithromycin (Abbott), cefalexin (União Química Farmacêutica Nacional), cefaclor (Eli Lilly), clindamycin (Sigma), chloramphenicol (Sigma) and tetracycline (Sigma). *S. pneumoniae* ATCC 49619 was used as a control. The MIC was defined as the lowest concentration of penicillin that completely inhibited growth, disregarding a single colony or a faint haze. The MIC₅₀ and MIC₉₀ were defined as the antimicrobial concentrations that inhibited growth of 50 and 90 % of the strains, respectively.

The minimal bactericidal concentration (MBC) of penicillin was determined for 105 GAS isolates by the broth macrodilution method as recommended by NCCLS. The MBC was defined as the lowest penicillin concentration that killed 99.9 % of the viable cells in the primary inoculum. Strains were considered to be penicillin tolerant when MBC/MIC ratios were 32 or higher and moderately tolerant when this ratio was 16 (van Asselt *et al.*, 1996). The MBC₅₀ and MBC₉₀ were defined as the antimicrobial concentrations that killed 50 % and 90 % of the strains, respectively. GAS K443 (a penicillin-tolerant isolate) was included as a control in the experiments for penicillin tolerance.

Genomic diversity. Agarose-inserted genomic DNA was prepared *in situ* for 96 GAS strains and cut with *Sma*I restriction enzyme as previously described (de Lencastre *et al.*, 1994), except that the GAS cell wall was lysed with 25U ml⁻¹ mutanolysin (Sigma). PFGE was carried out in a CHEF DR III apparatus using the following programme: initial forward time of 1 s, final forward time 30 s, during 23 h at 6.1 V cm⁻¹ at 11.3 °C. The gels were stained with ethidium bromide and photographed. Bacterial clones were defined as proposed by Tenover *et al.* (1995). The clone type was assigned with a capital letter or with a combination of two capital letters and the subtypes were distinguished by Arabic numerals.

RESULTS

We analysed the antimicrobial susceptibility and genetic diversity of GAS isolates obtained between 1978 and 1997 from healthy human carriers and patients in three different Brazilian cities. All 211 GAS isolates were susceptible to penicillin, cefalexin and cefaclor. MICs for penicillin ranged from 0.0025 to 0.02 mg l⁻¹ and both MIC₅₀ and MIC₉₀ values were 0.01 mg l⁻¹, except in 1990–1997 when the MIC₅₀ was 0.005 mg l⁻¹. The MIC₅₀/MIC₉₀ values for the cephalosporins were 0.5 mg l⁻¹.

Penicillin tolerance was investigated in 105 of the 211 isolates. No tolerance was detected, since the MBC/MIC ratio was ≥ 32 . However, four isolates were defined as being moderately tolerant, with an MBC/MIC ratio of 16. Although the MICs did not differ between the moderately tolerant and non-tolerant isolates, the geometric means of the MBCs varied significantly ($P < 0.05$) for the two categories (Table 2).

Table 3 summarizes the MIC₅₀ and MIC₉₀ values of the isolates tested for the other antimicrobials, which have been used as therapeutic alternatives to penicillin. Full resistance to erythromycin, clarithromycin, chloramphenicol or clindamycin was not detected among the isolates studied. One isolate showed intermediate resistance to erythromycin (MIC = 0.5 mg l⁻¹), four to chloramphenicol (MIC = 8 mg l⁻¹) and two to clindamycin (MIC = 0.05 mg l⁻¹). The MIC for tetracycline, unlike that for the other drugs, varied between 0.06 mg l⁻¹ and 64 mg l⁻¹, and the MIC₉₀ was 32 mg l⁻¹. Approximately one-half of the isolates examined were resistant to tetracycline (MIC ≥ 8 mg l⁻¹) and 3.8 % showed intermediate resistance to this drug (MIC = 4 mg l⁻¹). No significant change in antimicrobial-susceptibility patterns was detected during the 19-year period covered by this study.

The strains of GAS studied displayed extensive genetic diversity (Table 1; Fig. 1). In the random sample of 96 GAS isolates analysed by PFGE, a total of 60 different patterns was observed. Despite the clonal diversity displayed by these isolates, some PFGE patterns persisted for up to 18 years (Table 1; Fig. 2). Thus, four strains, which displayed a pattern assigned B (subtypes B₁ and B₂), were isolated over a period of 18 years from the oropharynx and from skin infections or abscesses. Another PFGE pattern displayed by four strains associated with the oropharynx or with skin infections, called F (subtypes F₁ and F₂), persisted for a period of 12 years. Similarly, PFGE pattern G was found in four strains within a period of 10 years. The same feature was observed for clone type I, which persisted for 13 years. The five strains belonging to pattern I (subtypes I₁ and I₂) were isolated from the oropharynx or from skin infections. Finally, we verified that a PFGE pattern, designated T (subtypes T₁ and T₂), isolated from skin, mucosal infections or the oropharynx, was found in six GAS isolates that persisted for 18 years (Table 1).

It is important to note that some specific clonal types were spread over different geographical areas. Thus, clonal types F,

Table 2. MIC and MBC values of penicillin for non-tolerant and moderately tolerant *S. pyogenes* strains

Tolerance	MIC (mg l ⁻¹)			MBC (mg l ⁻¹)		
	Mean	Range	50/90	Mean	Range	50/90
Non-tolerant (<i>n</i> = 101)	0.01	0.005–0.02	0.01/0.02	0.02	0.005–0.16	0.02/0.08
Moderately tolerant (<i>n</i> = 4)	0.01	0.005–0.02	0.01/0.02	0.19	0.08–0.32	0.16/0.32

Table 3. Antimicrobial susceptibility of 211 *Streptococcus pyogenes* isolates obtained in different Brazilian cities

All MIC values are in mg l⁻¹. For each antimicrobial, the range is given in parentheses; for tetracycline, the susceptibility profile (R, resistant; I, intermediate; S, susceptible) is given.

Year	No. isolates	Erythromycin (0.007–1 mg l ⁻¹) MIC ₅₀ /MIC ₉₀	Clarithromycin (0.004–0.25 mg l ⁻¹) MIC ₅₀ /MIC ₉₀	Clindamycin (0.003–1 mg l ⁻¹) MIC ₅₀ /MIC ₉₀	Tetracycline (0.03–128 mg l ⁻¹)			
					MIC ₅₀ /MIC ₉₀	R (%)	I (%)	S (%)
1978–1983	53	0.06/0.12	0.03/0.06	0.06/0.12	16/32	36 (67.9)	1 (1.9)	16 (30.2)
1984–1989	77	0.06/0.12	0.03/0.03	0.03/0.06	8/16	39 (50.6)	1 (1.3)	37 (48.1)
1990–1997	81	0.06/0.12	0.03/0.03	0.03/0.06	0.5/16	30 (37.0)	6 (7.4)	45 (55.6)
	211	0.06/0.12	0.03/0.06	0.03/0.06	4/32	105 (49.8)	8 (3.8)	98 (46.4)

Fig. 1. PFGE patterns of representative GAS isolates displaying genetic diversity.

H, I, J, T and AI were isolated from infected patients or healthy carriers that lived in different Brazilian states, separated by a distance of about 400 km (Table 1; Fig. 2).

A random sample of 37 tetracycline-resistant GAS isolates was analysed for clonality. Twenty-nine different patterns were identified, indicating that horizontal spread of the *tet* gene rather than vertical transmission was the major mechanism of spread of this resistant trait among the population studied.

DISCUSSION

Antimicrobial susceptibility

Antimicrobial resistance poses a significant threat to public health worldwide, with certain bacterial diseases already being untreatable with commercially available antimicrobials (Cohen, 1994). GAS infections are exceptional, in that penicillin has remained the antimicrobial of choice and no penicillin-resistant clinical isolates of GAS have been detected for more than five decades, despite the extensive and frequently indiscriminate use of β -lactams and of the *in vitro* selection of penicillin-resistant mutants (Tomasz & Muñoz, 1995).

Despite this susceptibility to penicillin, there have been claims of an increased number of treatment failures during penicillin treatment of GAS infections (Gerber, 1996). In addition, since the mid-1980s, there has been an apparent resurgence of severe, invasive disease caused by strains of GAS, as well as of acute rheumatic fever, a non-suppurative sequela of streptococcal pharyngitis (Stevens *et al.*, 1989; Veasy *et al.*, 1994). Suggested reasons for this reemergence

Fig. 2. PFGE of representative GAS strains displaying the same PFGE patterns, isolated at different times and recovered from different geographical areas.

include the possibility that there has been a substantial change in the susceptibility of the bacteria to commonly used antimicrobials (Gerber, 1996). Tolerance was also thought to explain the increased persistence of GAS after penicillin therapy in patients who presented with pharyngitis (Kim & Kaplan, 1985; van Asselt *et al.*, 1996). Continued vigilance is, therefore, needed to detect any change in the susceptibility pattern of GAS isolates to antimicrobials.

In this study, we found no significant change in the *in vitro* susceptibility of GAS isolates to penicillin. All 211 isolates were uniformly susceptible to very low concentrations of penicillin and to all the other antimicrobials tested, except tetracycline. In addition, none of the 105 GAS isolates tested was tolerant to penicillin, although four were found to be moderately tolerant. The incidence of penicillin tolerance reported in different studies varied widely and ranges from 0 to 100 % (Dünder & Babacan, 1997; Kim & Kaplan, 1985; Panzaru *et al.*, 1997; van Asselt *et al.*, 1996; Wittler *et al.*, 1990; Orrling *et al.*, 1996). Our results are in accordance with those reported by Wittler *et al.* (1990) and Orrling *et al.* (1996), who did not detect penicillin-tolerance among the GAS isolates that they studied. Strain differences, methodological variation and the criteria chosen to define tolerance may explain these discrepancies.

Erythromycin is used widely as an alternative to penicillin in the management of streptococcal pharyngitis in penicillin-allergic patients. Although erythromycin-resistant GAS isolates have been described in several countries (Cornaglia *et al.*, 1998; Nakae *et al.*, 1977; Orden *et al.*, 1998; Yan *et al.*, 2000), in our study none of the isolates tested was resistant to erythromycin and only one showed intermediate resistance. These findings are similar to those reported previously in Brazil and in other South American countries (Giglio *et al.*, 1996; Lopardo *et al.*, 1997; Teixeira *et al.*, 2001). In contrast, we verified that tetracycline resistance was relatively common, with an incidence of approximately 50 %. Similar results were reported in Brazil (Teixeira *et al.*, 2001) and

also in other countries (Maruyama *et al.*, 1979; Ripa *et al.*, 2001; Seppälä *et al.*, 1993). Although tetracycline has not been recommended for the therapy of GAS diseases, selective pressure from the intensive use of tetracycline to treat a variety of human and veterinary infections may have contributed to the emergence of this resistance among GAS isolates around the world.

Genetic diversity

Both phenotypic and genotypic methods have been used in the epidemiological surveillance of GAS. M serotyping is a well-established typing system with at least 80 recognized types, but its discriminatory power is considered to be poor because different genotypes may share the same M type (Nguyen *et al.*, 1997; Single & Martin, 1992). Genomic typing methods have rarely been used to characterize the epidemiology of GAS. Among these methods, PFGE of chromosomal DNA has been used with success (Bert *et al.*, 1997; Nguyen *et al.*, 1997), as it is able to distinguish between isolates within the same M serotype (Jasir *et al.*, 2000; Nguyen *et al.*, 1997). Although it is complex, PFGE is established as the most sensitive and specific system for bacterial typing. Until the present study, there have been no Brazilian data on the genetic diversity of isolates of *S. pyogenes* obtained from asymptomatic carriage or clinical infection in human populations. Thus, using this approach, we established the extensive genetic diversity among the GAS population studied. Considerable genetic diversity was previously reported in a study involving an urban area of low endemicity (Paris), where 18 unrelated clones, without a dominant type, were found in a group of 25 patients (Nguyen *et al.*, 1997). In contrast, two genetically unrelated, dominant clones were isolated from 35 of 52 patients (67 %) living in a semiclosed area, the Ile de la Réunion in France, where streptococcal infections were hyperendemic (Nguyen *et al.*, 1997). Clonality was also demonstrated in a population of 500 GAS clinical isolates from Belgium. Although 136 unrelated PFGE types were identified, two PFGE types predominated among

the population studied (Descheemaeker *et al.*, 2000). Although we were unable to establish any dominant PFGE patterns among the strains analysed, some PFGE clones were found to persist for up to 18 years. In addition, some clone types were spread over different cities in Brazil.

We also found identical PFGE patterns in GAS isolates obtained from different diseases, showing that strains displaying different genetic backgrounds have the ability to adhere, colonize and infect distinct human sites. Other authors have reported GAS isolates that display the same PFGE pattern as causes of both invasive and non-invasive disease (Descheemaeker *et al.*, 2000; Murase *et al.*, 1999; Nakashima *et al.*, 1997).

In addition to the extensive genetic diversity in the population of tetracycline-resistant GAS studied, the same clonal type (for example, clone type F) was found in both susceptible and resistant isolates. Identical results have been reported by others (Jasir *et al.*, 2000). These data suggest that tetracycline-resistant isolates were mostly disseminated among the collection of GAS isolates studied as a result of horizontal spread of the *tet* gene rather than of a specific resistant clone. The polyclonal nature of the resistant isolates has also been reported recently in other countries. Thus, the authors of a survey of 134 tetracycline-resistant GAS in Iran concluded that the high rate of tetracycline resistance that they found was due to multiclonal dissemination of the resistance rather than to epidemic spread of single clones (Jasir *et al.*, 2000). In contrast, more than 20 distinct PFGE types were recognized in a study with 207 tetracycline-resistant GAS in Italy. In this study, 79 % of the isolates fell into just four clusters, indicating that the majority of the strains in that population probably derived from the spread of a limited number of clones (Ripa *et al.*, 2001). Similarly, Jasir *et al.* (2001), using a smaller series of 50 GAS isolates (M77), found that all 11 isolates that displayed resistance to tetracycline were grouped in a single cluster.

In conclusion, GAS isolates obtained from Brazil remain susceptible to the great majority of antimicrobials used for the therapy of streptococcal infections, including erythromycin. Fifty per cent of GAS isolates studied were resistant to tetracycline. The PFGE analysis showed that, in the isolates studied, the *tet* gene was disseminated among isolates of different genetic backgrounds. Although the GAS isolates analysed were genetically very diverse, some specific clonal types could persist for at least 18 years. Strains with different genetic backgrounds were involved in different streptococcal diseases. Finally, the geographical spread of unique clone types was also found.

ACKNOWLEDGEMENTS

This work was supported in part by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Financiadora de Estudos e Projetos (FINEP/PRONEX). We are very grateful to Abbott Laboratórios do Brasil Ltda, União Química Farm-

acêutica Nacional S/A and Eli Lilly Laboratories for the gifts of clarithromycin, cefalexin and cefaclor, respectively. We thank Rachel Neves Soares Santos for her excellent technical assistance.

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