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International Journal of Antimicrobial Agents 26 (2005) 457–462

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Tracking methicillin-resistant *Staphylococcus aureus* clones in Colombian hospitals over 7 years (1996–2003): emergence of a new dominant clone

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Received 26 April 2005; accepted 20 August 2005

Abstract

Worldwide dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) clones is a well-characterised phenomenon. Two hundred isolates of MRSA recovered from 17 Colombian hospitals collected between 2001 and 2003 were characterised by pulsed-field gel electrophoresis (PFGE). A new dominant electrophoretic pattern unrelated to previously characterised clones in Colombia was detected in 137 (68.5%) of these isolates. Only 40 (20%) isolates still showed a pattern closely related to a previously described dominant clone. The new electrophoretic pattern was indistinguishable from a cluster of isolates recovered in Chile between 1996 and 1998. Isolates from this clonal cluster exhibited multidrug resistance but were susceptible to linezolid and glycopeptides. The results indicate a shift in the population genetics of Colombian MRSA and confirm dissemination of the Chilean clone for the first time.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Antibiotic resistance; Molecular epidemiology

1. Introduction

Within 2 years of the introduction of methicillin for clinical use, methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in England in 1961 [1]. Since then, the emergence and spread of MRSA has been documented in almost every continent and it is associated both with hospital and community-acquired infections [2]. Methicillin resistance is related to the expression of an altered penicillin-binding protein (PBP2a, encoded by the *mec*A gene) with reduced affinity for penicillins and other β-lactam antibiotics [1], but capable of effective transpeptidase activity for cell wall synthesis.

The recent emergence of multidrug-resistant isolates of MRSA (MDR-MRSA) exhibiting decreased susceptibility to glycopeptides has raised concern about the possibility of untreatable *S. aureus* infections arising from clones of MDR-MRSA [1,3–5]. As MRSA spreads through healthcare centres and the community all over the world, it is important to monitor its dissemination and also to identify potential geographical reservoirs of MDR clones [2,3].

Although pulse-field gel electrophoresis (PFGE) continues to be the gold standard technique for typing bacterial isolates, including MRSA [3,6–9], multiple DNA-based methods have been used to characterise *S. aureus* isolates and to track the dissemination of MRSA clones [3,6,10]. These methodologies include *Cla*I-*mec*A polymorphism analysis, Tn*554* insertion pattern, *spa*A typing, multilocus sequence typing (MLST) and staphylococcal chromosomal cassette (SCC) *mec* typing. Thus, the spread of major international MRSA clones throughout the world has been

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doi:10.1016/j.ijantimicag.2005.08.013

documented, designated the Iberian [2,3,6], Brazilian [2,3], New York–Japan [2,9] and Paediatric [2,11] clones.

In Colombia, the first molecular epidemiological study of MRSA included isolates from Bogotá city (1996–1998) and revealed the presence of a dominant clone that was a derivative of the 'Paediatric clone' (or PFGE pattern D) [12]. This clone was initially recovered from paediatric patients in Portugal, Poland and Argentina [2,11]. The Colombian isolates differed in that they were recovered from patients of all ages and were more resistant to antibiotics [12]. The aim of this study was to characterise the population genetics of a new collection of Colombian MRSA isolates recovered from 17 hospitals around the country (between 2001 and 2003) using PFGE. The new electrophoretic patterns were compared with previously characterised clones identified from 1996–1998 [12]. The results indicated the emergence of a new dominant clone in Colombian hospitals.

2. Materials and methods

2.1. Bacterial isolates

A total of 200 MRSA clinical isolates were collected from 17 clinical institutions from seven Colombian cities between March 2001 and March 2002 and from January to April 2003. All hospitals were tertiary care centres in different geographical areas of the country. The majority of isolates were recovered from Bogotá and Cali. The isolates were recovered from surgical wounds, blood, abdominal abscesses, fluid from infected joints, peritoneal fluid, bronchoalveolar lavage, pleural effusion, urine, cerebral spinal fluid and skull abscesses from individual patients in different wards [13]. Identification of MRSA isolates was confirmed by a polymerase chain reaction multiplex assay following the protocol of Martineau et al., which allows species-specific identification of MRSA and detection of *mec*A and 16S rRNA genes [14]. A previously characterised collection of 76 clinical isolates of MRSA recovered between 1996 and 1998 was included in the analysis (PFGE patterns and susceptibility testing).

2.2. Susceptibility testing

Susceptibility tests of each MRSA isolate recovered from March 2001 to March 2002 were performed using an agar dilution method with an inoculum of 10^5 colony-forming units per spot. Bacteria were plated on Mueller–Hinton agar (ICN Bio-medicals, Inc., Irvine, CA) supplemented with 2% NaCl and incubated at 35 °C. Minimal inhibitory concentrations were read after 20 h of incubation, or after 24 h for vancomycin, teicoplanin and oxacillin. A broth microdilution method was performed on all isolates recovered in 2003 according to the Center for Laboratory Standards Institute [15]. The following antimicrobial agents were tested: ciprofloxacin, chloramphenicol, erythromycin, clindamycin, gentamicin, linezolid, teicoplanin, tetracycline, sulphamethoxazole/trimethoprim (SXT), oxacillin, rifampicin and vancomycin [13]. All MRSA were screened for intermediate level resistance to vancomycin (VISA isolates) following published recommendations [16]. *Staphylococcus aureus* ATCC 29213 was used as the reference strain.

2.3. PFGE

Total DNA was extracted following previously described protocols [6,7]. After digestion with *Sma*I endonuclease, total DNA was separated in a CHEF-DR II System (Bio-Rad Laboratories, Hercules, CA) with the following conditions: block 1: run time 10 h, switch time 5–15 s and voltage 6 V/cm; and block 2: run time 13 h, switch time 15–60 s and voltage 6 V/cm. The gels were stained and photographed using standard methodology [17]. Cluster analysis of *Sma*I macrorestriction profiles was performed and analysed using the Dice similarity coefficient with a PFGE band similarity software (Diversity software; Bio-Rad Laboratories, Hercules, CA), using a cut-off of 75% as the criterion for cluster formation. Final PFGE interpretation was based on the criteria of Tenover et al. [18]. Briefly, isolates with identical banding pattern were considered as the same bacterial type, those presenting three or less bands of difference were considered as closely related, and isolates that differed by six or more bands were considered separate types. *Staphylococcus aureus* NCTC 8325-4 strain was used as a control strain to assess fragment size. The MRSA isolates HPV120 (representative of the Iberian clone), HSJ93 (Brazilian clone), HDE3 (Paediatric clone) and CHL93 (Chilean clone) were included for comparisons. The PFGE patterns were designated A (Iberian), B (Brazilian) and D (Paediatric), following the nomenclature described previously [12]. PFGE patterns F (Chilean) and X (unrelated to previous clones) originated from this study.

3. Results

3.1. Bacterial isolates and origin

Identification of all 200 clinical isolates was confirmed by microbiological and molecular methods. Most isolates were recovered from surgical wound infections, blood and joint aspirates. The cities Bogotá and Cali provided the majority of isolates (48% and 45%, respectively); these two cities are separated geographically by the Andes Mountains and are 300 miles apart.

3.2. PFGE clonal types

The PFGE patterns of *Sma*I DNA digests were analysed and grouped into five major types based on their banding pattern. As shown in Fig. 1, the majority of the Colombian clinical isolates showed a banding pattern (designated type

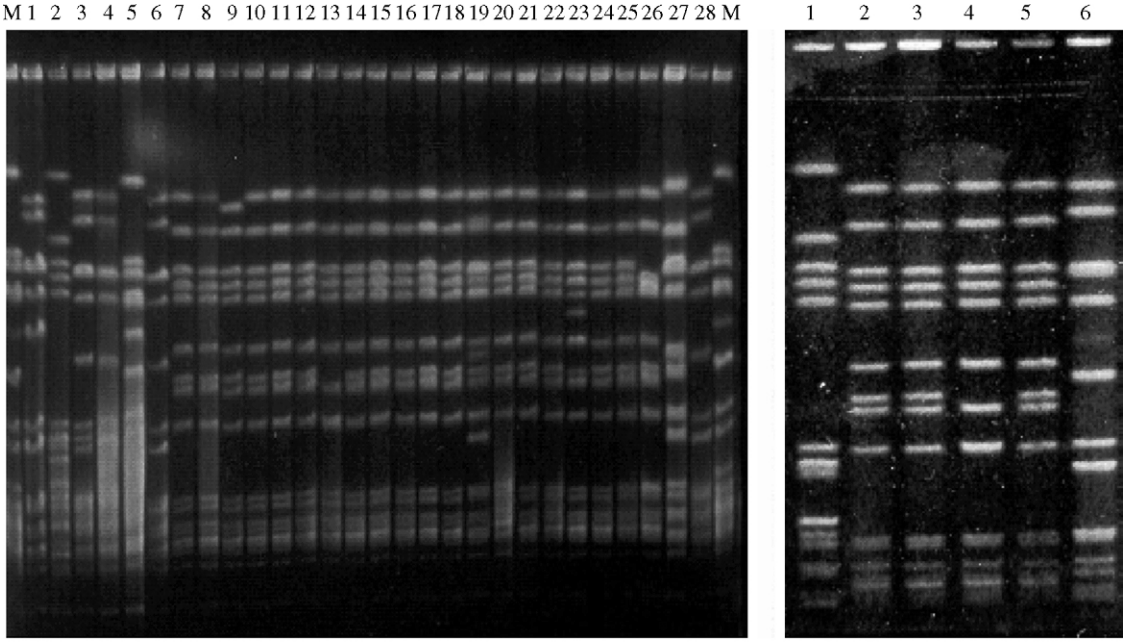

Fig. 1. Panel A: pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested genomic DNA from Colombian isolates and representative international clones (excluding the Chilean clone). Molecular size marker (lanes M and 5); isolate HPV120, Iberian clone (PFGE pattern A) (lane 1); isolate HSJ93, Brazilian clone (pattern B) (lane 2); isolates HDE3 and INS-32 from the Paediatric clone (pattern D) (lanes 3, 4 and 6); Colombian clinical isolates with PFGE pattern F (lanes 7–26); and Colombian clinical isolates with PFGE pattern D (lanes 27 and 28). Panel B: PFGE from Colombian isolates and representative isolates from the Chilean and Brazilian clones. Brazilian clone (lane 1); Colombian isolates exhibiting PFGE pattern F (lanes 2–4); isolate CHL93 (representative of Chilean clone) (lane 5); and Colombian isolate representative of pattern X (lane 6).

F) different from the previously described Iberian, Brazilian or Paediatric types designated A, B and D, respectively [3]. Among the 200 clinical isolates analysed, the dominant type F was present in 137 (68.5%) of these isolates (Fig. 2A). Within isolates belonging to PFGE pattern F, ten different subtypes (≤three bands difference) were identified in 17 isolates. This pattern had no genetic relatedness to the previously identified dominant 'Paediatric' clone from the city of Bogotá found between 1996 and 1998 (PFGE type D), as it presents more than six bands of difference (Fig. 1, panel A). In contrast, it shows an indistinguishable banding pattern to Chilean clinical isolates from 1996–1998 [8] (Fig. 1, panel B). Within PFGE type D, 40 isolates (20%) presented closely related patterns (designated D1 and D2) with two and three band differences, respectively. This type was found in cities other than Bogotá. Interestingly, eight isolates (4%) were grouped within the type B associated with the Brazilian clone and one strain exhibited a pattern associated with the Iberian clone (type A), which had not been previously reported in Colombia. The final 14 isolates showed patterns not related to the international circulating clones and were grouped as type X. Cluster analysis confirmed the PFGE results (Fig. 3).

3.3. Antimicrobial profiles

The majority of isolates (96%) exhibited multidrug resistance. They were all susceptible to vancomycin, teicoplanin and linezolid. Two isolates were susceptible to all antibiotics (belonging to PFGE patterns B and F; Table 1). Seven MRSA isolates were only resistant to a single antimicrobial agent (either tetracycline or ciprofloxacin) (Table 1). Five of the latter isolates belonged to PFGE pattern X (which has no correlation to previously described types) and two exhibited pattern B.

A substantial increase in the percentage of isolates resistant to ciprofloxacin and gentamicin was observed when isolates from 1996–1998 were compared with isolates recovered in 2001–2003 (58% vs. 98.5% and 58% vs. 92%, respectively) (Fig. 2B). In 1996–1998, resistance to rifampicin was found in 58% of isolates. Surprisingly, the percentage decreased substantially in 2001–2003 (to only 20% of all isolates). These characteristics appear to be related to the predominance of the clonal type F.

4. Discussion

The first molecular characterisation of MRSA in Colombia was performed with isolates recovered between 1996 and 1998 in one Colombian city [12]. These isolates were characterised by PFGE and hybridisation of *Cla*I restriction digests with *mec*A-and Tn*554*-specific probes. The results revealed that 75 of 76 MRSA isolates belonged to a single clonal cluster that had previously been described in Portugal, Poland and

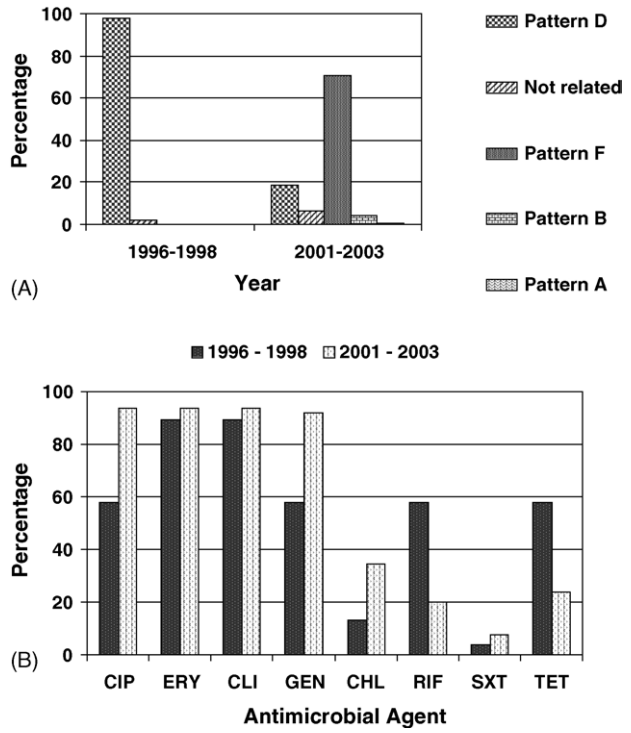

Fig. 2. (A) Frequency distribution of pulsed-field gel electrophoresis patterns of Colombian clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in two time periods. (B) Antimicrobial resistance profiles of Colombian MRSA clinical isolates recovered in two time periods. CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; CHL, chloramphenicol; RIF, rifampicin; SXT, sulphamethoxazole/trimethoprim; TET, tetracycline.

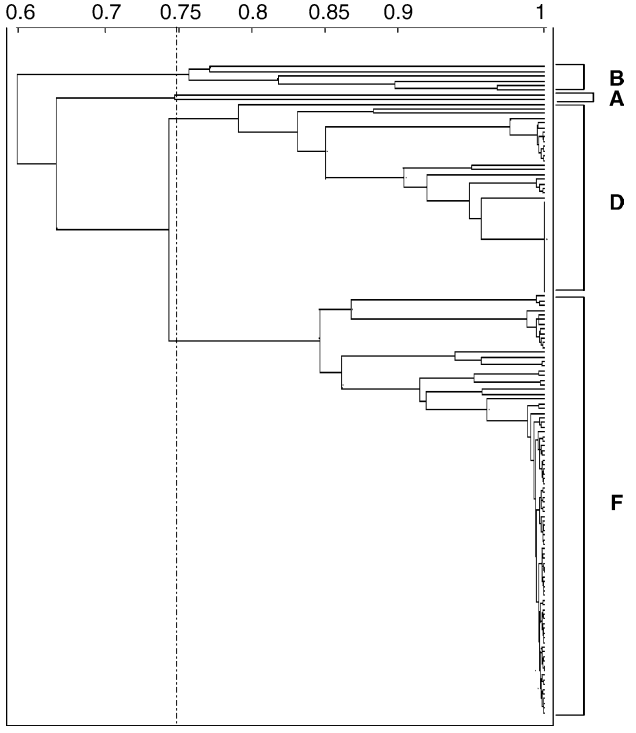

Fig. 3. Cluster analysis of Colombian methicillin-resistant *Staphylococcus aureus* isolates from 2001–2003. Four different clusters were observed and designated A, B, D and F, following pulsed-field gel electrophoresis patterns and comparison with representatives of the Iberian (pattern A), Brazilian (pattern B) and Paediatric (pattern D) clones, respectively.

Argentina (designated the Paediatric clone) with a particular PFGE pattern (D) [12]. In this work, we have continued to follow the molecular evolution of MRSA in Colombia with the study of a collection of 200 clinical MRSA isolates recovered over 3 years (2001–2003) using PFGE.

The most striking fact is that the previously dominant Paediatric clone has been replaced by a different clonal type over the span of 2 years. The new clonal type (designated type F) still predominates even if only isolates from Bogotá city are included in the analysis (the only city included in the first study in 1996–1998) (Fig. 2A). The reason for this clonal switch is unclear. A substantial subtype shift in isolates belonging to the Paediatric clone was noted in Colombian MRSA between 1996 and 1998 [12]: nine new subtypes appeared in 1997 and two additional subtypes in 1998 [12], indicating a constant evolution of the population genetics of Colombian MRSA. Moreover, a high frequency of relative genetic instability of the *mec* element that led to loss of the methicillin-resistant phenotype was noted in Colombian isolates from the Paediatric clone [12]. Nonetheless, this clone has remained dominant in several countries [2,5,11,12,19,20]. The emergence of a more stable *mec* element in type F might explain the success in dissemination: characterisation of the SCC*mec* in these isolates is currently underway.

Massive dissemination of the Brazilian clone (type B) has previously been documented in South America [2], even among very geographically distant cities. However, Colombian MRSA appears to behave differently from the rest of Latin America: from 1996–1998, the predominant clone circulating in South America was the Brazilian clone and its derivatives [8], whereas in Colombia it was the Paediatric clone. One would have expected an emergence of the Brazilian clone in Colombia replacing other clonal types. Unlike Brazil, Chile does not share a geographical border with Colombia and it was surprising to identify a predominant clone that initially emerged far from Colombia. This is the first evidence of dispersion of this clonal type and further analysis should be carried out to analyse whether dissemination is specific to Colombia. Our results support the hypothesis that successful spread of MRSA in hospitals not only depends on its invasive capacity but might be influenced by particular environmental conditions.

In terms of antibiotic susceptibilities, the type F clonal cluster differs from the previously characterised Paediatric clone in two main aspects: (i) a much higher percentage of isolates were resistant to ciprofloxacin and gentamicin (Fig. 2B); and (ii) resistance to rifampicin has decreased overall. On the other hand, a high percentage of isolates remained resistant to macrolides and lincosamides (likely to represent MLS_B-type resistance) and susceptibility to SXT continues to be high among Colombian MRSA isolates (more than 90%).

Table 1
Correlation between pulsed-field gel electrophoresis (PFGE) and antibiotic resistance patterns in Colombian methicillin-resistant *Staphylococcus aureus* between 2001 and 2003

PFGE pattern (no. of isolates)	Antimicrobial resistance
A (1)	CIP, ERY, CLI, GEN, CHL, TET, RIF
B (1)	None
B (1)	TET
B (1)	CIP
B (1)	CIP, ERY, CLI, GEN, CHL, TET, SXT
B (3)	CIP, ERY, CLI, GEN, TET, RIF, SXT
B (1)	ERY, CLI, TET
D (1)	CIP, ERY, CLI, GEN, TET, SXT
D (2)	CIP, ERY, CLI, GEN, TET, CHL, SXT
D (2)	CIP, ERY, CLI, GEN, TET, RIF, SXT
D (1)	CIP, ERY, CLI, TET, RIF
D (8)	CIP, ERY, CLI, GEN, RIF
D (1)	CIP, ERY, CLI, GEN, TET
D (12)	CIP, ERY, CLI, GEN
D (1)	CIP, GEN, RIF
D (4)	CIP, ERY, CLI, GEN, TET, RIF
D (1)	CIP, ERY, CLI, GEN, CHL, TET
D (4)	CIP, ERY, CLI, GEN, CHL
D (2)	CIP, ERY, CLI, GEN, CHL, TET, RIF
D (1)	CIP, ERY, CLI, GEN, CHL, RIF
F (1)	None
F (1)	CIP, CHL
F (1)	CIP, ERY, CLI
F (1)	ERY, CLI, GEN
F (2)	CIP, GEN, CHL
F (56)	CIP, ERY, CLI, GEN
F (1)	ERY, CLI, GEN, CHL
F (1)	ERY, CLI, CHL
F (2)	CIP, ERY, CLI, TET, RIF
F (1)	CIP, ERY, CLI, GEN, RIF
F (48)	CIP, ERY, CLI, GEN, CHL
F (3)	CIP, ERY, CLI, GEN, TET
F (4)	CIP, ERY, CLI, GEN, CHL, TET
F (1)	CIP, ERY, CLI, GEN, CHL, TET, RIF
F (2)	CIP, ERY, CLI, GEN, RIF, SXT
F (2)	CIP, ERY, CLI, GEN, CHL, SXT
F (1)	CIP, ERY, CLI, GEN, TET, SXT
F (2)	CIP, ERY, CLI, GEN, TET, RIF
F (2)	CIP, ERY, CLI, GEN, CHL, RIF
F (1)	CIP, ERY, CLI, GEN, CHL, TET, SXT
F (2)	CIP, ERY CLI, GEN, TET, RIF, SXT
F (2)	CIP, ERY, CLI, GEN, CHL, RIF, SXT
X (5)	TET
X (1)	GEN, TET, RIF
X (1)	ERY, CLI, TET
X (1)	CIP, ERY, CLI, TET
X (1)	CIP, ERY, CLI, GEN
X (1)	CIP, ERY, CLI, GEN, TET
X (1)	CIP, ERY, CLI, GEN, CHL
X (1)	CIP, ERY, CLI, TET, RIF
X (1)	CIP, ERY, CLI, GEN, RIF
X (1)	CIP, ERY, CLI, GEN, CHL, TET

CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; CHL, chloramphenicol; TET, tetracycline; RIF, rifampicin; SXT, sulphamethoxazole/trimethoprim.

Glycopeptide or linezolid resistance has not yet emerged in Colombia. These results indicate that combination therapy with SXT and rifampicin might be a useful alternative to vancomycin or linezolid for the treatment of MRSA infections in Colombia. Moreover, these antibiotics can be given as oral preparations and are relatively cheap. Recent data for combination oral therapy in methicillin-susceptible *S. aureus* indicated that oral combination therapy might be as effective as intravenous antibiotics for the treatment of staphylococcal infections [21]. Data for MRSA for this kind of therapy are lacking but its use is widespread. Our data also show evidence that aminoglycosides and fluoroquinolones are likely to be ineffective in the treatment of MRSA infection in Colombian hospitals. Gentamicin is currently recommended for the initial treatment of staphylococcal endocarditis [22]. The evidence of resistance to gentamicin might preclude its use in Colombia for this purpose.

In conclusion, this study confirms the presence of a new dominant clonal type circulating among Colombian hospitals and provides evidence for the first dissemination of the Chilean clonal type. This clone exhibits particular phenotypic characteristics in terms of antibiotic resistance to help guide empirical treatment of suspected MRSA infections in Colombia.

Acknowledgments

Partial support for this study was provided by the Fond de Péréquation (Hôpital Cantonal de Genève) grants from Suisse National Research Foundation (No. 3200BBO-108401 and No. PP00B—103002/1) and the Instituto Colombiano para el Desarrollo de la Ciencia y Tecnologia, 'Francisco Jose de Caldas', Code: 2104-04-12685. We thank H. de Lencastre, Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal and Rockefeller University, New York, USA, for the gift of PFGE control strains and gel photograph comparisons. We are grateful to Sasi Darhman from Geneva University Hospital and to Carlos Chiriboga from the Molecular Biology Laboratory, Universidad El Bosque, Bogotá, for technical assistance.

Clinical institutions that provided isolates for this study were: Hospital Universitario de Neiva from Neiva; Hospital Bocagrande from Cartagena; Hospital San Jerónimo from Montería; Hospital Pablo Tobón Uribe from Medellín; Hospital Universitario Ramón González Valencia from Bucaramanga; Hospital Universitario del Valle, Centro Médico Imbanaco and Fundación Valle del Lilli from Cali; Instituto de Seguros Sociales E.S.E. Clínica del Niño, Hospital Universitario Clínica San Rafael, Clínica Carlos Lleras Restrepo, Instituto Nacional de Cancerología, Hospital San Ignacio, Fundación Abood Shaio, Clínica Santa Rosa, Hospital Regional Simón Bolívar and Fundación Santa fe de Bogotá from Bogotá city.

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