Methicillin-resistant *Staphylococcus aureus* DNA electrophoretic pattern: temporal changes in an endemic hospital environment

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ABSTRACT

Objective. *To describe the analysis of geographical and temporal distribution of DNA profiles determined by pulsed-field gel electrophoresis (PFGE) of methicillin-resistant* Staphylococcus aureus *(MRSA) strains isolated from hospitalized patients in a tertiary care university hospital in Brazil.*

Methods. *Ninety-nine samples of MRSA obtained from 89 patients in the period 1999–2004 were studied. MRSA strains were isolated from central venous catheters (33 isolates) and bloodstream infections (66 strains). PFGE with 20 units of SmaI restriction endonuclease was used for genomic typing.*

Results. *Analysis of DNA PFGE of 99 strains of MRSA revealed 26 profiles and their respective related profiles. The mean time interval for detecting MRSA infection was 26 days from hospital admission. Forty-nine patients (57.6%) had a recent hospitalization. The DNA PFGE MRSA profiles were distributed in three clonal groups—I, II, and III—according to the period of time when the MRSA strains were isolated. DNA PFGE MRSA profiles were spread homogeneously through all hospital wards.*

Conclusions. *Changes in the distribution of DNA PFGE MRSA profiles were largely temporal, with clonal groups being replaced over time, without predominance in any hospital ward or any specific area of the hospital.*

Key words *Staphylococcus aureus*; methicillin-resistant *Staphylococcus aureus*; electrophoresis, gel, pulsed-field; drug resistance, microbial; Brazil.

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Staphylococcus aureus is a pathogen responsible for a variety of community- and hospital-acquired infections; it also colonizes skin and nares of healthy individuals (1). *S. aureus* resistance to β-lactam antibiotics started soon after the introduction of penicillin in clinical practices in the mid-1940s; the most common mechanism is production of penicillinase induced by the *bla* gene, which is usually carried on a plasmid (2). The first description of methicillin-resistant *S. aureus* (MRSA) appeared at about the same time penicillinase-stable β-lactams became available. The main mechanism of methicillin resistance is mediated

by the acquired PBP2a, encoded by the *mecA* gene.

MRSA represents a serious threat to public health throughout the world, spreading fast and showing a great diversity of pandemic clones with relevant virulence and antimicrobial resistance (3). MRSA strains were mainly isolated in hospitals and in ambulatory health care centers (4). Recently, however, MRSA infections emerged, causing severe illness in community scenarios (5).

Molecular typing methods have been applied to help researchers map the spread and evolution of MRSA clones, including pulsed-field gel electrophoresis (PFGE), multilocus sequencing strain, and staphylococcal cassette chromosome *mec* typing (6, 7). PFGE is still considered a standard reference molecular technique for analyzing dissemination of hospital- and community-acquired MRSA and has proved to be one of the most discriminatory methods (8). It has been an excellent laboratory tool for emergency identification of new clones (9).

This study aims to describe the analysis of geographical and temporal distribution of DNA profiles, determined by PFGE, of MRSA strains isolated from hospitalized patients in a tertiary care university hospital.

MATERIALS AND METHODS

The study was conducted in a 400-bed tertiary care, university hospital (Hospital and Clinics HC-UNICAMP) in Campinas, Sao Paulo, Brazil, which provides all major medical services and is the reference hospital for 5 million inhabitants. A descriptive study was performed in a collection of 99 MRSA isolates obtained from 89 patients who were hospitalized from January 1999 to February 2004. MRSA strains were isolated from central venous catheter (33 isolates) and bloodstream (66 isolates) infections. Eighty isolates were individually obtained from 80 patients. A second or third isolate was included in the study, if there had been a minimum interval of 10 days. Nine patients had more than one MRSA isolate.

The isolates were collected in the presence of clinical signs and symptoms of infection. The results from the growth of > 15 colony-forming units from a 5-cm segment of the central venous catheter tip by semiquantitative (roll plate) cultures were considered positive. Blood samples were collected and cultivated by means of the Bactec® automated system. The clinical pathology laboratory had previously identified the MRSA strains and stored them in 10% skim milk, at –20°C, in the molecular epidemiology and infectious diseases laboratory.

The strains were inoculated on blood agar plates and *S. aureus* was confirmed with the Staphy test commercial kit (Probac, Sao Paulo, Brazil). Oxacillin resistance was confirmed by means of the disc diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards using 1-µg oxacillin and 30-µg cefoxitin discs (10).

Genomic DNA preparations for PFGE were done as described by Goering and Duensing (11) with modifications by Branchini et al. (12) using 20 units of *Sma*I restriction endonuclease (Gibco Life Technologies, Grand Island, New York, United States of America). Restriction fragments were separated by means of the CHEF-DR® III (Bio-Rad, Hercules, California, United States) electrophoresis system. Pulse time ranged from 5 to 35 seconds for 18 hours at 6 V/cm. A DNA ladder was used as a molecular weight marker. The gel was stained with ethidium bromide and photographed.

The genetic relationship between two given strains was estimated after images were captured with a digital imaging system (Bio-Capt version 99, Biogene software, Vilbert Loumart, France). The PFGE pattern dendogram was generated by using the Dice coefficient of similarity (CS) (13). Isolates were considered to originate from the same clone if CS = 1. Isolates were considered related if CS < 1 and ≥ 0.90. Isolates were considered as having a different profile if CS < 0.90.

Clinical data were obtained by revising patient records using a standardized form. The following variables were analyzed: age, gender, date and duration of hospitalization, surgical procedure, HIV status, outcome, day care surgical procedure, prior hospitalization if ≤ 1 year (recent) or ≥ 1 year (late), interval (days) of hospitalization, surgical procedures, and recent and late prior hospitalization until a positive MRSA culture was reported.

RESULTS

Eighty-nine patients with documented MRSA infections (blood culture or catheter-related infection) were included in the study; 61 (68.5%) were males from 20 to 50 years old, and 48 (56.4%) had had a surgical procedure. HIV serology was performed in 85 patients and 84.7% had a negative result. The mortality rate in the studied group was 63.5%.

Patients were in different units of the hospital, including clinical and surgical emergencies, bone marrow transplant, hematology, pediatrics, adult and pediatric intensive care wards, oncology, rheumatology, neurosurgery, orthopedics, internal medicine, infectious diseases, nephrology, cardiology, pneumology, gastric surgery, and AIDS day care center. Forty-nine (57.6%) patients had a prior hospitalization; among them, 38 (44.7%) had a hospitalization < 1 year before and 11 (12.9%) had been hospitalized > 1 year before (Table 1).

Analysis of the DNA PFGE profile of 99 MRSA strains showed 26 profiles, which were identified with numbers from 1 to 26, and their respective related profiles were named with lowercase letters from a to g (Table 2). Patients with more than one sample evaluated showed a different DNA PFGE profile.

The DNA PFGE profiles were distributed in three clonal groups—I, II, and III—according to the time when the MRSA strains were isolated. Clonal

TABLE 1. Variables and mean time to MRSA isolation, Campinas, Brazil, 1999–2004

Variable	Mean time, days
Hospital admission (present hospitalization)	25
Surgical procedure during hospitalization	21
Recent hospital discharge (< 1 year)	97
Late hospital discharge (> 1 year)	1 715
Outpatient surgery	141

Note: MRSA: methicillin-resistant *Staphylococcus aureus*.

TABLE 2. Distribution of DNA PFGE profiles and related profiles of 99 MRSA strains, and number of samples per profile, Campinas, Brazil, 1994–2004

DNA PFGE profiles (number of samples)	Related profiles (number of samples)
1 (3)	a (1), b (2), c (3), d (1), e (1), f (1), g (1)
2 (5)	a (2), b (1), c (1)
3 (3)	a (1), b (1), c (1)
4 (2)	a (2), b (1), c (1), d (2)
6 (1)	a (2)
8 (2)	a (1), b (1), c (1)
9 (1)	a (1), b (1)
10 (3)	a (1)
11 (1)	a (1)
12 (2)	a (1), b (1), c (2), d (1)
18 (1)	a (1), b (1), c (2)
21 (2)	a (1), b (1), c (1), d (1), e (1)
23 (1)	a (1)
5 (1), 7 (1), 13 (1), 14 (1), 15 (1), 16 (1), 17 (2), 19 (1), 20 (1), 24 (1), 25 (1), 26 (1)	. . .

Note: PFGE: pulsed-field gel electrophoresis, MRSA: methicillin-resistant *Staphylococcus aureus*, . . . : not applicable.

group I was composed mainly of MRSA strains isolated from January 1999 to April 2002 (DNA PFGE profiles 1–7), clonal group II consisted of isolates predominantly recovered from June 2001 to October 2003 (DNA PFGE profiles 8–20), and clonal group III included isolates from February 2003 to February 2004 (DNA PFGE profiles 21–26). DNA PFGE profile 4 was recovered during the entire study period (Figure 1).

DNA PFGE MRSA profiles were spread homogeneously across all hospital wards (Table 3). No specific localization of DNA PFGE profile with regard to the physical location of hospital wards was observed.

DISCUSSION

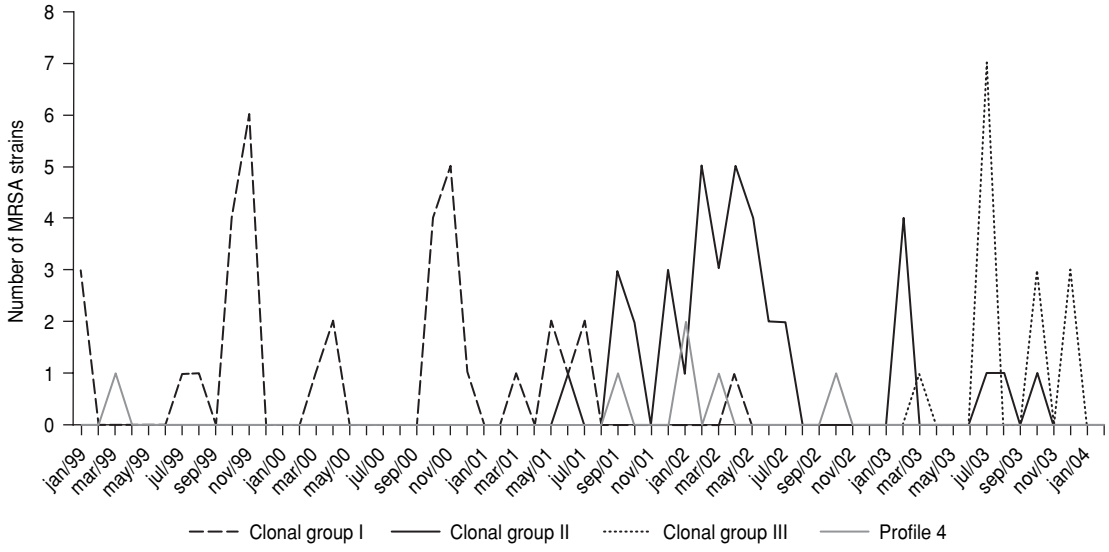
MRSA has been a major concern causing colonization and infections in hospitalized patients worldwide. Hospital infections with MRSA are reported to be caused by strains belonging to a single clone or to clones related to an endemic one (14). A large diversity of MRSA DNA profiles was identified by PFGE during this 6-year study. This study observed that changes in the distribution of DNA PFGE MRSA profiles were largely temporal, with clonal groups being replaced over time, without predominance in any hospital ward or in any specific area of the hospital. Of note, DNA PFGE profile 4 remained present throughout the study period, although it was not a predominant pattern.

Most patients in this study had been previously exposed to a hospital environment either by prior hospitalization or by outpatient surgery. Therefore, despite the mean time of about 3 weeks from hospital admission or inpatient surgery to MRSA detection, whether they had become colonized earlier on is not known. In that case, the DNA PFGE profile would represent the prevalent strain in periods other than the studied period.

A previous study conducted in the same hospital by Padoveze and Branchini (15) demonstrated a high similarity of DNA PFGE MRSA profiles. This study was carried out in the 1990s in two infectious disease wards and mainly included AIDS patients. A later study performed in our institution demonstrated an increasing DNA PFGE profile diversity compared with the previous study (14).

These studies may support a better understanding of the hospital epidemiology by MRSA strains and suggest a progression toward a greater diversity in DNA PFGE profile while MRSA remains

FIGURE 1. Temporal distribution of clonal groups I, II, and III and profile 4 determined by PFGE typing of MRSA strains, Campinas, Brazil, 1999–2004



Note: PFGE: pulsed-field gel electrophoresis, MRSA: methicillin-resistant *Staphylococcus aureus.*

TABLE 3. Distribution of MRSA clonal groups and DNA PFGE profile 4 in hospital wards, Campinas, Brazil, 1999–2004

Hospital ward	Clonal group and DNA PFGE profile 4[a]
Emergency room	I and profile 4[a]
Adult intensive care unit	I, II, III, and profile 4[a]
Bone marrow transplant	I and II
Pediatric intensive care unit	I and II
Pediatric	I
Oncology	II
Rheumatology	III
Neurosurgery	I, II, III, and profile 4[a]
Traumatology	I, II, and profile 4[a]
Orthopedics	I
Hematology	I, II, and III
Nephrology	I and III
Pneumology	I
Infectious diseases	I and II
Internal medicine	I, II, III, and profile 4[a]
Gastric surgery	I and II
Cardiology	Profile 4[a]
AIDS day care center	I, II, III, and profile 4[a]

Note: MRSA: methicillin-resistant *Staphylococcus aureus*, PFGE: pulsed-field gel electrophoresis.
[a] Including DNA PFGE-related profiles.

endemic in our hospital. The diversity and replacement of DNA PFGE MRSA profiles observed over time in this study might be explained by microevolution of the pathogen or by strain competition to adapt in the hospital environment.

Several studies using different molecular typing methods have demonstrated the diversity of MRSA genomic patterns, either in MRSA strains from the same hospital unit or among genotypes disseminated locally, nationally, and internationally. This fact supports the hypothesis that the greater the geographical spread the greater is the genomic evolution (16).

Trinidad et al. (17) reported a high variability in MRSA-related profiles of the Brazilian endemic clone (BEC), where the predominant profile was observed in 15% of the strains. Oliveira (18) reported that 70% of the strains of a study conducted in hospitals in different regions of Brazil belonged to the BEC profile. Da Silva Coimbra et al. (19) reported that 49% of MRSA isolates from three hospitals in Argentina had the profile of the BEC. In a German hospital, Ghebremedhin et al. (20) reported greater heterogeneity among MRSA strains during a 1-year study. Changing patterns of MRSA were also detected in a 16-year study in Portugal (21). Blanc et al. (22) identified MRSA clone replacement by other emerging clones, suggesting a rapid change.

This study was limited to evaluating the DNA profile defined by PFGE of samples obtained from blood- and catheter-related infections. For a better understanding of the characteristics of clonal replacement, appropriate additional research is necessary, including staphylococcal cassette chromosome *mec* typing of all MRSA infection and colonization samples.

In conclusion, this study showed that the distribution of DNA MRSA profiles determined by PFGE was temporally rather than geographically defined in the hospital, demonstrating that temporal changes in the electrophoretic pattern can occur in an endemic environment.

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RESUMEN

Patrón electroforético del ADN de *Staphylococcus aureus* resistente a la meticilina: cambios temporales en un medio hospitalario endémico

Objetivo. Analizar la distribución geográfica y temporal de los perfiles de ADN determinados mediante electroforesis en gel de campo pulsado (PFGE) de cepas de *Staphylococcus aureus* resistente a la meticilina (SARM) aisladas de pacientes internados en un hospital universitario de atención terciaria en el Brasil.

Métodos. Se estudiaron 99 muestras de SARM obtenidas 89 de pacientes en el período 1999–2004. Las cepas de SARM se aislaron de infecciones de catéteres venosos centrales (33 aislados) y del torrente sanguíneo (66 cepas). Para la tipificación genómica se empleó PFGE con 20 unidades de endonucleasa de restricción *Sma*I.

Resultados. El análisis del ADN de 99 cepas de SARM mediante PFGE reveló 26 perfiles, con sus respectivos perfiles relacionados. El intervalo medio de detección de la infección por SARM fue de 26 días desde el ingreso al hospital. En 49 pacientes (57,6%) había habido una hospitalización previa reciente. Los perfiles de ADN de SARM determinados mediante PFGE se distribuyeron en tres grupos clonales —I, II y III— según el período en el que se aislaron las cepas de SARM. Estos perfiles de ADN se encontraban distribuidos de manera homogénea en todos los servicios del hospital.

Conclusiones. Los cambios en la distribución de los perfiles de ADN de SARM determinados mediante PFGE fueron en gran medida temporales, con reemplazo de los grupos clonales con el transcurso del tiempo, y sin predominio en ningún servicio ni área específica del hospital.

Palabras clave *Staphylococcus aureus*; *Staphylococcus aureus* resistente a meticilina; electroforesis en gel de campo pulsado; farmacorresistencia microbiana; Brasil.