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Use of molecular epidemiology to monitor the nosocomial dissemination of methicillin-resistant *Staphylococcus aureus* in a University Hospital from 1991 to 2001

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been the cause of major outbreaks and epidemics among hospitalized patients, with high mortality and morbidity rates. We studied the genomic diversity of MRSA strains isolated from patients with nosocomial infection in a University Hospital from 1991 to 2001. The study consisted of two periods: period I, from 1991 to 1993 and period II from 1995 to 2001. DNA was typed by pulsed-field gel electrophoresis and the similarity among the MRSA strains was determined by cluster analysis. During period I, 73 strains presented five distinctive DNA profiles: A, B, C, D, and E. Profile A was the most frequent DNA pattern and was identified in 55 (75.3%) strains; three closely related and four possibly related profiles were also identified. During period II, 80 (68.8%) of 117 strains showed the same endemic profile A identified during period I, 18 (13.7%) closely related profiles and 18 (13.7%) possibly related profiles and, only one strain presented an unrelated profile. Cluster analysis showed a 96% coefficient of similarity between profile A from period I and profile A from period II, which were considered to be from the same clone. The molecular monitoring of MRSA strains permitted the determination of the clonal dissemination and the maintenance of a dominant endemic strain during a 10-year period and the presence of closely and possibly related patterns for endemic profile A. However, further studies are necessary to improve the understanding of the dissemination of the endemic profile in this hospital.

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- Molecular epidemiology
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens. It causes major outbreaks and represents a severe clinical threat to patients worldwide (1-3), and its frequency has increased in the past 20 years (4). The high mortality and morbidity rates of MRSA infections associated with the potential for intra-hospital dissemination are of great concern to the medical staff and to the infection control team (5,6).

MRSA is highly transmissible among hospitalized patients and the infected or colonized patients are the main reservoir of the bacteria in hospitals worldwide (5). The hospital epidemiology of nosocomial MRSA infections has particular characteristics in each institution and varies according to the complexity and size of the hospitals (7).

MRSA has been one of the major causes of hospital infections in American hospitals, with an increase in percentage from 2.4 to 29% between 1975 and 1991, and currently reaching about 40%. In England, the percentage increased from 1.5 to 13.2% between 1989 and 1995 (8-13). In Brazil, the prevalence of MRSA is high, especially in large and teaching hospitals, ranging from 26.6 to 70%, and MRSA is considered to be the main pathogen causing hospital outbreaks (14-18).

The use of molecular techniques is a valuable resource for the understanding of the hospital epidemiology of these infections and is of help in the application of efficient control measures (19,20). Monitoring the hospital distribution of MRSA by application of DNA-based molecular typing methods has significantly increased the resolution of epidemiological analysis (21,22). Restriction fragment length polymorphism analysis of genomic DNA using pulsed-field gel electrophoresis has been considered to be a reliable DNA-based method for differentiating MRSA strains and has proved to be superior to most of all other molecular typing methods due to its high discriminatory power and reproducibility (23-27).

The purpose of the present investigation was to study the molecular epidemiology of MRSA strains isolated from patients with nosocomial infection hospitalized at the University Hospital of Universidade Estadual de Campinas (Unicamp) over a period of ten years.

Patients and Methods

Study design

The University Hospital of Unicamp is located in the State of São Paulo and is a public, tertiary care teaching hospital with 403 beds, and is the reference center for a population of about 4 million inhabitants.

The ten-year study consisted of two periods: period I from April 1991 to February 1993, and period II from May 1995 to August 2001. The bacterial strains were obtained from patients with hospital infections defined by an infection control practitioner using the CDC definitions for hospital infections (28). All bacterial isolates were first identified by the clinical microbiology laboratory and were stored at -70ºC in 8.0 ml of 10% skim milk. All isolates were obtained from patients hospitalized by different medical specialties of the University Hospital and were randomly included in the study.

Processing of bacterial isolates and susceptibility test

The MRSA isolates included in the study were cultivated on a 5% blood agar plate and identified and tested for susceptibility to antimicrobial agents by the disk diffusion method (29). Readings were obtained within 20 to 24 h and the isolates were classified as susceptible or resistant according to National Committee for Clinical Laboratory Standards (30) recommendations. The antimicrobial

agents evaluated were as follows: oxacillin, ampicillin, penicillin G, cephalothin, gentamicin, amikacin, netilmycin, tetracycline, chloramphenicol, clindamycin, sulfamethoxazole-trimethoprim, imipenen, erythromycin, tobramycin, and vancomycin. All isolates were resistant to oxacillin and susceptible to vancomycin.

Genomic DNA typing

MRSA isolates were grown overnight in brain heart infusion and DNA extraction was performed in agarose plugs as described by Goering and Duesing (for review, see Ref. 16), with modifications. DNA was digested with 20 U *Sma*I (CCC↓GGG) (Gibco Life Technologies) and two thirds of each digested agarose plug was inserted into slots in a 1% agarose gel and run in 0.5X TBE buffer (45 mM Tris, pH 8.0, 45 mM boric acid, and 1 mM EDTA) for 18 h at 6 V/cm. The pulse time ranged from 5 to 35 s. A DNA ladder was used as a molecular weight marker. The gels were stained with ethidium bromide and photographed under UV light. The genetic relatedness among the strains was interpreted by the method of Tenover et al. (23) for bacterial strain typing by pulsed-field gel electrophoresis. Isolates with identical or related pulsed-field gel electrophoresis patterns were considered to derive from a common clone.

The genetic relationship among the pulsed-field gel electrophoresis patterns was also analyzed by computer software after capturing the autoradiographic images with an IS-1000 digital imaging system (Bio-Capt MW, version 99; M&S Instruments Trading Inc.). The dendrogram of the pulsed-field gel electrophoresis patterns was generated using a Dice coefficient (Biogene software; Vilbert-Loumart, France). The dendrogram does not represent the genetic relatedness among the strains according to Tenover et al. (23), but was a graphic representation of the similarity.

Results

Bacterial isolates

Seventy-three MRSA strains isolated from 58 patients during period I and 117 strains isolated from 94 patients during period II were studied. The sources of bacterial isolates are shown in Table 1.

Genomic DNA analysis

Period I. The molecular study by pulsed-field gel electrophoresis after restriction enzyme hydrolysis of the 73 outbreak strains with *Sma*I showed 5 genomic profiles: A, B, C, D, and E. Profile A was identified in this study as the endemic strain and included 55 (75.3%) isolates. Profile A included two categories of genetic relatedness profiles: a closely related subtype represented by 3 strains (4.1%; Figure 1: lanes 3, 4 and 14) and a possibly related subtype represented by 4 strains (5.8%; Figure 1: lanes 2, 9, 12, 13). The endemic profile A is shown in lanes 5, 6 and 7 of Figure 1.

Period II. The molecular study of genomic DNA showed that 80 (68.8%) of the 117 MRSA isolates presented profile A, 18 (13.7%) presented possibly related subtype profiles and 18 (13.7%) closely related subtype profiles, and 1 strain showed profile B which was recognized during period I. Pro-

Table 1. Sites of isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in patients hospitalized in a University Hospital.

Site of isolation	Infection (No. and % of cases)	
	Period I	Period II
Blood	4 (5.4)	45 (38.5)
Lower respiratory tract secretion	20 (27.4)	18 (15.9)
Surgical wound	23 (31.5)	14 (11.9)
Central venous catheter	13 (17.8)	15 (12.9)
Urine	–	14 (11.9)
Other	13 (17.8)	11 (9.4)

Some patients had more than one site of MRSA infection. Period I = 1991-1993; period II = 1998-2001.

file A was also the predominant genomic DNA pattern that was identified during period I as the endemic strain (Figure 2). Figure 3 describes the genetic relationship among the strains from periods I and II.

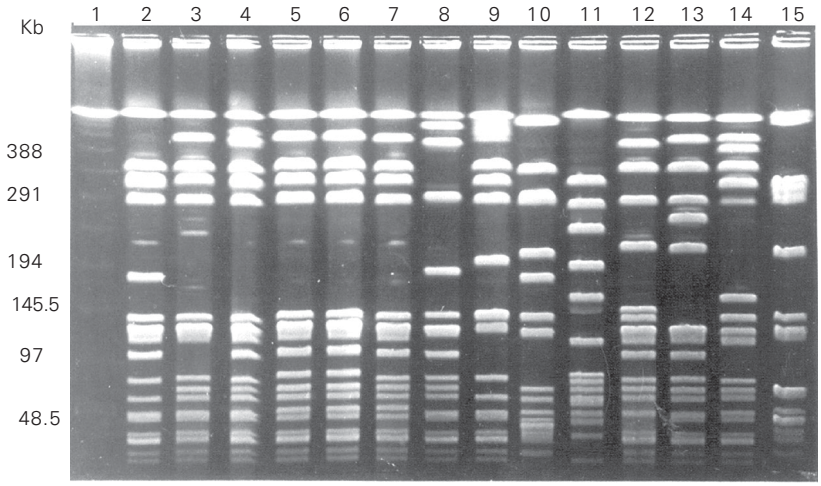

Figure 1. Pulsed-field gel electrophoresis profiles of genomic DNA from methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from patients with hospital infection from April 1991 to February 1993 (period I). *Lane 1*: Molecular weight marker (λ ladder); *lanes 2*, *9*, *12,* and *13*: MRSA profile possibly related to the endemic profile A strain; *lanes 3*, *4,* and *14*: MRSA profile closely related to the endemic strain; *lanes 5*, *6,* and *7*: profile A (endemic strain); *lanes 8*, *10*, *11*, and *15*: profiles E, D, C, and B, respectively.

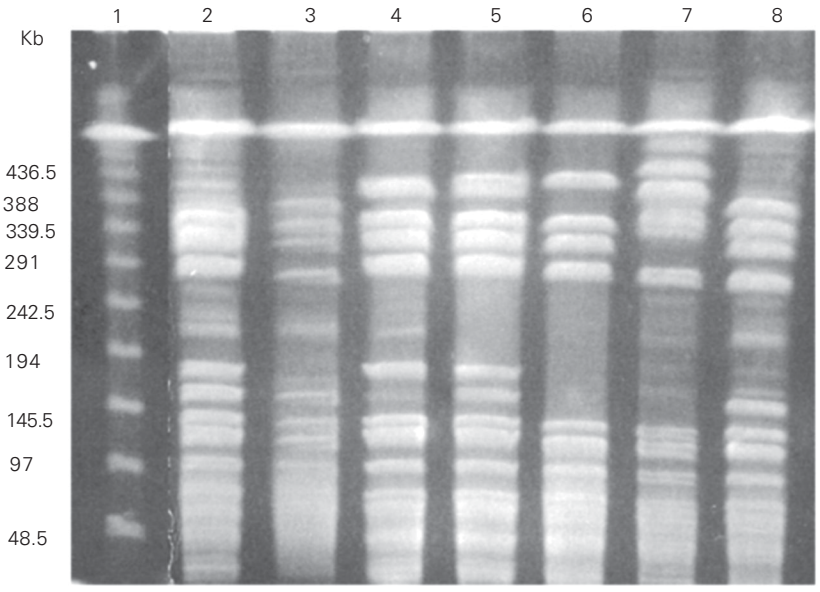

Figure 2. Pulsed-field gel electrophoresis profiles of genomic DNA from methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from patients with hospital infection from May 1995 to August 2001 (period II). *Lane 1*: Molecular weight marker (λ ladder); *lanes 3*, *4,* and *5*: MRSA profile closely related to the endemic strain; *lanes 2*, *7,* and *8*: MRSA profile possibly related to the endemic strain; *lane 6*, profile A, endemic strain.

Discussion

MRSA is recognized as a highly transmissible pathogen causing infection or colonization in hospitalized patients which persists over time in the hospital setting (31). In the present study, the molecular epidemiology of hospital infection caused by MRSA showed that an endemic strain, profile A, was present in a University Hospital for 10 years. During period I (1991-1993), 73% of the MRSA were derived form the same clone. However, closely similar and possibly related strains were also recognized and four unrelated profiles were detected during period I. During period II (1995-2001), the same endemic profile A strain accounted for 68.8% of the isolates genotyped. However, the number of similar strains probably related to the MRSA endemic profile A increased and only one unrelated strain was detected during period II.

These results suggest that the adaptability of endemic profile A in our hospital was high and prolonged. Endemic profile A was first detected in 1993 and Sader et al. (14) soon demonstrated the dissemination of this strain among hospitals in the city of São Paulo. Texeira et al. (32) analyzed MRSA strains from different geographic areas in Brazil and identified the clonal dissemination of this endemic profile A in hospital from different parts of the country. All of these studies were performed with MRSA strains obtained during a limited period of time. Our study consisted of two periods within a 10-year survey and showed the ability of this endemic strain to persist for long periods of time in our hospital and the disappearance of some clones that were present during period I. Only closely similar and possibly related strains were detected during period II, even though the endemic strain was still the most prevalent strain causing hospital infections.

However, it has not been possible to

determine why only the endemic strain and its derived clones remained in the hospital. The epidemiologic typing of MRSA strains was particularly helpful, showing that most strains were derived from relatively few clones (15,31,33).

Computer analysis of the endemic profile A identified in period I and profile A from period II showed 96% of similarity and they were considered to be the same profile A originating from the common MRSA clone widely recognized in São Paulo and other Brazilian hospitals (14,18,34).

A recent study demonstrated a low mutation rate among MRSA strains during a hospital outbreak (1995-2001), leading to a better understanding of the significance and spreading of MRSA in the hospitals. The study suggested the persistence of some MRSA strains as well as the rapid turnover of MRSA in large teaching hospitals and in a general University Hospital of tertiary reference (5).

Previous studies have shown the inter-hospital clonal dissemination of MRSA strains (4,35-37) and have demonstrated that once an endemic MRSA strain is introduced into a hospital, it is very difficult to eradicate it, with a consequent increased incidence of nosocomial MRSA infections (36).

Pulsed-field gel electrophoresis is a good method of high reproducibility and resolving power for epidemiologic differentiation of MRSA isolates (38). The present results demonstrate the potential utility of pulsed-field gel electrophoresis in the epidemiological analysis of the strains. However, it is a slow and time-consuming procedure that requires specifically trained personnel and sophisticated equipment (39,40). The ability of pulsed-field gel electrophoresis to differentiate MRSA strains makes it one of the most appropriate typing methods for investigating the molecular epidemiology of nosocomial MRSA infection (3,4,19,40).

Although it has not been possible to elucidate why only the endemic profile A strain and its derived clones remained in our hospital, we suggest that low adherence to infection control, such as hand hygiene and environmental

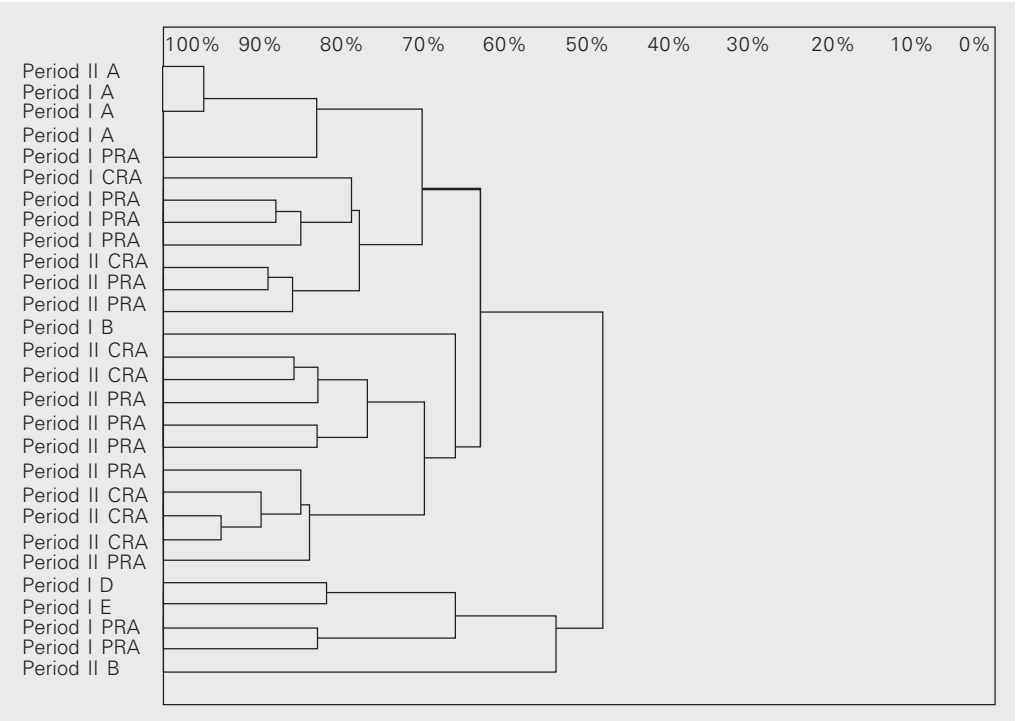

Figure 3. Dendrogram of the methicillin-resistant *Staphylococcus aureus* (MRSA) strains detected by pulsed-field gel electrophoresis during periods I and II, and profiles A, B, D, and E. The values were generated from the Dice coefficient showing the similarity of MRSA strains isolated from April 1991 to February 1993 (period I) and from May 1995 to August 2001 (period II). CRA = closely related to profile A; PRA = possibly related to profile A.

cleaning might play an important role. Further studies on the virulence and adherence factors of MRSA profile A in addition to controlled epidemiology studies involving infection control practices can lead to a better understanding of the molecular epidemiology of MRSA infections in our hospital.

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