

***Staphylococcus aureus* outbreak in the intensive care unit of the largest public hospital in Quito, Ecuador**

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Summary. *Staphylococcus aureus* is a frequent cause of nosocomial pneumonia and bacteremia worldwide. Classical and molecular epidemiology approaches were used to study a *S. aureus* outbreak in the intensive care unit (ICU) of one of the largest public hospitals in Quito. *Staphylococcus aureus* isolates from 17 patients and 19 potential carriers from the staff were collected from March 2007 to February 2008 and analyzed by pulsed-field gel electrophoresis (PFGE) to determine their clonal relationships. During this period the hospital reported 16 cases of hospital-acquired staphylococcal pneumonia and an apparent outbreak occurred from June to September 2007. DNA from these isolates formed six different PFGE patterns: four clonal groups, and two groups of clonally related isolates. Molecular typing failed to identify any staphylococcal reservoir among staff members. The current study suggested that a staphylococcal outbreak that occurred in the summer of 2007 was caused by different bacterial clones, although some clones were shared by two patients. Historical analysis of the staphylococcal infections in the ICU showed a higher incidence during the summer months, which coincided with the programmed personnel shift. This observation suggests that outbreaks might be produced by the introduction of improperly trained personnel. [Int Microbiol 2013; 16(2):81-86]

Keywords: *Staphylococcus aureus* · staphylococcal pneumonia · nosocomial outbreaks · MRSA

Introduction

Nosocomial infections are serious public health problems especially in developing countries, where the rates are 3- to 20-times higher than in industrialized countries [25]. These

infections increase mortality, morbidity, and treatment costs [23]. *Staphylococcus aureus*, while a member of the normal microbiota of the nasopharynx, can also produce lower respiratory tract infections, bacteremia (the second most frequent infection), and sepsis [4,16]. Virulence and antibiotic resistance in *S. aureus* is enhanced by horizontal DNA transfer [8]. The presence of *Staphylococcus aureus* carriers among hospital staff in intensive care, dialysis, and surgical units increases the risk of nosocomial infections two- to three-fold [12,18].

The emergence of resistance to cephalosporins in different bacteria [7] and, especially, outbreaks of methicillin-resistant *S. aureus* (MRSA) have become increasingly widespread in hospitals [12]. There also has been an increasing number of

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nosocomial and community-acquired MRSA infections that carry a high mortality. In fact, a report published in 2008 showed that, in the USA, mortality caused by MRSA infections was similar to the combined mortality associated with AIDS, tuberculosis, and viral hepatitis [3]

Surveillance is critical in the fight against nosocomial infections because it allows an assessment of the true impact of these infections and their associated risk factors, thereby guiding decision-making regarding prevention and control measures [11,19]. Molecular typing is an important epidemiological tool for studying the genetic lineage of pathogens in order to establish their patterns of dissemination. Genotyping assays have traced nosocomial outbreaks of *S. aureus* to a limited number of bacterial clones [1,17,21]. These are endemically recognized bacteria with epidemic behaviors.

This report describes an outbreak of staphylococcal infections in the intensive care unit (ICU) of the largest public hospital in Quito, Ecuador. The main objective of the study was to determine the factors involved in the occurrence of a staphylococcal outbreak in this hospital.

Materials and methods

Sample collection. The study was carried out in the Carlos Andrade Marín Hospital in Quito, a 300-bed tertiary care center. The 18-bed ICU admits critically ill patients. This study was reviewed and approved by the Bioethics Committee of the University of San Francisco de Quito and by the Bioethics Committee of the aforementioned hospital. During a 1-year period (March 2007 to February 2008), we analyzed 17 isolates collected from patients with hospital-acquired *S. aureus* pneumonia. Information about the cause of admission, co-morbidities, length of hospital stay, and bed location

Table 1. Electropherotype and antimicrobial profiles of *Staphylococcus aureus* isolates obtained from patients with pneumonia

Isolate ^a	Date of Isolation	Type	Antibiotic pattern ^b	PFGE pattern ^c
P1	May-07	MRSA	A	I
P2	June-07	MSSA	B	II
P3	June -07	MSSA	C	III
P4	July-07	MRSA	D	IVa
P5	July -07	MRSA	D	V
P6	July -07	MRSA	D	V
P7	Aug-07	MSSA	E	VI
P8	Aug -07	MRSA	D	IVb
P9	Aug -07	MSSA	E	VII
P10	Aug -07	MRSA	D	IVa
P11	Aug -07	MSSA	F	VIII
P12	Aug -07	MSSA	E	IXa
P13	Sept-07	MSSA	G	IXb
P14	Oct-07	MSSA	H	IXb
P15	Nov-08	MRSA	D	X
P16	Dec-08	MRSA	D	X
P17	Feb-08	MSSA	C	IXb

^aAll isolates were cultured from tracheal fluid except for P9 and P16, which were cultured from blood. Most isolates were obtained from patients with mechanic ventilation except for P7, P10, P11, P12. All isolates originated from hospital-acquired pneumonia except for P7, which was obtained from a community-acquired pneumonia.

^bLetters indicate antibiotic sensitivity profiles: (A) resistant to ceftazidime, erythromycin, norfloxacin, and penicillin; (B) no resistance to any of the antibiotics tested; (C) resistant to erythromycin; (D) resistant to ceftazidime, erythromycin, clindamycin, norfloxacin, and penicillin; (E) resistant to penicillin; (F) intermediate resistant to penicillin; (G) resistant to penicillin and norfloxacin; (H) resistant to erythromycin, clindamycin, and penicillin.

^cRoman numbers (I to X) indicate electropherotypes (see Fig. 2).

was also obtained. In the same hospital and during the same period of time, we collected 51 nasal swabs from ICU healthcare workers (14 medical doctors, 15 nurses, 10 nursing assistants, 5 physiotherapists, 1 social worker, 1 administrative staff person, 3 cleaning staff, and 2 technical staff members).

Bacterial isolates. Seventeen *S. aureus* isolates (one bacterial colony per patient) from in-patients with pneumonia were collected between March 2007 and February 2008 from the Carlos Andrade Marín Hospital in Quito. In addition, 19 strains were obtained from nasal samples of 51 healthcare workers employed in the ICU. The samples were cultivated in mannitol salt agar (MSA) and Baird-Parker agar, and were subjected to coagulase and DNase tests. The isolates were stored at -80°C until analyzed. Isolates from patients were labeled P1 to P17 according to the chronological order of isolation. Additionally, *S. aureus* isolates were obtained from the nasopharynx (nasal swab) of the 51 ICU healthcare workers tested.

Antimicrobial sensitivity tests. Antibiotic susceptibility tests were carried out by the disk diffusion method using Mueller-Hinton agar. The antimicrobial agents tested included cefoxitin 30- μg disk (FOX), erythromycin 15- μg disk (E), clindamycin 2- μg disk (DA), norfloxacin 10- μg disk (NOR), and penicillin (P) 10-IU disk. To determine the antimicrobial susceptibility profile to methicillin we used cefoxitin disks and a breakpoint of ≤ 19 mm as indicative of oxacillin resistance, following the standards of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards) [5]. Isolates displaying similar antibiotic sensitivity profiles were designated with a letter (A–H), as shown in Table 1.

Pulsed-field gel electrophoresis. Isolates were analyzed by pulsed-field gel electrophoresis (PFGE). A sample of each *S. aureus* isolate was plated on brain-heart infusion agar and incubated overnight at 37°C . Agarose

plugs were prepared by mixing 200 μl of a bacterial suspension in 75 mM NaCl–25 mM EDTA (pH 7.5) buffer (optical density of 0.63 ± 0.02 , measuring the absorbance at 610 nm) with 200 μl of a molten solution of 1 % BioRad PFGE agarose in TBE 0.5 \times , 1 % SDS solution containing 4 μg of lysostaphin (Sigma-Aldrich, Oakville, Ontario, Canada). Cells were lysed in situ at 37°C for 4 h in lysis buffer (6 mM Tris-HCl [pH 8.0], 1 M NaCl, 100 mM EDTA, 0.5 % Brij-58, 8 μg of lysostaphin, 0.5 % sodium lauroylsarcosine). Cells in plugs were lysed overnight at 50°C in 15 ml of lysis buffer (50 mM Tris, 50 mM EDTA, 1 % Sarkosyl) containing 0.8 mg of proteinase K (Bio-Rad Laboratories, Hercules, CA, USA). The plugs were washed with sterile water and TE and each plug was digested overnight at 30°C in 100 μl of React 4 Buffer containing 0.1 mg/ml BSA and 10 U of the restriction enzyme *Sma*I. The slices were loaded into a 1 % agarose gel, and DNA was separated by PFGE using a CHEF DRII system (Bio-Rad) and TBE 0.5 \times buffer with pulse times of 5–40 s at 6 V/cm for 21 h at 14°C . Concatameric bacteriophage lambda DNA molecules (New England Biolabs, Ipswich, USA) were used as the molecular weight standard [15]. The PFGE patterns of the clinical isolates were compared and clonal relatedness was established based on the recommendations of Tenover et al. [22], followed by a clustering analysis using the UPGMA algorithm with the DICE coefficient.

Results

Bacterial isolates. Seventeen *S. aureus* strains (eight methicillin-resistant *S. aureus*, MRSA, and nine methicillin-sensitive) were isolated from patients during the 1-year study

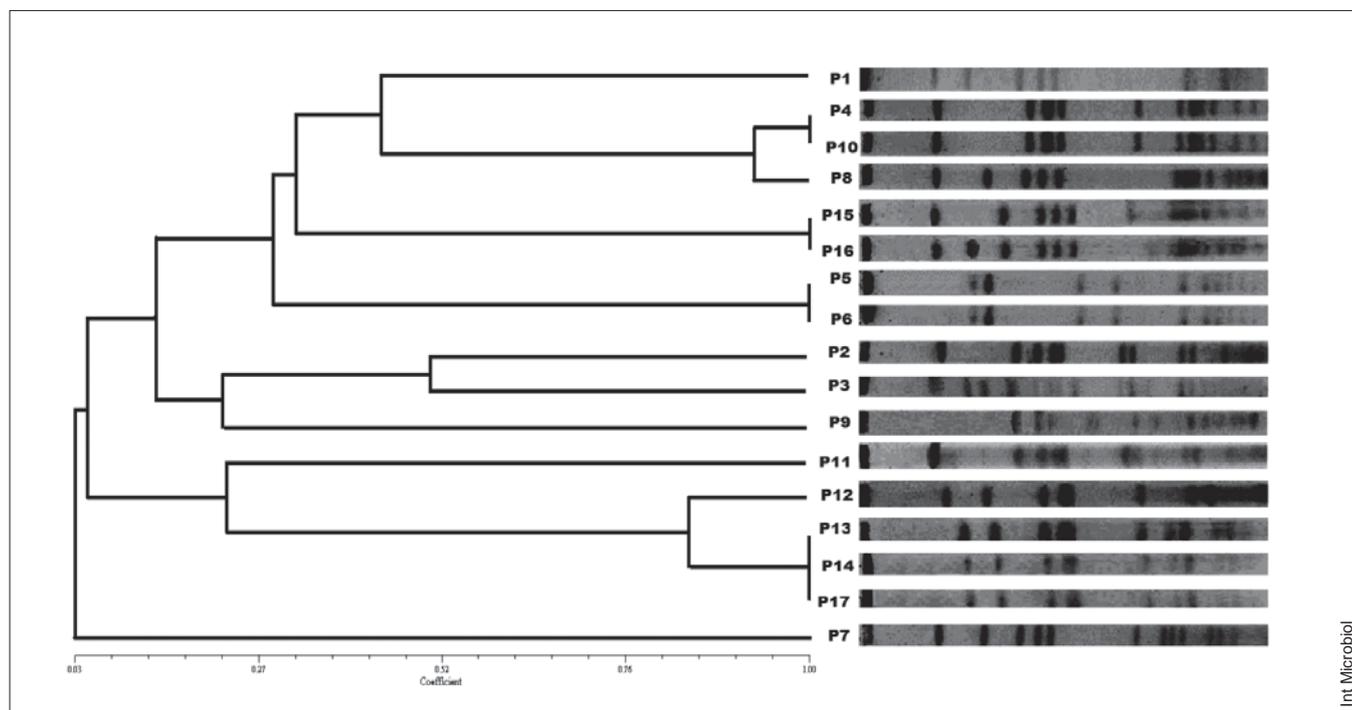


Fig. 1. UPGMA tree constructed with the PFGE profiles obtained from the staphylococcal isolates collected from 17 patients (P1–P17). Distances indicate the number of differences between bands. The DICE coefficient was used; P7, a strain isolated from a patient with community-acquired pneumonia, was regarded as an unrelated isolate. (See Table 1.)

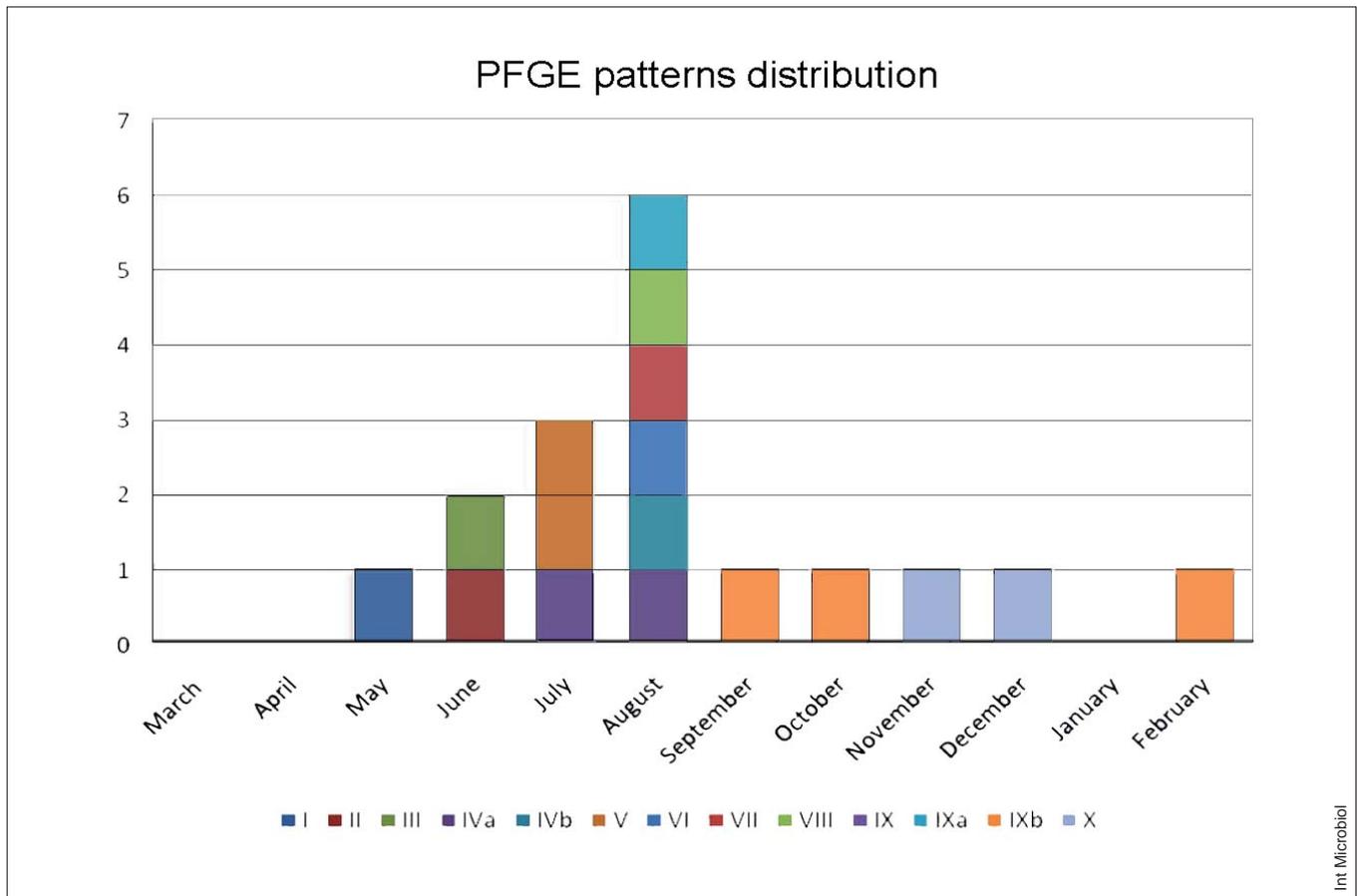


Fig. 2. Time distribution of *Staphylococcus aureus* PFGE patterns. The vertical axes indicate the number of staphylococcal infections. Roman numbers indicate electrophenotypes. (See Table 1 and Fig. 1.)

period. The characteristics of the strains are summarized in Table 1. During the 12-month investigation, the most common nosocomial infection was *S. aureus* ventilator-associated pneumonia (14 patients). The most common cause of admission of patients suffering pneumonia was severe traumatic brain injury (9 patients). An apparent outbreak, consisting of 11 cases of hospital-acquired pneumonia, occurred from July to September 2007 (Fig. 2). Nineteen *S. aureus* isolates were obtained from the 51 ICU healthcare workers from the same hospital.

Clonal analysis of isolates. The isolates associated with infections belonged to six PFGE profiles; four groups of isolates had identical PFGE patterns, and two isolates had similar but not identical profiles. The isolates with identical PFGE patterns were as follows: P4 and P10, belonging to PFGE pattern IVa (isolated in July and August 2007); P5 and P6, belonging to PFGE pattern V (isolated in July 2007); P15

and P16, belonging to PFGE pattern X (isolated in November and December 2007); and isolates P13, P14, and P17, belonging to PFGE pattern IXb (isolated in September, October 2007 and February 2008, respectively) (Fig. 1). The profile of P8 was similar but not identical to PFGE pattern IVa and the profile of P12 was similar but not identical to PFGE pattern IXb (Fig. 1).

Isolates P13, P14, and P17 had identical PFGE patterns (IXb) but their antimicrobial sensitivity profiles differed (Table 1). An isolate from community-acquired pneumonia (isolate P7) was used as the unrelated isolate (Fig. 1). In the phylogenetic analysis, all relationships were supported by the UPGMA algorithm with the DICE coefficient (Fig. 1). The distribution of PFGE patterns over time is consistent with the circulation of a variety of clones during the outbreak occurring between July 2007 and February 2008 (Fig. 2). Although staff members carried *S. aureus*, none of their PFGE patterns was found in the pneumonia outbreak.

Discussion

This report analyzes an outbreak of *S. aureus* that took place from July to September 2007 in the ICU of the largest public hospital in Quito. During this period, most cases of ventilator-associated pneumonia detected in the hospital were caused by *S. aureus*. The bacterium is a common cause of infections on ICUs in other parts of the world [10]. Molecular characterization of the isolates from patients showed that six different clonal groups circulated among ICU patients during this period and that some of the patients were infected with isolates with identical PFGE profiles (Figs. 1,2). However, there was no apparent relationship between the *S. aureus* isolates from patients and those from the personnel (data not shown). By contrast, previous studies had reported that healthcare workers carried bacteria infecting patients [2,13,14,20,24] and that outbreaks are usually caused by a limited number of clones [1,6,17,21]. Our data suggest that, in the Quito hospital, bacteria were transferred between patients, although the small number of samples from healthcare workers in this study may have hampered detection of the same clones in healthy carriers.

The summer outbreak coincided with a personnel rotation. Hospital records indicated that an outbreak of *S. aureus* had occurred during the same period in 2006. From the genotyping data and the time frame during which most of the cases occurred, we speculate that the cause of the outbreaks could have been the presence in the ICU of improperly trained personnel, who might have facilitated the cross-contamination of ventilation equipment. One clone (PFGE pattern IXb) was isolated from cases occurring from September 2007 to February 2008, suggesting that some strains would be carried by other patients or even personnel during this time. However, we failed to identify carriers among staff personnel (we were only able to sample 80 % of the staff, once). Additionally, inconsistencies between PFGE patterns and antibiotic resistance profiles suggest that this clone was subject to horizontal gene transfer during that period.

According to the recommendations of the World Health Organization (WHO), nosocomial infections can be prevented by appropriate isolation, sterilization, staff training, and epidemiologic surveillance [9]. In Carlos Andrade Marín Hospital, however, nosocomial infections were not reported periodically to the infection control committee. Antibiotic prophylaxis was administered to 15 of the 17 patients with acquired staphylococcal pneumonia, which indicates that therapy was unsuccessful. In addition, due to the increase in the number of cases during the summer, the personnel work-

ing on the ICU during those months should be adequately trained to prevent the dissemination of infections.

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Competing interests. None declared.

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