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A comparison of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* reveals no clinical and epidemiological but molecular differences

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ABSTRACT

Most studies on *Staphylococcus aureus* have focused on the molecular epidemiology of methicillin-resistant *S. aureus* (MRSA) infections. In contrast, little information is available regarding the molecular epidemiology of currently circulating methicillin-susceptible *S. aureus* (MSSA) isolates in hospital settings, an epoch when the epidemiology of *S. aureus* has undergone significant changes. We conducted a cross-sectional study to compare the clinical, epidemiological, and genetic characteristics of MSSA and MRSA isolates at 3 tertiary-care hospitals in Medellín, Colombia, from February 2008 to June 2010. The infections were classified according to the Centers for Disease Control and Prevention (CDC) definitions. Genotypic analysis included *spa* typing, multilocus sequence typing (MLST) and staphylococcal cassette chromosome (*mec*) (SCC*mec*) typing. A total of 810 patients was enrolled. One hundred infections (12.3%) were classified as community-associated (31 CA-MSSA, 69 CA-MRSA), 379 (46.8%) as healthcare-associated community-onset (136 HACO-MSSA, 243 HACO-MRSA), and 331 (40.9%) as healthcare-associated hospital-onset (104 HAHO-MSSA, 227 HAHO-MRSA). Genotype analyses showed a higher diversity and a more varied *spa* type repertoire in MSSA than in MRSA strains. Most of the clinical-epidemiological characteristics and risk factors evaluated did not allow for discriminating MRSA- from MSSA-infected patients. The lack of equivalence among the genetic backgrounds of the major MSSA and MRSA clones would suggest that the MRSA clones are imported instead of arising from successful MSSA clones. This study emphasizes the importance of local surveillance to create public awareness on the changing *S. aureus* epidemiology.

Introduction

Staphylococcus aureus is a major human pathogen being responsible for a wide variety of diseases, ranging from superficial skin infections to life threatening conditions such as bacteremia, endocarditis, pneumonia, or toxic shock syndrome (Lowy, 1998). The epidemiology of *S. aureus* changed mainly due to the emergence of methicillin resistance in 1961. Methicillin-resistant *S. aureus* (MRSA) infections were increasingly common in hospitals worldwide and those at higher risk were hospitalized patients or residents of long-term care facilities. This type of infection was denominated healthcare-associated MRSA (HA-MRSA) (Deurenberg and Stobberingh, 2008). But, MRSA also emerged causing infection in the community (community-associated MRSA, CA-MRSA), affecting patients who had never been hospitalized nor had known risk factors for MRSA infection (Deleo et al., 2010; Deurenberg and Stobberingh, 2008). In the present decade, increasing evidence suggests that CA-MRSA strains are infiltrating healthcare settings in many countries all over the world (Popovich et al., 2008). In South America including Colombia, the USA300-related MRSA

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strains are now reported causing nosocomial infections (Jimenez et al., 2012; Reyes et al., 2009). In fact, CA-MRSA infection has become endemic and more prevalent than community-associated methicillin-susceptible *S. aureus* (CA-MSSA) infection (Frazee et al., 2005; King et al., 2006; Moran et al., 2005; Sattler et al., 2002). In South America, most studies have focused on the description of the molecular epidemiology of MRSA infections in hospitals and in the community. As a consequence, there is little information regarding the molecular characterization of MSSA infections in these settings (Van Dijk et al., 2002; Vivoni et al., 2006). Considering the changing epidemiology of *S. aureus*, this study was conducted to compare the clinical, epidemiological, and molecular features of currently circulating MSSA and MRSA strains in 3 hospitals of Medellín, Colombia. Understanding the epidemiology of MSSA and MRSA infection, particularly in developing countries where the knowledge of *S. aureus* transmission dynamics is probably limited, is fundamental for devising effective prevention and control strategies.

Material and methods

Institutional review board approval

The study protocol was approved by the Bioethics Committee for Human Research at Universidad de Antioquia (CBEIH-SIU) (approval No. 0841150). An informed consent to participate in the study was signed by participants, parents, and/or guardians.

Study population

An observational cross-sectional study was conducted from February 2008 to June 2010, at 3 tertiary care hospitals of varying sizes. Hospital A is a large 648-bed university hospital, hospital B is a 380-bed medium-size tertiary care center, and hospital C is a 140-bed cardiology hospital. All institutions are located in Medellín, the second largest city in Colombia.

S. aureus isolate collection

Patients were prospectively identified by following the results of *S. aureus* clinical cultures as reported by the microbiology laboratory. Only the first isolate from each patient was evaluated. The MRSA collected correspond to all the isolates obtained during the time of the study and were described previously (Jimenez et al., 2012). Considering that the prevalence of MSSA is higher, a sample was defined and the sample size calculated based on the MSSA prevalence during 2007 within each institution. The MSSA isolates included were randomly selected each month, from February 2008 to June 2010, using a table of random numbers according to records of each participating institution.

Clinical and epidemiological data

Clinical and epidemiological data from each participant were obtained from medical records. Information included demographic aspects, medical history, antimicrobial use, hospitalization, comorbidities, type of infection, treatment, and length of hospital stay.

First, a comparison of clinical and epidemiological characteristics between MSSA and MRSA was performed. Then, the variables considered being risk factors for the acquisition of MRSA were also compared. To further strengthen the comparisons, the standard epidemiological definitions established by the Centers for Disease Control and Prevention (CDC) for the classification of the MRSA infections in community-associated (CA-MRSA) or healthcare-associated (HA-MRSA), were applied in order to assess the CA- or HA- status of MSSA infections (Klevens et al., 2007). Healthcare-associated infections were further classified as either community-onset (HACO) or hospital-onset (HAHO). Infections were defined as HACO if (i) a positive *S. aureus* culture was obtained within the first 48 h of hospital admission, and (ii) at least one of the following healthcare-associated risk factors was present: presence of an invasive device at the time of admission, history of *S. aureus* infection or history of surgery, hospitalization, dialysis or intensive care unit (ICU) admission, and use of antibiotics during the preceding year. Infections were HAHO if (i) a positive *S. aureus* culture was obtained within 48 h after hospital admission, and (ii) at least one of the above-mentioned risk factors was present. Lastly, infections were defined as community-associated (CA) if (i) a positive *S. aureus* culture was obtained during the first 48 h of hospital admission without healthcare-associated risk factors (Klevens et al., 2007).

Strains and antibiotic susceptibility

Identification of *S. aureus* was conducted by standard laboratory methods based on colony morphology in sheep blood agar and positive catalase and coagulase tests (Becker and von Eiff, 2011). Antibiotic susceptibilities of *S. aureus* isolates were assessed in accordance with Clinical Laboratory Standards Institute guidelines (CLSI, 2009) using a Vitek 2 instrument (bioMérieux, Marcy l'Etoile, France). The antibiotics tested included clindamycin, erythromycin, gentamicin, linezolid, moxifloxacin, oxacillin, rifampin, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, and vancomycin. The *S. aureus* ATCC 29213 strain was used for quality control. Presence of the species-specific *nuc* and *fem*A genes as well as the *mec*A gene (determinant of methicillin resistance) was verified by polymerase chain reaction (PCR) as previously described (Brakstad et al., 1992; Mehrotra et al., 2000).

Strain typing: spa typing (spa), multilocus sequence typing (MLST) and SCCmec typing

In all isolates, the protein A gene polymorphic region (*spa*) was amplified and sequenced (Shopsin et al., 1999). Corresponding *spa* types were assigned using eGenomics software (Mathema et al., 2008; Shopsin et al., 1999), and Ridom *spa* types were subsequently assigned using the *spa* typing website (http://www.spaserver.ridom.de/) developed by Ridom GmbH and curated by SeqNet.org (http://www.SeqNet.org/) (Harmsen et al., 2003). MLST was performed on a subset of the most prevalent and representative *spa* types (64 isolates) using the methodology described by Enright et al. (2000). The allele numbers and sequence types (STs) were assigned using the database maintained at http://saureus.mlst.net/, while clonal complexes (CCs) were inferred using eBURST analysis (Feil et al., 2004). Clonal complexes for all remaining strains were inferred by *spa* repeat pattern analysis (Mathema et al., 2008; Strommenger et al., 2006) or by referring to the Ridom SpaServer website. For MRSA isolates, the SCC*mec* types and subtypes were determined using a set of multiplex PCR reactions (Kondo et al., 2007).

Statistical analyses

Comparisons of clinical, epidemiological, and molecular characteristics, as well as the risk factors, were carried out between MSSA- and MRSA-infected patients and among the different groups of infections obtained after applying CDC criteria. Categorical variables were compared using Chi-square test or Fisher's exact test or Student's *t* and Mann–Whitney *U* tests for continuous variables. p values ≤ 0.05 were considered statistically significant. Statistical analyses were carried out using the software package SPSS® v15.0.