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Isolation of community-acquired methicillin-resistant *Staphylococcus aureus* in healthy carriers in a Mexican community

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SUMMARY

Objectives: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) clones are spreading rapidly among the population in many regions worldwide. Little information is available on CA-MRSA in Mexico. The aim of this study was to identify CA-MRSA strains in the nose and throat of healthy people in a Mexican community.

Methods: A total of 131 MRSA strains from the nose and throat obtained from healthy people in Mexico City were characterized. The genes *mecA*, *lukS-PV/lukF-PV*, and ACME-*arcA* were detected by PCR. Staphylococcal cassette chromosome *mec* (SCC*mec*), pulsed-field gel electrophoresis (PFGE), and *spa* typing were performed.

Results: Bacteria that had a Panton–Valentine leukocidin (PVL)-positive gene and SCC*mec* type IV or V were designated as CA-MRSA strains. We found that 21.4% of MRSA strains were CA-MRSA and that the percentage of CA-MRSA strains was similar in the nose and the throat. A great diversity of profiles was found in the strains identified by PFGE pattern and *spa* typing. Only one strain similar to the USA300 genotype was found; this strain carried the ACME-*arcA* gene.

Conclusions: CA-MRSA strains were detected in the nose and throat of healthy people. We identified a high level of genetic diversity among CA-MRSA strains in healthy people of Mexico City, which were different from the USA and pandemic clone profiles.

1. Introduction

Staphylococcus aureus, particularly methicillin-resistant *S. aureus* (MRSA), is one of the most important nosocomial pathogens; however, in the early 1990s, infections in healthy individuals emerged among communities in Australia.[1] These clones were called community-acquired/associated MRSA (CA-MRSA).

CA-MRSA may cause many types of infection, ranging from mild skin infections to severe abscesses, sepsis, and necrotizing pneumonia.[2] The most severe manifestations of disease may be fatal, such as pneumonia, which occurs in approximately 1–2% of CA-MRSA infections.[3]

Molecular typing studies have shown that CA-MRSA differs from hospital-associated MRSA (HA-MRSA):[4,5] these strains belong to distinct genetic lineages and usually carry the staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, V, or VII[6] and specific virulence factors, such as Panton–Valentine leukocidin (PVL).[7] CA-MRSA has also been shown to have higher expression levels of toxins, phenol soluble modulins (PSMs), and hemolysins.[2] The USA300 clone can harbor the arginine catabolic mobile element (ACME), and the *arc* gene cluster within the ACME may function as a virulence or strain survival factor.[8,9] In general, CA-MRSA is more virulent compared to HA-MRSA due to the presence of various virulence factors.[10]

CA-MRSA clones have been spreading rapidly in communities. The CA-MRSA epidemic has led to an overall increase in MRSA infections in the USA.[11,12] The clone predominating in the USA is USA300,[12,13] whereas CA-MRSA infections in other parts of the world are generally caused by other clones.

Today, five major PVL-positive CA-MRSA clones are disseminating worldwide: the ST1 clone is observed in Asia, Europe, and the USA, the ST8 clone in Europe and the USA, the ST30 clone in Australia, Europe, and South America, the ST59 clone in Asia and the USA, and the ST80 clone in Asia, Europe, and the Middle-East.[10]

CA-MRSA has started to replace HA-MRSA in healthcare facilities, especially in the USA and Taiwan where the CA-MRSA prevalence is high.[14,15]

Staphylococci are ubiquitous colonizers of human epithelia and mucous membranes and are opportunistic pathogens. About a

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third of the population is colonized with *S. aureus*, mainly in the nose; there is a clear correlation between nasal colonization and infection, indicating that most infections originate from colonizing strains.[16,17]

Nasal colonization is the most significant predictor of invasive disease; however, in some studies, nearly half of patients carrying *S. aureus* are colonized extranasally.[18] The throat in particular may be selectively colonized and escape current routine screening programs.[19–21]

In a previous work, we investigated the presence of *S. aureus* in the throat and anterior nares of healthy volunteers in Mexico City. We found that 59.8% of the volunteers carried *S. aureus*, and of these 8.6% had MRSA strains.[22]

The aim of this study was to identify CA-MRSA strains in these same healthy people.

2. Materials and methods

2.1. Study population

Volunteers were recruited from schools and factories in Mexico City. None of the volunteers had recently been hospitalized. Volunteers were screened for nasal and throat carriage. The age of the subjects ranged from 1 to 96 years (mean age 21 years); 54% were females and 46% were males.[22]

Samples were collected in accordance with relevant guidelines for ethical research design, confidentiality, and the protection of human subjects. The protocol was reviewed and approved by the ethics and biosafety committees of the Universidad Autónoma Metropolitana-Xochimilco. All participants provided their informed consent. Great care was taken to ensure that all people understood that they would participate as volunteers; no academic or economic incentives were offered.

2.2. Bacterial isolates

A total of 131 MRSA strains from 1039 *S. aureus* strains obtained from healthy people in Mexico City were characterized: 65 strains were from the nose (N) and 66 strains were from the throat (F). Of these, 50 strains were exclusively from nasal carriers, 51 strains were exclusively from throat carriers, and 30 strains were from nasal and throat carriers.[22]

2.3. Detection of the mecA gene, PVL genes, and the ACME-arcA gene

mecA PCR was performed as described by Oliveira and de Lencastre.[23] The *lukS-PV/lukF-PV* genes (which encode PVL) were detected by PCR as described by Lina et al.[24] The ACME-*arcA* gene PCR was performed as described previously.[25] *S. aureus* NRS643 was used as positive control for these PCR assays.

2.4. SCCmec typing

SCC*mec* types were determined using two previously described sets of multiplex PCR reactions.[23,26] MRSA strains used as positive controls for SCC*mec* types were ATCC BAA44 (Iberian MRSA clone), ATCC BAA41 (New York MRSA clone), ATCC BAA39 (Hungarian MRSA clone), and ATCC BAA1680 (USA300). Several *mecA* amplicons were sequenced and compared to sequences listed on the website SCC*mec* (http://www.staphylococcus.net).

2.5. Pulsed-field gel electrophoresis (PFGE)

PFGE following *Sma*I digestion was performed according to a protocol described elsewhere.[27] DNA fragment patterns were normalized using *S. aureus* strain NCTC8325. Samples were run on a CHEF-DR II system (Bio-Rad). Gels were photographed and digitized using Bio-Rad Gel Doc.

PFGE patterns were analyzed with Gene Tool software and Gene Directory software (Syngene, UK). Percent similarities were obtained from the unweighted pair group with mathematical average (UPMGA) based on Dice coefficients. Band position tolerance was set at 1.25%. The PFGE patterns were compared using the criteria of Tenover et al.[28] for bacterial strain typing, and a similarity coefficient >75% was selected to define possibly related isolates.

All MRSA isolate PFGE patterns were compared with the PFGE patterns of the following reference strains: NRS642 (USA100), NRS651 (USA200), NRS643 (USA300), NRS123 (USA400), NRS648 (USA600), NRS386 (USA700), NRS653 (USA800), NRS652 (USA1000), NRS7450 (USA1000, SCC*mec* V), ATCC BAA44 (Iberian MRSA clone), ATCC BAA41 (New York MRSA clone), and ATCC BAA39 (Hungarian MRSA clone).

2.6. spa typing

The polymorphic X region of the protein A gene (*spa*) was amplified and sequenced as described previously.[29] Corresponding *spa*-types were assigned using the SPA Searcher website (http://seqtools.com). Ridom *spa*-types were subsequently assigned using the *spa* typing website developed by Ridom GmbH (http://spaserver.ridom.de/).

2.7. Statistical analysis

The Chi-square test was applied. Data were analyzed using JMP 9.0 statistical package (SAS Institute Inc., Cary, NC, USA). A critical value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Characterization of MRSA strains

Of the 131 MRSA isolates analyzed by multiplex PCR, the SCC*mec* types of 129 (98.5%) could be determined. Four different SCC*mec* types and a subtype were detected, including II, III, IV, IVa, and V. The majority of the typeable MRSA isolates harbored SCC*mec* type IV (66, 50.4%), followed by SCC*mec* type II (31, 23.6%) and SCC*mec* type IVa (28, 21.4%). The remaining SCC*mec* types were present at very low frequencies, including types V (3, 2.3%) and III (1, 0.8%); SCC*mec* type I was not detected at all (Table 1).

The genes for PVL (*lukS-PV/lukF-PV*) were present in isolates harboring SCC*mec* types II, IV, IVa, and V, and were most frequently observed in isolates harboring SCC*mec* type IV (22, 67%), followed by SCC*mec* type IVa (5, 15%) and SCC*mec* type II (5, 15%), and SCC*mec* type V (1, 3%) (Table 1).

The isolates were classified as CA-MRSA when they presented the SCC*mec* type IV, or IVa, or V, and the *lukS-PV/lukF-PV* genes. Twenty-eight CA-MRSA strains were found. This corresponds to 21.4% (28/131) of MRSA strains or 2.6% of all isolates (28/1039).

Similar numbers of strains with different SCC*mec* types and that were PVL-positive were found in isolates exclusively from nasal carriers and isolates exclusively from throat carriers; no statistical significance was observed ($p = 0.2382$) (Table 1). Therefore, the percentage of CA-MRSA strains is similar in both niches: 22.4% (11/49) in exclusive nasal carriers and 17.6% (9/51) in exclusive throat carriers ($p = 0.8657$). The percentage was slightly higher – 25.8% (8/31) – in isolates from nasal and throat carriers (Table 1).

The ACME-*arcA* gene was detected in only four CA-MRSA strains (106F, 1251F, 1668F, and 1668N).