# Nosocomial infections caused by community-associated methicillin-resistant *Staphylococcus aureus* in Colombia

Carlos Arturo Alvarez, MD, MSc, DTM&H,[a] Nancy Yomayusa, MD,[b] Aura Lucia Leal, MD, MSc,[c] Jaime Moreno, MSc,[d] Sebastian Mendez-Alvarez, PhD,[e,f] Milciades Ibañez, MSc,[g,h] and Natasha Vanegas, PhD[i]
Bogotá, Columbia, and Tenerife, Spain

***Background:*** Community-associated methicillin-resistant *Staphylococcus aureus* strains (CA-MRSA) have emerged as the causative agent of health care-associated infections.
***Methods:*** An observational and prospective study was carried out in 5 hospitals in Bogotá, Colombia; severe MRSA infections were identified, and their origin led to classification as health care-associated (HA-MRSA), community-associated, or nosocomial infections. MRSA isolates were analyzed by SCC*mec*, pulsed-field gel electrophoresis, multilocus sequence typing, and virulence factors.
***Results:*** Twenty-six (10.4%) CA-MRSA nosocomial infection-causing strains (eg, USA300) were detected in 250 MRSA infection isolates in mainly primary bacteremia and surgical site infections. The mortality related to nosocomial infection by CA-MRSA was 27%.
***Conclusion:*** The presence of nosocomial infection by CA-MRSA in Colombia was confirmed.
***Key Words:*** Methicillin-resistant *Staphylococcus aureus*; Colombia; nosocomial infections.



Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Latin America has been described in Brazil, Uruguay, Colombia, and Argentina.[1,2] CA-MRSA has begun to replace typical hospital-acquired isolates during recent years, especially in the United States and Taiwan where CA-MRSA prevalence (52%) is very high.[3] Colombian CA-MRSA isolates (such as ST8) have recently been confirmed and are clonally related to USA300.[4] The presence of CA-MRSA causing nosocomial infection in several Colombian institutions was confirmed in this study.

From the Departamento de Enfermedades Infecciosas[a] and Departamento de Medicina Interna,[b] Facultad de Medicina, UNISANITAS, Clinica Reina Sofia, Bogotá, Colombia; Facultad de Medicina, Universidad Nacional de Colombia, Bogotá, Colombia[c]; Departamento de Microbiologia, Instituto Nacional de Salud, Bogotá, Colombia[d]; Laboratorio de Biología Molecular, Instituto de Investigación, Hospital Universitario Nuestra Señora de Candelaria, Tenerife, Spain[e]; Departamento de Microbiología, Universidad de La Laguna, Tenerife, Spain[f]; Departamento Medicina Comunitaria, Facultad de Medicina, Universidad El Bosque, Bogotá, Colombia[g]; Facultad de Medicina UNISANITAS, Clinica Reina Sofia, Bogotá, Colombia[h]; and Laboratorio de Genética Molecular Bacteriana.[i]

Address correspondence to Carlos Arturo Alvarez, MD, MSc, DTM&H, Calle 122 No. 11D-71 Apto 412, Bogotá, Colombia. E-mail: calvarem@gmail.com.

Conflicts of interest: None to report.

0196-6553/$36.00



doi:10.1016/j.ajic.2009.05.013

## METHODS

### Demographic characteristics and epidemiologic and clinical classification criteria

An observational and prospective study was carried out between June 2006 and December 2007; patients requiring hospitalization who were older than 18 years and suffering from MRSA infection and who had been attended at 5 third-level hospitals in Bogotá, Colombia, were included in the study. Once the isolates had been confirmed, they were classified by using previously described clinical and epidemiologic criteria as health care-associated MRSA (HA-MRSA), community-associated, or nosocomial infections.[5,6] To summarize, categorization required a patient's clinical history to be evaluated, as well as the timing of specimen collection for clinical cultures. Infection in a patient was likely to have been acquired during their hospital stay without any evidence having been presented that infection was incubating or present on admission.

### Antimicrobial identification and susceptibility tests

The presence of MRSA was confirmed by simultaneous amplification of *nuc* and *mecA* genes. Automated systems (MicroScan combo positive 25 WalkAway plus

System; Dade Behring, West Sacramento, CA) were used for the antimicrobial susceptibility tests; these were confirmed by the agar dilution method, following Clinical Laboratory Standards Institute recommendations (2008).[7]

## Classifying SCC*mec* elements and detecting virulence factor encoding genes

SCC*mec* type and subtype were detected by previously described multiple poymerase chain reaction.[8] The presence of *lukF-PV* and *lukS-PV* genes (Panton Valentine leucocidin [PVL]) was also determined, as was that of 14 genes encoding SEA-SEQ enterotoxins, and of *icaA*, *icaB*, *icaC*, *icaD*, and *icaR* genes (forming part of the *ica* operon, related to biofilm); *fnbA*, *fnbB*, *fib*, *clfA*, *clfB*, *eta*, *etb*, and *tst* genes (encoding fibronectin and fibbrinogen binding proteins, clumping factor, exfoliative toxins, and toxic shock syndrome, respectively).

## Pulsed-field gel electrophoresis and multilocus sequence typing

The methodology described by Cruz et al was used for MRSA pulsed-field gel electrophoresis (PFGE)[9]; Dice coefficient-based methodology was used for the analysis (greater than 85% similarity), and multilocus sequence typing was performed using the technique described by Enright et al.[10] PFGE was carried out on all isolates, and then multilocus sequence typing was applied to at least 1 isolate from each PFGE type.

## RESULTS

Two hundred fifty MRSA infections (66 [26.4%] with CA-MRSA) were confirmed during the study period. Table 1 describes the clinical and demographic characteristics of the 26 patients suffering from nosocomial infection in which the presence of CA-MRSA strain was confirmed; the remaining 40 CA-MRSA isolates were considered to be due to community-associated infections. CA-MRSA nosocomial infections occurred in people older than 60 years and were especially related to surgical site infection and bacteriemia; there was a 27% infection-related mortality rate.

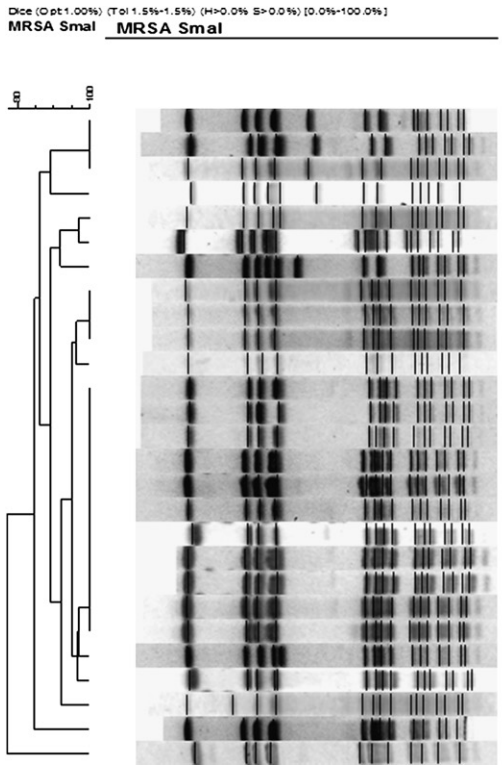
CA-MRSA nosocomial infections showed (COL70, COL 86, COL99, COL593, COL614) to be temporal and geographically related in 5 patients of the health centers. A close genetic relationship between isolates was confirmed by PFGE, possible cross transmission being considered (Fig 1).

Two similar strains of CA-MRSA nosocomial infections were found in 2 different health centers. Each strain showed a temporal and geographic relationship between 3 (COL70, COL86, COL99) and 2 (COL593, COL614) patients, respectively. A close genetic relationship between isolates was confirmed by PFGE, possible cross transmission being considered (Fig 1).

**Table 1.** The demographic, clinical, and epidemiologic characteristics of patients suffering from hospital-onset CA-MRSA infection

| Characteristics | Hospital-onset CA-MRSA infection, n (%) |
|---|---|
| Sex | |
| Male | 12 (48) |
| Age groups, yr | |
| <40 | 6 (23) |
| 40-49 | 3 (12) |
| 50-59 | 4 (15) |
| ≥60 | 13 (50) |
| Comorbidity | |
| Basic chronic disease | 19 (73) |
| Diabetes | 3 (12) |
| Chronic obstructive pulmonary disease | 2 (8) |
| Solid neoplasia | 5 (19) |
| Chronic renal disease | 4 (15) |
| Other basic pathologies | 15 (57.7) |
| Hospital factors | |
| Central catheter | 5 (19) |
| Peripheral catheter | 13 (50) |
| Arterial catheter | 2 (8) |
| Urinary tract catheter | 10 (38) |
| Nasal gastric tube | 2 (8) |
| Hemodialysis | 4 (15) |
| Mechanical ventilation | 3 (12) |
| Surgery | 19 (73) |
| Infection diagnosis | |
| Laboratory-confirmed bacteriemia | 9 (35) |
| Surgical site infection | |
| Organ space | 5 (19) |
| Deep | 5 (19) |
| Superficial incisional | 3 (12) |
| Other | 4 (15) |
| McCabe index | |
| Rapidly fatal | 5 (26) |
| Ultimately fatal | 14 (73) |
| Appropriate initial antibiotic therapy | 13 (50) |
| Outcomes | |
| Management in ICU | 6 (23) |
| Improvement | 15 (58) |
| No improvement | 1 (4) |
| Relapse | 3 (11) |
| Death | 7 (27) |

Figure 1 shows the molecular characteristics of the isolates analyzed in this study. PFGE patterns revealed 12 different pulsotypes having ST8 similarity coefficients ranging from 87% to 92% (compared with the USA300-0114 clone). Most isolates presented SCC*mec* IVc (96%), found for the first time in Colombia; 30.8% of these being only resistant to β-lactamase antibiotics, and 53.8% exhibited additional tetracycline resistance. Regarding the presence of virulence




| Isolate | Resistance | PFGE | SCC*mec* | *LukS/F* | *ica* | *fnbA/B* | *clfA/B* | *sek* | *seq* | *eta* |
|---|---|---|---|---|---|---|---|---|---|---|
| .COL42 | OXA | U4 | IVc | + | + | + | + | + | + | + |
| .COL305 | OXA,ERY | U4 | IVc | + | + | + | + | + | + | + |
| .COL402 | OXA,ERY,CLIN | U4 | IVc | + | + | + | + | + | + | + |
| .COL523 | OXA | U20 | IVa | + | + | + | + | + | + | + |
| .COL404 | OXA,TET | U12 | IVc | - | + | + | + | + | + | - |
| .COL70 | OXA | U14 | IVc | + | + | + | + | + | + | - |
| .USA300 | OXA,ERY,TET | U | IVa | + | + | + | + | + | + | + |
| .COL418 | OXA | U2 | IVc | + | - | - | + | - | - | + |
| .COL593 | OXA,TET | U2 | IVc | - | + | + | + | + | + | - |
| .COL614 | OXA | U2 | IVc | - | + | + | + | + | + | - |
| .COL359 | OXA,GEN,CIP,ERY,TET | U5 | IVc | + | + | - | + | + | + | + |
| .COL332 | OXA,TET | U1 | IVc | + | + | - | + | - | - | + |
| .COL344 | OXA,TET | U1 | IVc | + | + | + | + | + | + | - |
| .COL352 | OXA,TET | U1 | IVc | + | + | + | + | + | + | + |
| .COL68 | OXA | U1 | IVc | + | + | + | + | + | + | + |
| .COL71 | OXA,TET | U1 | IVc | + | + | + | + | + | + | + |
| .COL257 | OXA,CIP | U1 | IVc | + | + | + | + | + | + | + |
| .COL86 | OXA,CIP | U1 | IVc | + | + | + | + | + | + | + |
| .COL129 | OXA | U1 | IVc | + | + | + | + | + | + | - |
| .COL196 | OXA,ERY,CLIN | U1 | IVc | + | + | + | + | - | + | - |
| .COL126 | OXA,TET | U1 | IVc | + | + | + | + | + | + | - |
| .COL135 | OXA,TET | U1 | IVc | + | + | - | + | - | - | + |
| .COL242 | OXA,TET | U8 | IVc | + | + | + | + | + | + | - |
| .COL99 | OXA | U9 | IVc | + | + | + | + | + | + | - |
| .COL414 | OXA,TET | U3 | IVc | + | + | + | + | + | + | - |
| .COL241 | OXA,CIP,TET | U17 | IVc | + | + | + | + | + | + | + |
| .COL555 | OXA,GEN,ERY,TET,CLIN | U7 | IVc | - | + | + | + | - | - | + |

**Fig 1.** Microbiologic and molecular characteristics of the health care-associated infection MRSA isolates. This dendogram shows the clinical isolates' close genetic relationship to the USA300-0114 clone (U). Antimicrobial resistance: OXA, oxacilin; ERY, erythromycin; CLIN, clindamycin; TET, tetracycline; CIP, ciprofloxacin; GEN, gentamicin. The presence of genes encoding different virulence factors. *lukS/F*, Panton Valentine leucocidin; *ica*; ica operon; *fnbA/B*, fibronectin-binding proteins; clfA/B, clumping factor; sek and seq, enterotoxins K and Q respectively; ETA, exfoliative toxin A.

factors, 85% of the isolates carried *fnbA/B* and *LukS/F-PV* genes; 77% and 81% *sek* and *seq* genes, respectively; and 100% *clfA/B* genes. Interestingly, the *eta* gene was only detected in 15 isolates (57.7%). However, 96% of the isolates had the *ica* operon genes needed for biofilm formation; in this case, phenotypical characteristics were not determined.

## DISCUSSION

Buitrago et al[11] used phenotype time series analysis to describe the possible circulation and presence of CA-MRSA in invasive infections in 33 Colombian hospitals during the period 2001-2007; they found that the annual MRSA percentage remained constant during the 7 years of surveillance (45.1% on average) but that there was an increase in the MRSA strains' multisusceptible phenotype. Our findings were convincing, not just in terms of the molecular confirmation of the presence of CA-MRSA and associating it with nosocomial infection, but in demonstrating that it spreads and could lead to outbreaks.

Even though severe CA-MRSA infection presence and transmission have already been described,[12,13] more CA-MRSA strains were related to nosocomial infection in this study than those described by Benoit et al[13] in Uruguay (39.4% compared with 21%); CA-MRSA could quickly replace HA-MRSA in Colombian hospitals. These findings are concerning because MRSA and nosocomial infection prevalence in developing countries hospitals is greater than in the United States,[14] probably because of resource-limited settings and less adherence to control measures, thus facilitating dissemination. Rosenthal et al[14] have described device-associated nosocomial infection rates as being higher in the rest of the world than in the United States (80.8% compared with 48.1%), with their study also showing high MRSA prevalence in intensive care units in Latin America, Asia, Africa, and Europe. This has created a new challenge for the infection committees of hospitals with limited resources. When patients colonized with CA-MRSA are admitted (without suitable screening and the pertinent isolates being taken), it is possible that endemic CA-MRSA may be generated within a particular

hospital or from a patient coming from the community. Based on recently circulated recommendations for preventing MRSA transmission,[15] the proposed measures must be established in Colombia, and the most cost-effective ones prioritized (adherence to handwashing protocols, evaluating MRSA-related risks, monitoring programs, and others), ensuring that they are rapidly implemented. However, the presence of CA-MRSA could be suspected from a particular patient's phenotype profile, given that most hospitals in resource-limited settings are not actively screening MRSA on admission. In this case, the presence of oxacillin and tetracycline or oxacillin resistance could only be considered as a CA-MRSA movement marker.

It should be stressed that CA-MRSA is highly virulent (especially because PVL toxin genes have an 85% virulence factor) and that biofilm can be formed, thereby playing a possible role in bacterial adhesion and in evading the host's immune system. The infection-associated mortality reached 27% in this study, and 11% of the patients presented a relapse even though there was no direct relationship with any of the virulence factors. It is worth noting that CA-MRSA virulence could be greater than that for classic MRSA and that the mortality rate could increase in spite of suitable antibiotic management in compromised hosts (older people, those having had prior surgery, the critically ill, those suffering severe infections, and in those in whom invasive devices are used). Although some of the detected cases involved superficial skin and soft tissue infections, several invasive infections were identified (9 bacteriemia cases), thereby hindering their management and worsening the outcome. One of this report's limitations lies in the fact that it was not possible to identify whether a patient had already become colonized with CA-MRSA before being admitted or whether he/she had acquired it during his/her stay in a health care institution. Hospital-associated CA-MRSA infections have thus emerged in Colombia, thereby requiring that measures be taken for preventing their dissemination and improving the administration of appropriate initial antibiotic therapy.

The authors would like to thank the Departamento Administrativo de Ciencia, Tecnologia e Innovación (COLCIENCIAS) of Colombia for financial support (Grant 1308-O4-18054), and hospital infection control staff in participating Hospitals (San José, El Tunal, Reina Sofía, San Pedro Claver and Palermo).

## References

1. Alvarez CA, Barrientes OJ, Leal AL, Contreras GA, Barrero L, Rincón S, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Colombia. Emerg Infect Dis 2006;12:2000-1.
2. Gardella N, von Specht M, Cuirolo A, Rosato A, Gutkind G, Mollerach M. Community-associated methicillin-resistant *Staphylococcus aureus*, eastern Argentina. Diagn Microbiol Infect Dis 2008;62:343-7.
3. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? Clin Infect Dis 2008;46:787-94.
4. Arias CA, Rincon S, Chowdhury S, Martínez E, Coronell W, Reyes J, et al. MRSA USA300 clone and VREF: a US-Colombian connection? N Engl J Med 2008;13:2177-9.
5. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988;16:128-40.
6. Cohen AL, Calfee D, Fridkin SK, Huang SS, Jernigan JA, Lautenbach E, et al. Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/HICPAC positionpaper. Infect Control Hosp Epidemiol 2007;10:901-13.
7. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Eighteenth informational supplement. Vol. 28. M100-S18. National Committee for Clinical Laboratory Standards. Wayne, Pennsylvania. 2008.
8. Milheirico C, Oliveira DC, Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant *Staphylococcus aureus*: "SCCmec IV multiplex". J Antimicrob Chemother 2007;60:42-8.
9. Cruz C, Moreno J, Renzoni A, Hidalgo M, Reyes J, Schrenzel J, et al. Tracking methicillin-resistant *Staphylococcus aureus* clones in Colombian hospitals over 7 years (1996-2003): emergence of a new dominant clone. Int J Antimicrob Agents 2005;26:457-62.
10. Enright M, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008-15.
11. Buitrago G, Castillo JS, Leal AL, Sánchez R, Alvarez CA. Methicillin-resistant *Staphylococcus aureus* community-acquired phenotype spread in hospitals in Bogota, Colombia. Clin Microbiol Infect 2008; 14(Suppl 1):S411.
12. Klevens VD, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* in the United States. JAMA 2007;298:1763-71.
13. Benoit SR, Estivariz C, Mogdasy C, Pedreira W, Galiana A, Galiana A, et al. Community strains of methicillin-resistant *Staphylococcus aureus* as potential cause of healthcare-associated infections, Uruguay, 2002-2004. Emerg Infect Dis 2008;14:1216-23.
14. Rosenthal VD, Maki DG, Mehta AC, Alvarez-Moreno CA, Leblebicioglu H, Higuera FL, et al. International Nosocomial Infection Control Consortium report, data summary for 2002-2007. Am J Infect Control 2008;36:627-37.
15. Calfee DP, Salgado CD, Classen D, Arias KM, Podgorny K, Anderson DJ, et al. Strategies to prevent transmission of methicillin-resistant *Staphylococcus aureus* in acute care hospitals. Infect Control Hosp Epidemiol 2008;29(Suppl 1):S62-80.