Infection, Genetics and Evolution 14 (2013) 401–405

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Methicillin-resistant *Staphylococcus aureus* ST30-SCC*mec* IVc clone as the major cause of community-acquired invasive infections in Argentina

S. Fernandez [a,1], L. de Vedia [b,1], M.J. Lopez Furst [c], N. Gardella [a], S. Di Gregorio [a], M.C. Ganaha [e], S. Prieto [f], E. Carbone [g], N. Lista [b], F. Rotrying [h], M.E. Stryjewski [d], M. Mollerach [a,*]

[a] *Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina*
[b] *Hospital J F Muñiz, CABA, Argentina*
[c] *Sanatorio Mendez, CABA, Argentina*
[d] *Department of Medicine and Division of Infectious Diseases, Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC), CABA, Argentina*
[e] *Hospital Vicente Lopez y Planes, Gral Rodríguez, Provincia de Buenos Aires, Argentina*
[f] *Hospital Nuestra Señora de Luján, Provincia de Buenos Aires, Argentina*
[g] *Hospital Aeronáutico, CABA, Argentina*
[h] *Hospital UAI, CABA, Argentina*

ARTICLE INFO

Article history:
Received 5 October 2012
Received in revised form 18 December 2012
Accepted 19 December 2012
Available online 20 January 2013

Keywords:
Methicillin-resistance
Community-associated *Staphylococcus aureus*
CA-MRSA
MRSA invasive infection
Molecular epidemiology

ABSTRACT

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have become a major concern worldwide. We conducted a prospective multicenter study of invasive CA-MRSA to evaluate clinical features and genotype of strains causing invasive infections in Argentina. A total of 55 patients with invasive CA-MRSA infections were included. Most patients (60%) had bloodstream infections, 42% required admission to intensive care unit and 16% died. No CA-MRSA isolates were multiresistant (resistant $\geqslant$3 classes of antibiotics). All isolates carried Panton-Valentine leukocidin (PVL) genes and staphylococcal cassette chromosome (SCC*mec*) type IV. The majority CA-MRSA strains belonged to ST30 and had identical pulsed-field gel electrophoresis (PFGE) patterns, qualifying as a clonal dissemination of a highly transmissible strain. The main clone recovered from patients with CA-MRSA invasive infections was genotyped as pulsed-field gel electrophoresis type C-ST30, SCC*mec* type IVc-*spa* type 019, PVL positive. It has become predominant and replaced the previously described CA-MRSA clone (PFGE type A, ST5, SCC*mec* type IV, *spa* type 311).

1. Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have occurred worldwide, primarily in healthcare settings. Over the last 15 years, there have been increasing reports of patients with little or no healthcare contact suffering from MRSA infection. Defined now as community-acquired MRSA (CA-MRSA) this pathogen has reached epidemic proportions. While most CA-MRSA infections involve skin and skin structures (Stryjewski and Chambers, 2008), the invasive nature of CA-MRSA has also been observed in patients with necrotizing pneumonia, severe sepsis, osteomyelitis, meningitis and death (David and Daum, 2010; Deleo et al., 2010; Deurenberg and Stobberingh, 2009).

CA-MRSA strains are phylogenetically distinct from traditional hospital associated (HA-MRSA) clones. CA-MRSA isolates generally exhibit SCC*mec* IV or V, a narrow range of drug resistance, and commonly carry Panton-Valentine leukocidin (PVL) genes, rarely identified in HA-MRSA isolates (Ma et al., 2002; Naimi et al., 2003). Presence of PVL genes in *S. aureus* isolates has been associated with abscess formation, primary skin infections (Lina et al., 1999), severe necrotizing pneumonia, and increased complications of hematogenous osteomyelitis; however, the role of PVL in the pathogenesis of *S. aureus* infections has not been fully elucidated.

CA-MRSA clones seem to have a delineated (but dynamic) geographical distribution (David and Daum, 2010). In the United States, USA400 clone (ST1-IVa) was the most prevalent clone before 2001 (Stemper et al., 2004) and remains a common cause of community-onset disease among indigenous populations in Alaska and the Pacific Northwest. A second epidemic CA-MRSA clone, the USA300 (ST8-IVa, t008, PVL + and ACME-I) emerged between 1999 and 2001 and now causes most of the CA-MRSA infections in the United States (Kennedy et al., 2008). The European clone (ST80-IVc) was found to be predominant between 2004 and 2006 in Europe. However, the frequency of USA300 and related clones has been increasing in the last two years, representing a changing

* Corresponding author. Address: Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Ciudad Autónoma de Buenos Aires, Argentina. Tel.: +54 11 4964 8285; fax: +54 11 4964 8274.
E-mail address: mmollera@ffyb.uba.ar (M. Mollerach).
[1] These authors contributed equally to this paper.

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2012.12.018

trend in the epidemiology of CA-MRSA in that continent (Rolo et al., 2012). Moreover, recently it was demonstrated that MRSA clones causing invasive infection differ considerably between countries exhibiting a regional distribution in Europe (Grundmann et al., 2010).

The Southwest Pacific clone (ST30-IV) has mostly been described in Australia and New Zealand, where it is also known as the Western Samoan phage pattern (Nimmo et al., 2006; Smith and Cook, 2005). Moreover, 45 distinct clones of CA-MRSA have been identified in Australia; many of these are related to recognized MRSA lineages, but only three clones have successfully adapted to the Western Australian community environment (Coombs et al., 2011).

In Argentina, a PVL-positive epidemic CA-MRSA clone has been previously described (CAA clone: Pulsotype A, ST 5, SCC*mec* IV, *spa* type 311, PVL positive) as the predominant clone causing both invasive and non invasive MRSA infections (Gardella et al., 2008; Sola et al., 2008; von Specht et al., 2006).

However, spread of some epidemic clones into other regions has produced some displacement of previously circulating strains indicating that successful lineages may have competitive advantages which may be key in the evolution of this pathogen (Amorim et al., 2007; Gardella et al., 2005). Recent reports describing CA-MRSA clones invading healthcare settings underscore such potential advantages (Maree et al., 2007; Sola et al., 2012).

In our country we did not have recent, prospective, multicenter, clinically based studies in patients with invasive CA-MRSA infections. The aim of this study was to describe the clinical and molecular epidemiology of current invasive infections caused by CA-MRSA in adolescent and adult patients in Argentina.

2. Material and methods

2.1. Study design

A prospective, multicenter, observational study was designed to evaluate clinical and molecular features of invasive CA-MRSA infections in Argentina between March 2010 and December 2011. Patients were enrolled in 11 participating hospitals located in the central region of the country: Buenos Aires City ($N = 5$), Buenos Aires Province ($N = 5$) and Santa Fe ($N = 1$). The study was reviewed by each Institutional Review Board (IRB) and informed consent form obtained if requested by the IRB.

2.2. Patient selection and definitions

Patients were enrolled if they were $\geqslant$14 year-old and had invasive MRSA infection (see definition below). Patients were excluded if they had any of the following during the last 12 months: hospitalization, dialysis, surgery, presence of catheters or percutaneous medical devices, or residence in a long-term care facility. Community acquired MRSA infection was defined by a positive MRSA culture at the time of hospital admission or within the 48 h after hospital admission. Invasive MRSA infection was defined by the isolation of MRSA from a normally sterile body site.

2.3. Data collection

Clinical and demographic information was obtained using clinical report forms. Data collected included social–economical variables, comorbidities, use of prior antibiotic, clinical presentation, main laboratory results at baseline, source of isolate (e.g. blood, joint fluid, pleura), and outcomes.

Management decisions were made on an individual basis by physicians at each participating institution, following local policies and standards. Follow-up data was intended to be obtained up to 90 days after the end of treatment.

2.4. Microbiological studies

The isolates were identified at the participating hospitals as *S. aureus* on the basis of conventional diagnostic procedures. All MRSA isolates were stored at −20 °C and shipped to a central laboratory (Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires). Antimicrobial susceptibility testing was performed by diffusion methods according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2009).

2.5. PCR amplification of mecA, lukS/F-PV, SCCmec and agr typing

Detection of the *mec*A and PVL coding genes (*lukS/F-PV*) was performed after extraction of genomic DNA as previously described (von Specht et al., 2006). SCC*mec* types were determined by PCR with a simplified version of Kondo's typing system, including M-PCR-1 and M-PCR-2 (Kondo et al., 2007). SCC*mec* type IV was further sub-typed using published primers (Milheirico et al., 2007). In specific cases, SCC*mec* typing was also performed as recommended by Oliveira et al. (Oliveira and de Lencastre, 2002).

The *agr* locus was genotyped by multiplex PCR as it was described before (Gilot et al., 2002).

2.6. Typing methods

Genotyping analysis was conducted using *spa* typing (Harmsen et al., 2003) and Pulsed-Field Gel Electrophoresis (PFGE) with *Sma*I as previously described (Chung et al., 2000). Comparison of the PFGE fingerprints was performed by the unweighted pair-group method clustering analysis (UPGMA), applying the Dice correlation coefficient. MRSA clones previously described in Argentina were included in PFGE pattern analysis (Gardella et al., 2008). Representative isolates of the major pulsotypes were studied by Multilocus Sequence Typing (MLST) (Enright et al., 2000).

2.7. Statistical analysis

Descriptive statistics were used to summarize the characteristics of the patients. Continuous variables were expressed as medians and interquartile range, and categorical variables as percentages.

3. Results

3.1. Clinical features

A total of 55 patients were included in the study. Most patients were males, the median age was 29 years-old and only 16% had diabetes. More than half of patients had medical evaluation and/or received antibiotics within 30 days prior to the admission. Most common infections were bloodstream and/or pulmonary. Around 40% of patients required ICU. Demographic and clinical variables are displayed in Table 1. Vancomycin was the most commonly prescribed antibiotic and almost two thirds of patients were treated with $\geqslant$2 antibacterials (Table 2). Most patients were cured or improving at the end of therapy and death was documented in 16% of patients.

3.2. Antimicrobial susceptibility

Antibiotic susceptibility testing revealed resistance to oxacillin (100%) Resistance to erythromycin (9%), clindamycin (6%),