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Original article

Antimicrobial resistance, virulence factors and genetic lineages of hospital-onset methicillin-resistant *Staphylococcus aureus* isolates detected in a hospital in Zaragoza

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ABSTRACT

Introduction: MRSA population dynamics is undergoing significant changes, and for this reason it is important to know which clones are circulating in our nosocomial environment.
Materials and methods: A total of 118 MRSA isolates were collected from clinical samples from patients with previous hospital or healthcare contact (named as hospital-onset MRSA (HO-MRSA)) during a one year period. Susceptibility testing was performed by disk diffusion and microdilution. The presence of resistance genes and virulence factors were tested by PCR. All isolates were typed by SCC*mec*, *spa* and *agr* typing. PFGE and MLST were applied to a selection of them.
Results: Eighty-three HO-MRSA isolates (70.3%) were resistant to any antibiotic included in the macrolide–lincosamide–streptogramin B group. Among these isolates, the M phenotype was the most frequent (73.5%). One hundred and seven of HO-MRSA isolates (90.7%) showed aminoglycoside resistance. The combination *aac*(6′)-Ie-*aph*(2″)-Ia + *ant*(4′)-Ia genes was the most frequent (22.4%). Tetracycline resistance rates in HO-MRSA isolates were low (3.4%), although a high level of mupirocin resistance was observed (25.4%). Most of the HO-MRSA isolates (approximately 90%) showed SCC*mec* type IVc and *agr* type II. Fifteen unrelated pulsotypes were identified. CC5 was the most prevalent (88.1%), followed by CC8 (5.9%), CC22 (2.5%), CC398 (2.5%) and CC1 (0.8%).
Conclusion: CC5/ST125/t067 lineage was the most frequent. This lineage was related to aminoglycoside resistance, and to a lesser extent, with macrolide resistance. The presence of international clones as EMRSA-15 (CC22/ST22), European clones as CC5/ST228, community clones related to CC1 or CC8 and livestock associated clones, as CC398, were observed in a low percentage.

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Resistencia a antibióticos, factores de virulencia y líneas clonales de *Staphylococcus aureus* resistente a la meticilina de origen hospitalario en un hospital de Zaragoza

Palabras clave:
Origen hospitalario
SARM
Líneas clonales
Genes de resistencia
Tipado molecular

R E S U M E N

Introducción: Las dinámicas poblacionales de SARM están experimentando cambios significativos en los últimos tiempos. Por ello es importante conocer qué líneas clonales circulan en nuestro ambiente hospitalario.
Materiales y métodos: Durante un año, se seleccionaron 118 SARM de muestras clínicas de pacientes con contacto previo con el ambiente hospitalario (SARM de origen hospitalario [SARM-OH]). Las pruebas de sensibilidad se realizaron mediante difusión con discos y microdilución. La presencia de genes de resistencia y factores de virulencia fueron estudiados mediante PCR. Se estableció el tipo de SCC*mec*, *spa* y *agr* en todos los aislados, y en una selección se estudió su relación genética por PFGE y MLST.
Resultados: Ochenta y tres SARM-OH (70,3%) fueron resistentes a al menos un antibiótico del grupo de los macrólidos-lincosamidas-estreptograminas B. Entre estos, el fenotipo M fue el más frecuente (73,5%). Ciento siete aislamientos (90,7%) mostraron resistencia a aminoglucósidos. La combinación *aac*(6′)-Ie-*aph*(2″)-Ia + *ant*(4′)-Ia fue la más frecuente (22,4%). Las tasas de resistencia a tetraciclinas detectadas fueron bajas (3,4%). Se observó un 25,4% de resistencia de alto nivel a mupirocina. Aproximadamente un 90% de SARM-OH mostraron SCC*mec* tipo IVc y *agr* tipo II. Se identificaron 15 pulsotipos no relacionados. El CC5 fue el más prevalente (88,1%) seguido de CC8 (5,9%), CC22 (2,5%), CC398 (2,5%) y CC1 (0,8%).
Conclusión: La línea clonal CC5/ST125/t067 fue la más habitual. Esta línea se relacionó con resistencia a aminoglucósidos, y, en menor medida, con macrólidos. La presencia de clones internacionales como EMRSA-15 (CC22/ST22), clones europeos como CC5/ST228, clones comunitarios relacionados con CC1 o CC8 y clones asociados al ganado, como el CC398, se observaron en un bajo porcentaje.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of human bacterial infections worldwide.[1] Currently, MRSA is now endemic in many hospitals and healthcare facilities in industrialized countries.[2] The prevalence of MRSA among European countries varies considerably being significantly lower in northern countries (approximately 1 or 2%) than in other European countries (up to 45%).[3]

In recent years, polyclonal emergence of new clones has been described. Pandemic spread of different hospital-associated MRSA (HA-MRSA) clones, as ST239/SCC*mec*III-IV, EMRSA-16 or ST36/SCC*mec*II (CC30) and ST125/SCC*mec*IV (CC5) and community-associated MRSA (CA-MRSA) clones, as ST1/SCC*mec*IV (CC1), USA 300 or ST8/SCC*mec*IV (CC8), ST30/SCC*mec*IV (CC30) has been observed.[4–6] To date, livestock-associated MRSA (LA-MRSA) clones has not been frequently identified in hospitals or nursing homes in Europe, although the spread seems to be dependent on the region and the intensity of pig farming.[7]

In Spain in early twenties, the dominant clone was Iberian clone (ST247/SCC*mec*I) but over the years this clone has reduced its presence.[8] Currently, the most widespread HA-MRSA clone is ST125/SCC*mec*IV/t067 which is an allelic variant of ST5 pediatric clone.[9]

MRSA population dynamics is undergoing significant changes due to demographic variations such as immigration and the development of sophisticated and complex health systems (i.e. care of patients with severe underlying diseases and invasive devices in day hospitals, residency in a long-term care facility. . .).[4] For this reason it is more difficult to establish the epidemiology of MRSA infections. The aim of the study was to analyze the molecular epidemiology, clonal lineages, resistance mechanisms and virulence traits of hospital and healthcare associated MRSA clones to understand the epidemiology of these infections.

Materials and methods

Setting

The study was performed in the University Teaching Hospital "Lozano Blesa" (Zaragoza, Spain), with 803 beds attending a population of 286,774 inhabitants with 29,506 annual admissions. The study was conducted between July 2009 and July 2010.

Bacterial isolates and patient information

A total of 118 MRSA isolates were collected in our institution from clinical samples belonged to patients with previous hospital or healthcare contact (named as hospital or healthcare onset MRSA, HO-MRSA). HO-MRSA was defined as an isolate cultured from a clinical specimens obtained $\geq$72 h after patient's hospital admission or whose sources of isolation were associated with one of the following hospital or healthcare risk factors: history of previous hospitalization, surgeries or dialysis, residency in a long-term care facility, the presence of medical devices or previous MRSA infection or colonization.

One isolate per patient was included. For each patient the following data were collected: gender, age, nationality, source of the culture sample and underlying disease.

Identification and antimicrobial susceptibility testing

The identification was performed using WIDER I System (Francisco Soria-Melguizo, Madrid, Spain). Susceptibility testing to cefoxitin, erythromycin, azithromycin, spiramycin, clindamycin, pristinamycin, chloramphenicol, tetracycline, minocycline, streptomycin, gentamicin, tobramycin, amikacin, kanamycin and netilmicin (Mast Diagnostics, Bootle, UK and Bio-Rad, Marnes La Coquette, France) was performed by disk diffusion and interpreted