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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 SCCmec, 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 SCCmec 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

R E S U M E N

Palabras clave:

Origen hospitalario
SARM
Líneas clonales
Genes de resistencia
Tipado molecular

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 SCCmec, *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 SCCmec 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.

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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/SCCmecIII-IV, EMRSA-16 or ST36/SCCmecII (CC30) and ST125/SCCmecIV (CC5) and community-associated MRSA (CA-MRSA) clones, as ST1/SCCmecIV (CC1), USA 300 or ST8/SCCmecIV (CC8), ST30/SCCmecIV (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/SCCmecI) but over the years this clone has reduced its presence.⁸ Currently, the most widespread HA-MRSA clone is ST125/SCCmecIV/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 ≥ 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

according to CLSI guidelines¹⁰ and the Société Française de Microbiologie breakpoints (<http://www.sfm-microbiologie.org>). Mupirocin susceptibility testing was confirmed by E-test strip method.

Resistance genotypes and virulence factors

The presence of *mecA*, *aac*(6′)-Ie-*aph*(2′′)-Ia, *aph*(3′)-IIIa, *ant*(4′)-Ia, *erm*(A), *erm*(B), *erm*(C), *msr*(A), *vga*(A), *vga*(C), *lnu*(A), *tet*(K), *tet*(M), *tet*(L) and *mupA* resistance genes was tested by PCR as previously described.^{11,12} The presence of virulence genes encoding the Pantan Valentine leukocidin (PVL) (*lukF/lukS*-PV), TSST-1 (*tst*), Exfoliative Toxin A (ETA) (*eta*), B (ETB) (*etb*) was investigated by PCR.¹² Positive and negative controls were included in all PCRs.

Molecular typing of isolates

All MRSA isolates were typed (SCCmec, *spa* and *agr* typing) as previously described.¹²

Pulsed field gel electrophoresis (PFGE) using *Sma*I and *Eag*I restriction enzymes (New England Biolabs, Hertfordshire, United Kingdom) was performed in the isolates with different *spa* types, isolates with the same *spa* type but with different antibiotic resistance profile and mupirocin or tetracycline resistance.¹³ *Eag*I restriction enzyme was used in tetracycline resistant isolates because presumably could be related to the CC398 which are non-typeable by *Sma*I. Profiles were analyzed with GelCompar II® software (Applied Maths, Kortrijk, Belgium). Dendrograms were generated by the unweighted pair-group method using arithmetic averages, based on the Dice similarity coefficient with a 1.0% band position tolerance.¹⁴ PFGE patterns were assigned into pulsotypes (named with capital letters) and subtypes (named with numbers). Different pulsotypes were considered if the similarity coefficient was <80%. Different subtypes were considered when the similarity coefficient oscillates in the 80–95% interval.

Sixteen selected MRSA isolates (isolates represented of each PFGE type and the isolate with the new *spa* type (t10034)) were characterized by MLST amplifying fragments of *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL* housekeeping loci.¹⁵ All amplicons were sequenced and compared with the MLST database in order to know the sequence type (ST). The assignment of Clonal Complexes (CC) was achieved according to the ST determined (in 16 isolates). In those cases in which STs were not determined, CCs were presumably assigned according to the *spa* type (in the remaining 102 isolates).

Results

Overview and epidemiological data

A total of 118 HO-MRSA isolates were collected from clinical samples during one year. These isolates belonged to 72 male (61.1%) and 46 female (38.9%). The median age and standard deviation of these patients was 75.27 ± 15.06 years old (range 1–96). These isolates were mostly recovered from skin lesions and wounds (60%). The remaining isolates were obtained from lower respiratory tract samples (20%), urine samples (8%), blood (5%), abscesses (4%) and upper respiratory samples (2%). These isolates were more frequently found in primary care (20.3%), internal medicine (18.6%), vascular surgery (14.4%), emergency department (8.5%), pulmonology (6.7%) and intensive care unit (5.9%).

The predominant risks factors during the year prior to MRSA isolation were hospitalization (71.9%), residency in a long-term care facility (38%), surgery (38%), medical devices (38%), dialysis (2.5%) or previous MRSA infection or colonization (2.5%).

Most common underlying diseases (often combined) were cardiovascular (80.5%), diabetes (25.3%), and neoplastic disease (27.1%). Other underlying diseases were found in 9.3% of the patients.

Susceptibility testing, detection of antimicrobial resistance genes and virulence factors

All isolates showed resistance to betalactam antibiotics tested (cefoxitin, oxacillin, and amoxicillin-clavulanate) and *mecA* gene was amplified in all of them. Table 1 shows the characteristics of 118 HO-MRSA isolates including their phenotype and genotype resistance.

Eighty-three HO-MRSA isolates (70.3%) were resistant to any antibiotic included in the macrolide-lincosamide-streptogramin B (MLS_B) group. The rates of erythromycin and azithromycin resistance were high (68.6% in both cases). Among erythromycin resistant isolates, the M phenotype was detected in 73.5% of them encoded by *msr*(A) (71.1%) and *msr*(A)+*erm*(B) genes (2.4%). Constitutive resistance (cMLS_B phenotype) was observed in 16.9% of erythromycin resistant isolates encoded by *erm*(C) gene (7.2%), *erm*(A) gene (2.4%), *erm*(A)+*erm*(B) genes (3.6%) and *msr*(A)+*erm*(C) genes in 3.6% of the isolates. Inducible resistance (iMLS_B phenotype) encoded by *erm*(C) was detected in 8.4% of resistant isolates. Finally, the L phenotype was observed in 1.2% of erythromycin resistant isolates encoded by *lnu*(A) gene.

One hundred and seven of HO-MRSA isolates (90.7%) showed resistance to any antibiotics included in the aminoglycoside family, mainly kanamycin (89%), tobramycin (72.9%) and amikacin (53.4%). *ant*(4′)-Ia gene was the most frequently detected (49.5%), followed by *aph*(3′)-IIIa (11.2%) and *aac*(6′)-Ie-*aph*(2′′)-Ia genes (3.7%). One-third of isolates (33.6%) showed combinations of several resistance genes. The combination *aac*(6′)-Ie-*aph*(2′′)-Ia + *ant*(4′)-Ia genes was the most frequent (22.4%). In two isolates, which showed only streptomycin resistance, we could not determine the resistance gene profile.

Tetracycline resistance rates in HO-MRSA isolates were low (3.4%). Four isolates showed tetracycline resistance encoded by *tet*(K) and *tet*(M) + *tet*(K) genes. One isolate exhibited tetracycline and minocycline resistance encoded by *tet*(M).

Mupirocin MICs ≥256 mg/L were found in 30 isolates (25.4%) (the rest of them showed MICs <8 mg/L). High mupirocin MICs were confirmed by E-test strip method showing high level mupirocin resistance (MIC > 512 mg/L). This resistance was encoded by *mupA* gene.

The genes encoding the PVL were detected in 3 of our 118 HO-MRSA isolates (2.5%). Only one isolate with *tst* gene was detected. This isolate, obtained from a respiratory specimen (sputum) from HIV patient, carry *erm*(C) and *ant*(4′)-Ia genes and was included in ST5/*spa*t9298/SCCmecI/*agr*II profile. Genes encoding A and B exfoliatins were not detected in any isolate.

Molecular typing of isolates

Most of the HO-MRSA isolates (90%) showed SCCmec type IVc. Other SCCmec found were: SCCmec type I (4.2%), SCCmec type IVh (2.5%), SCCmec type V (2.5%) and SCCmec type IVa (0.8%) (Table 1).

Highly diverse *spa* types were identified among HO-MRSA. The most frequently detected was *spa* type t067 (61%) followed by t2220 (7.6%), t2226 (6.7%), t002 (5.1%), t008 (4.2%), t041 (4.2%), t011 (2.5%) and t032 (1.7%). Sporadically *spa* types t068, t127, t1302, t190, t306, t4978 and t9298 were detected. A new *spa* type, *spa* t10034, was identified in this study which was subsequently included in Ridom StaphType® software. This new *spa* type belongs to ST5.

Table 1

Antimicrobial resistance, virulence genes and genetic lineages of 118 HO-MRSA isolates.

CC	ST (n) number of isolates	spa type (n) number of isolates	SCCmec	agr	PVL/TSST	PGFE profile (n) number of isolates	Resistance profile* (n) number of isolates	Resistance genes
5	ND	067 (72)	IVc	II	–	pA ₃ (1)	ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₄ (1)	ERY, AZM, SPI, CLI, TOB, KAN	<i>erm</i> (C), <i>ant</i> (4')-Ia
	ND					pA ₅ (2)	ERY, AZM, SPI, CLI, TOB, AMI, KAN	<i>erm</i> (C), <i>ant</i> (4')-Ia
	ND						TOB, KAN, CLO	<i>ant</i> (4')-Ia
	ND					pA ₇ (2)	ERY, AZM, GEN, TOB, AMI, KAN	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2'')
	ND						TOB, AMI, KAN	<i>ant</i> (4')-Ia
	ND					pA ₈ (1)	ERY, AZM, SPI, CLI, TOB, AMI, KAN	<i>erm</i> (C), <i>ant</i> (4')-Ia
	ND					pA ₉ (2)	ERY, AZM, KAN	<i>msr</i> (A), <i>ant</i> (4')-Ia
	ND						GEN, TOB, AMI, KAN, MUP	<i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₁₀ (1)	TOB, AMI, KAN	<i>ant</i> (4')-Ia
	ND					pA ₁₁ (1)	ERY, AZM, SPI, CLI, TOB, AMI, KAN, STR, MUP	<i>erm</i> (C), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₁₂ (2)	ERY, AZM, SPI, CLI, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>erm</i> (C), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, STR, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₁₃ (6)	ERY, AZM, SPI, CLI, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>erm</i> (C), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP (2)	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, NET, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND						GEN, TOB, AMI, KAN, STR, NET, MUP	<i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₁₄ (2)	ERY, AZM, TOB, AMI, KAN, STR	<i>msr</i> (A), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa
	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₁₇ (1)	TOB, KAN	<i>ant</i> (4')-Ia
	ND					pA ₁₈ (1)	–	–
	ND					pA ₁₉ (2)	GEN, TOB, AMI, KAN, MUP	<i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, STR, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa, <i>mupA</i>
	ND					pA ₂₀ (1)	ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa, <i>mupA</i>
	ND					pA ₂₁ (1)	ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pA ₂₂ (3)	ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>mupA</i>
	125					pB ₁ (1)	ERY, AZM, KAN, STR, CLO	<i>msr</i> (A), <i>aph</i> (3')-IIIa
	ND						ERY, AZM, TOB, AMI, KAN, STR, CLO	<i>msr</i> (A), <i>ant</i> (4')-Ia
	ND					pC ₃ (2)	ERY, AZM, TOB, KAN	<i>msr</i> (A), <i>ant</i> (4')-Ia
	ND						ERY, AZM, KAN (2)	<i>msr</i> (A), <i>aph</i> (3')-IIIa
	ND					pC ₄ (3)	ERY, AZM, TOB, AMI, KAN	<i>msr</i> (A), <i>erm</i> (B), <i>ant</i> (4')-Ia
	ND						GEN, TOB, KAN, MUP	<i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND					pC ₅ (1)	ERY, AZM, TOB, AMI, KAN	<i>msr</i> (A), <i>ant</i> (4')-Ia
	ND					pC ₆ (1)	ERY, AZM, TOB, AMI, KAN	<i>msr</i> (A), <i>ant</i> (4')-Ia
	ND					pC ₇ (1)	ERY, AZM, TOB, AMI, KAN, CLO	<i>msr</i> (A), <i>ant</i> (4')-Ia
	125					pD ₁ (1)	ERY, AZM, SPI, CLI, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>erm</i> (C), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	125						ERY, SPI, STR, CLO	<i>erm</i> (C)
	ND					pE ₁ (1)	ERY, AZM, GEN, TOB, AMI, KAN	<i>erm</i> (C), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia
	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP (2)	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>mupA</i>
	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr</i> (A), <i>aac</i> (6')-Ie- <i>aph</i> (2''), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa, <i>mupA</i>
	ND					ND (31)		

Table 1 (Continued)

CC	ST (n) number of isolates	spa type (n) number of isolates	SCCmec	agr	PVL/TSST	PGFE profile (n) number of isolates	Resistance profile* (n) number of isolates	Resistance genes
8	ND						ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr(A)</i> , <i>erm(B)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>aph(3')-IIIa</i> , <i>mupA</i>
	ND						ERY, AZM, KAN (2)	<i>msr(A)</i> , <i>aph(3')-IIIa</i>
	ND						ERY, AZM, KAN (2)	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND						ERY, AZM, SPI, CLI, TOB, KAN	<i>erm(C)</i> , <i>ant(4')-Ia</i>
	ND						ERY, AZM, TOB, AMI, KAN (12)	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND						ERY, AZM, TOB, AMI, KAN	<i>msr(A)</i> , <i>ant(4')-Ia</i> , <i>aph(3')-IIIa</i>
	ND						ERY, AZM, TOB, AMI, KAN, CLO	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND						ERY, AZM, TOB, AMI, KAN, STR	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND						ERY, AZM, TOB, KAN (2)	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND						TOB, AMI, KAN (4)	<i>ant(4')-Ia</i>
	ND					pA ₁₆ (1)	ERY, AZM, KAN, MUP	<i>msr(A)</i> , <i>aph(3')-IIIa</i> , <i>mupA</i>
	125					pC ₁ (2)	ERY, AZM, KAN, STR	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND	2220 (9)	IVc	II	–	pC ₅ (1)	ERY, AZM, AMI, KAN	<i>msr(A)</i> , <i>ant(4')-Ia</i>
	ND					pC ₈ (1)	ERY, AZM, KAN	<i>msr(A)</i> , <i>aph(3')-IIIa</i>
	ND					ND (4)	ERY, AZM, KAN	<i>msr(A)</i> , <i>aph(3')-IIIa</i>
	ND					pA ₄ (1)	TOB, AMI, KAN, STR	<i>ant(4')-Ia</i>
	ND					pA ₅ (1)	GEN, TOB, AMI, KAN, MUP	<i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>mupA</i>
	ND	2226 (8)	IVc	II	–	pA ₆ (2)	GEN, TOB, AMI, KAN, MUP	<i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>mupA</i>
	ND					pA ₁₄ (1)	ERY, AZM, GEN, TOB, AMI, KAN, MUP	<i>msr(A)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>mupA</i>
	ND					pA ₁₅ (1)	TOB, KAN	<i>ant(4')-Ia</i>
	ND					ND (2)	TOB, KAN	<i>ant(4')-Ia</i>
	125	4978	IVc	II	–	pG ₁ (1)	ERY, AZM, KAN, CLO	<i>msr(A)</i> , <i>aph(3')-IIIa</i>
	146 (1)					pA ₁ (2)	ERY, AZM, TOB, AMI, KAN	<i>erm(C)</i> , <i>ant(4')-Ia</i>
	ND					pA ₁₇ (1)	–	–
	ND	002 (6)	IVc	II	–	pA ₁₈ (1)	–	–
	ND					ND (2)	–	–
	228						TOB, AMI, KAN, STR	<i>ant(4')-Ia</i>
	ND						ERY, AZM, SPI, CLI, GEN, TOB, KAN, STR	<i>erm(A)</i> , <i>erm(B)</i> , <i>aac(6')-Ie-aph(2'')</i>
	ND					pF ₁ (3)	ERY, AZM, SPI, CLI, GEN, TOB, KAN, STR	<i>erm(A)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>aph(3')-IIIa</i>
	ND	041 (5)	I	II	–		ERY, AZM, SPI, CLI, GEN, TOB, KAN, STR	<i>erm(A)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i>
	ND					pF ₂ (1)	ERY, AZM, SPI, CLI, GEN, TOB, KAN, STR	<i>erm(A)</i> , <i>erm(B)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>aph(3')-IIIa</i>
	ND		IVc			pF ₁ (1)	ERY, AZM, SPI, CLI, GEN, TOB, KAN, STR	<i>erm(A)</i> , <i>erm(B)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>aph(3')-IIIa</i>
	ND	306	IVc			pA ₂ (1)	TOB, KAN	<i>ant(4')-Ia</i>
	5	9298	I	II	TSST+	pH ₁ (1)	ERY, AZM, TOB, KAN	<i>erm(C)</i> , <i>ant(4')-Ia</i>
	5	10,034	IVc		–	pC ₂ (1)	ERY, AZM, GEN, TOB, AMI, KAN	<i>msr(A)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i>
8	8 (1)				PVL+	pI ₁ (2)	–	–
	8				–	pK ₁ (1)	TOB, AMI, KAN	<i>ant(4')-Ia</i>
	ND	008 (5)	IVc	I	–	pK ₂ (1)	TOB, KAN	<i>ant(4')-Ia</i>
	8				PVL+	pJ ₁ (1)	–	–
	8	68	IVc	I	–	pL ₁ (1)	–	–
22	ND	190	IVc	I	–	pK ₃ (1)	TOB, KAN	<i>ant(4')-Ia</i>
	22 (1)	032 (2)	IVh	I	–	pM ₁ (1)	ERY, AZM, SPI, CLI	<i>erm(C)</i>
	ND					pM ₃ (1)	ERY, AZM	<i>erm(C)</i>
	ND	1302	IVh	I	–	pM ₂ (1)	–	–
1	1	127	IVa	III	–	pO ₁ (1)	ERY, AZM, KAN, STR, TET	<i>erm(C)</i> , <i>ant(4')-Ia</i> , <i>tet(K)</i>
398	398 (1)					pN ₁ (2)	STR, TET	<i>tet(K)</i> , <i>tet(M)</i>
	ND	011 (3)	V	I	–		GEN, TOB, KAN, TET, MIN	<i>aac(6')-Ie-aph(2'')</i> , <i>tet(M)</i>
	ND					pN ₂ (1)	CLI, GEN, TOB, KAN, STR, TET	<i>lnu(A)</i> , <i>aac(6')-Ie-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>tet(M)</i>

ND: not determined; PVL: Pantón–Valentine leukocidin; TSST: toxic shock syndrome toxin.

* ERY: erythromycin, AZM: azithromycin, SPI: spiramycin, CLI: clindamycin, GEN: gentamicin, TOB: tobramycin, AMI: amikacin, KAN: kanamycin, STR: streptomycin, NET: netilmicin, TET: tetracycline, MIN: minocycline, MUP: mupirocin.

Amplification of the hypervariable region of the *agr* locus showed that *agr* group II was predominant (88.1%), followed by *agr* group I (11.1%) and *agr* group III (0.8%).

In addition, 79 MRSA isolates were studied by PFGE. A high genetic variability was found, although some clusters were observed. Fifteen unrelated pulsotypes were identified (named pulsotypes pA–pO). Pulsotypes pA₁₃ (6 isolates), pA₂₂ (3 isolates) and pC₄ (3 isolates) were the most frequent (Table 1).

Sixteen isolates were further characterized by MLST being ST125 the predominant (5 isolates) followed by ST8 (4), ST5 (2), ST1, ST22, ST146, ST228 and ST398.

Distribution in CC was established according to the ST or presumably assigned according to the *spa* type. CC5 was the most prevalent (88.1%) followed by CC8 (5.9%), CC22 (2.5%), CC398 (2.5%) and CC1 (0.8%).

Discussion

In Spain, MRSA prevalence has remained stable in recent years. During the course of this study, MRSA prevalence in our hospital was 30.9%, consistent with other resistance rates found in different Spanish hospitals.^{16,17} It has been observed that some MRSA clones tend to occur more frequently in certain countries or areas¹⁸ while other clones spread worldwide, threatening public health.¹⁹ Controlling the infection in hospitals or healthcare centers could reduce the overall prevalence of MRSA, for this reason, it is important to know which clones are circulating in our nosocomial environment.

Studies conducted in Spain showed that in our country, a small number of international clones have spread.⁴ The predominant Iberian clone before 1996 in Spain was replaced by CC5/ST228/SCCmecI clone. In our study, five strains belonged to CC5/ST228/*spat*041/SCCmecI/*agr*II profile (one of them possessed SCCmec type IVc instead of SCCmec type I). These strains showed high homology in PFGE patterns (pulsotypes pF₁ and pF₂) and constant resistance pattern (*erm*(A) ± *erm*(B) + *aac*(6')-Ie-*aph*(2'')-Ia + *aph*(3')-IIIa). However, the presence of this clone in Spain has been steadily declining, being replaced by international EMRSA-16 clone (CC30/ST36/SCCmecII), very common in the UK.^{4,6} Currently, this clone has an irregular representation in different institutions while CC5/ST125/SCCmecIV has become more prevalent.¹⁶ High percentage of our isolates belonged to this CC5/ST125/SCCmecIV. This high proportion is mainly due to the large number of isolates belonging to *spa* type t067, although other *spa* types were found (Table 1). Approximately one-quarter (22.8%) of isolates included in *spa* type t067 were associated with tobramycin and erythromycin resistance mediated by *ant*(4')-Ia and *msr*(A) genes, respectively. Moreover, most of the high level mupirocin resistance isolates that are circulating in our hospital environment belonged to CC5/ST125/t067.

In a previous study conducted by our group, we observed that isolates belonged to CC5/ST125/t067 were predominant in community areas.¹² This finding suggests a great exchange of genetic lineages from hospital or healthcare services to the community environment.

Other HA-MRSA clones were detected in our hospital environment. Three strains included in CC5/ST125/*spat*2226/SCCmecIVc/*agr*II profile belonged to patients admitted to the same nursing home. Isolates belonging to ST5 were sporadic in our study. Three strains were included in CC22 (EMRSA-15), a British clone first detected in 1999 in Spain, always detected in a very low percentage (<2%).⁶

The *tst* gene was detected in one isolate (belonging to CC5/ST5/*spat*9298/SCCmecI/*agr*II). This strain seems to be related with TSST-1 positive ST5 Geraldine clone

(ST5/*spat*002/SCCmecI/*agr*II) frequently found in hospital and community environment in France.²⁰

However, we not only observed HA-MRSA clones circulating by our hospital or healthcare environment. The presence of typically community clones (CC8 and CC1) and livestock associated clones (CC398) was observed. Most of the CA-MRSA strains circulating in our country were included in USA300 clone or its Latin American variant mainly due to the large population exchanges with American continent in recent years.^{21,22} The presence of this clone was low (five isolates) in our hospital environment. Three isolates, belonging to Bolivian patients, contained the *lukF/lukS-PV* genes. Two of them were recovered from a patient with a breast abscess and her daughter that showed cellulite in her right inframandibular area. CC1 includes several strains of CA-MRSA,²³ but this CC also has been detected in animals.²⁴ One of our isolates, obtained from a Romanian patient with a previous history of animal contact, belonged to CC1 (ST1/*spat*127/SCCmecIVa PVL negative).

In our healthcare environment, we observed the presence of CC-related to animals as CC398. These strains were recovered from different specimens (sputum, urine and surgical wound) and belonged to patients with no history of previous contact with animals (one of them was living in a rural area with a high density of farms, but we could not confirm animal contact because the patient died). The relationship between tetracycline resistance and CC398 strains was already suggested.²⁵ Resistance is mainly related to *tet*(M) gene, but not excluded other genes as *tet*(K) or *tet*(L), or even a combination of both, as we have found in one of our isolates.⁷ An unusual resistance profile (clindamycin resistance but erythromycin susceptibility) has been described in ST398 isolates associated with the presence of *vga*(A) and *vga*(C) or *lnu*(A) genes.^{26,27} This unusual profile was found in our study encoded by *lnu*(A) gene.

In conclusion, in our healthcare environment, most of the strains belong to CC5, where ST125/t067 lineage was the most important. 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 (mainly carried by nonnative patients) and livestock associated clones, as CC398, were observed in a low percentage.

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Conflict of interest

The authors declare that they have no conflict of interest.

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