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Genotypic and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) clones with high-level mupirocin resistance.

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Abstract

A high proportion of methicillin-resistant *Staphylococcus aureus* isolates recovered in one year period showed high-level mupirocin-resistance (HLMUPR-MRSA) in our environment (27.2%). HLMUPR-MRSA isolates were mainly collected from skin and soft tissue samples, and diabetes was the main related comorbidity condition. These isolates were more frequently found in vascular surgery. HLMUPR-MRSA were more resistant to aminoglycosides than mupirocin-susceptible MRSA, linked to the presence of bifunctional and/or nucleotidyltransferase enzymes with/without macrolide resistance associated with the *msr(A)* gene. Most of HLMUPR-MRSA isolates belonged to ST125/t067. Nine IS257-*ileS2* amplification patterns (p3 was the most frequent) were observed in HLMUPR-MRSA isolates, suggesting the presence of several mupirocin-resistance-carrying plasmids in our environment and promoting the emergence of mupirocin resistance. The presence of the same IS257-*ileS2* amplification pattern p3 in 65% of HLMUPR-MRSA, all of them ST125/t067, suggests a clonal spread in our hospital and community environment which could explain the high prevalence of HLMUPR-MRSA during the study period. An outbreak situation or an increase in mupirocin consumption was not observed.

Keywords: high-level mupirocin resistance; MRSA; clonal lineages; resistance genes.

1 **Introduction**

2 Mupirocin, also known as pseudomonic acid A, is a topical antibiotic that was originally
3 isolated from *Pseudomonas fluorescens*. It is used for decolonization of nasal carriers of
4 methicillin-resistant *Staphylococcus aureus* (MRSA) (Sutherland et al., 1985). The increased
5 use of this antibiotic has been accompanied by outbreaks of mupirocin-resistant MRSA
6 (Schmitz and Jones, 1997; Simor et al., 2007).

7 Mupirocin is an analogue of isoleucine that inhibits protein synthesis by competitively binding
8 to the enzyme isoleucyl-tRNA synthetase (Yanagisawa et al., 1994). The high-level mupirocin-
9 resistant isolates show a MIC greater than 512 mg/L. This resistance is mediated by the
10 acquisition of a plasmid containing the *ileS2* gene that encodes an alternative isoleucyl-tRNA
11 synthetase enzyme and is generally flanked by copies of the insertion sequence IS257 (Pérez-
12 Roth et al., 2010; Woodford et al., 1998).

13 The objective of the study was to identify the prevalence, clonal lineages, resistance
14 mechanisms and virulence genes of high-level mupirocin-resistant MRSA (HLMUPR-MRSA)
15 isolates recovered from inpatients and outpatients in our institution during one year.

16 **Material and Methods**

17 One hundred forty-seven MRSA isolates were collected from clinical samples in the University
18 Teaching Hospital “Lozano Blesa” (Zaragoza, Spain) from July 2009 to July 2010. Only one
19 isolate per patient was included. The study was conducted retrospectively. Clinical records of all
20 patients were reviewed. For each patient the following data were collected: gender, age, medical
21 service, source of the culture sample and comorbid conditions during the year prior to MRSA
22 isolation (dialysis, diabetes, vascular disease, malignancy, hospitalization, MRSA isolation,
23 medical devices, contact with healthcare settings and surgery). To establish the possible
24 community origin of MRSA isolates, CDC criteria were followed, considering in this category
25 the isolates from outpatients with no hospitalization history during the last year, or from patients
26 within their first 48 h after admission, who presented no other established risk factors for

27 MRSA infection, such as a sojourn in a long-term care facility, dialysis, surgery, indwelling
28 permanent catheters, or medical devices. Antibiotic consumption in the year prior to isolation
29 was studied only in patients who carried HLMUPR-MRSA. Mupirocin consumption data in our
30 hospital were obtained from pharmacy records.

31 Identification and susceptibility testing was carried out by WIDER®I System (Francisco Soria-
32 Melguizo, Madrid, Spain) and disk diffusion method and interpreted according to the CLSI
33 guidelines (Clinical and Laboratory Standards Institute (CLSI), 2014). High mupirocin
34 MICs (mupirocin MICs ≥ 256 mg/L) were confirmed by E-test strip method.

35 The main genes that encode resistance to macrolides, lincosamide, streptogramin type B,
36 aminoglycosides, tetracycline and mupirocin were investigated by PCR (Aarestrup et al., 2000;
37 Aktas et al., 2007; Choi et al., 2003; Larsen et al., 2008; Lina et al., 1999; Udo et al., 2001).
38 Clinical strains usually harbor a complex mixture of resistance plasmids, and laborious time
39 consuming methodologies are required to associate HLMUPR-MRSA with a plasmid type
40 (Leski et al., 1999; Pérez-Roth et al., 2006). To enable the monitoring of HLMUP resistance
41 dissemination in staphylococci, we used a rapid typing method for strain characterization based
42 on the heterogeneous IS257-*ileS2* spacer regions (amplification of up- and down- stream IS257-
43 *ileS2* spacer regions) found on *ileS2*-encoding plasmids. Four PCR reactions were performed, as
44 previously described (Pérez-Roth et al., 2011), on all mupirocin-resistant isolates.

45 All isolates were typed by *spa* typing (Larsen et al., 2008). Only HLMUPR-MRSA isolates
46 were typed by SCC*mec*, *agr* typing, PFGE using *Sma*I and MLST as previously described
47 (González-Domínguez et al., 2012). PFGE profiles were analyzed with GelCompar II®
48 software (Applied Maths, Kortrijk, Belgium). Dendrograms were generated by the unweighted
49 pair-group method using arithmetic averages, based on the Dice similarity coefficient with a
50 1.0% band position tolerance. PFGE patterns were assigned into pulsotypes (named with capital
51 letters) and subtypes (named with numbers in subscript). Different pulsotypes were considered
52 if the similarity coefficient was <80%. Different subtypes were considered when the similarity

53 coefficient oscillates in the 80–95% interval. HLMUPR-MRSA isolates were screened for
54 virulence genes encoding Panton-Valentine leukocidin (PVL) (*lukS-PV* and *lukF-PV* genes),
55 exfoliative toxins ETA and ETB (*eta* and *etb* genes) and toxic shock syndrome toxin (TSST) (*tst*
56 gene) (Jarraud et al., 2002; Larsen et al., 2008).

57 Statistical significance was calculated for comparison of proportions using the chi-square test
58 with Yates' correction. A p -value ≤ 0.05 was considered statistically significant (SPSS V15.0;
59 SPSS, Chicago, IL).

60 **Results and discussion**

61 During the course of this study, MRSA prevalence in our hospital was 30.9%, consistent with
62 other resistance rates found in different Spanish hospitals (Cuevas et al., 2008; González-
63 Domínguez et al., 2015; Lozano et al., 2013). Forty MRSA isolates showed HLMUPR,
64 representing 27.2% of the studied isolates (mupirocin MICs ≥ 256 mg/L). This percentage is
65 higher than that found in other Spanish hospital (Daskalaki et al., 2009). Other isolates were
66 mupirocin-susceptible (i.e. MIC, <8 mg/L).

67 Mupirocin resistance appears to emerge easily in health centers with unrestricted policies that
68 allow widespread use of mupirocin for long periods (Lee et al., 2011). Between 2009 and 2010,
69 mupirocin consumption in our hospital stayed in approximately 1250 units per year (the
70 consumption in the community could not be established). This mupirocin consumption has
71 remained stable over the years. A guideline for surveillance and control of MRSA in hospitals
72 was published in Spain in 2008 (Rodríguez-Baño et al., 2008). Active surveillance cultures to
73 detect asymptomatic MRSA colonization is recommended in all guidelines for control and
74 prevention of MRSA. In general, the guidelines recommends screening the patients at high risk
75 of colonization (previously colonized, patients with multiple hospital or healthcare facilities
76 admissions with high prevalence of MRSA). In our hospital, mupirocin 2% nasal ointment is
77 used for decolonization of nasal carriers of MRSA after positive MRSA screening.

78 No statistically significant differences were found between patients with HLMUPR-MRSA and
79 mupirocin-susceptible MRSA isolates, regarding to age (median: 75 years versus 72 years;
80 $p=0.124$), male gender (50.0% versus 59.8%; $p=0.285$), community onset (25.0% versus 17.7%;
81 $p=0.326$).

82 In relation to comorbidity conditions associated with patients included in our study, diabetes
83 was more frequently found in patients with HLMUPR-MRSA than mupirocin-susceptible
84 MRSA isolates (35.0% versus 18.7%; $p=0.037$); while the presence of malignancy (10.0%
85 versus 25.2%; $p=0.044$) or medical devices (12.5% versus 30.8%; $p=0.024$) were more related
86 with patients who were infected with mupirocin-susceptible MRSA isolates.

87 HLMUPR-MRSA were more frequently collected from skin and soft tissue samples than
88 mupirocin-susceptible MRSA isolates (72.5% versus 27.1%; $p<0.001$). This association
89 between HLMUPR-MRSA and skin and soft tissue infections has also been described by other
90 authors (Daskalaki et al., 2009). HLMUPR-MRSA were more frequently found in vascular
91 surgery service than mupirocin-susceptible MRSA (30.0% versus 7.5%; $p<0.001$). No
92 significant differences in the prevalence of other medical services were found. HLMUPR-
93 MRSA were less frequently collected from lower respiratory tract samples than mupirocin-
94 susceptible MRSA isolates (2.5% versus 25.2%; $p=0.002$).

95 During the year prior to isolation, the antibiotics most frequently prescribed in patients who
96 carried these HLMUPR-MRSA isolates were beta-lactams (57.5%) and mupirocin (30%). These
97 results were not obtained in the group of mupirocin-susceptible MRSA isolates.

98 An aminoglycoside resistance phenotype was more frequently observed among HLMUPR-
99 MRSA than mupirocin-susceptible MRSA isolates (gentamicin: 85.0% versus 11.2%;
100 $p<0.001$, kanamycin: 97.5% versus 82.2%; $p=0.016$, tobramycin: 92.5% versus 64.5%;
101 $p=0.001$, amikacin: 85.0% versus 42.0%; $p<0.001$, streptomycin: 12.5% versus 19.6%;
102 $p=0.314$, netilmicin: 7.5% versus 0%; $p=0.019$) (Figure 1). Aminoglycoside resistance was

103 encoded by *aac(6′)-Ie-aph(2′′)-Ia* (85.0% versus 11.2%; $p<0.001$) and *ant(4′)-Ia* (85.0%
104 versus 64.5%; $p=0.016$) genes. A high rate of macrolide resistance was observed, but no
105 significant differences were found between high-level mupirocin-resistant and mupirocin-
106 susceptible isolates (erythromycin: 75.0% versus 58.8%; $p=0.07$, azithromycin: 75.0% versus
107 58.8%; $p=0.07$, spiramycin: 17.5% versus 13.1%; $p=0.496$, clindamycin: 17.5% versus 12.1%;
108 $p=0.4$). The difference in the prevalence of erythromycin and clindamycin resistance is due to
109 the high percentage of the M phenotype that implicates erythromycin-resistant but clindamycin-
110 susceptible pattern. Macrolide resistance by efflux due to the *msr(A)* gene is related with this
111 phenotype. The main macrolide resistance gene found in our HLMUPR-MRSA isolates was
112 *msr(A)* (67.5% versus 43.9%; $p=0.011$). None of HLMUPR-MRSA isolates showed
113 tetracycline resistance. Three predominant resistance gene profiles were found in HLMUPR-
114 MRSA isolates compared with mupirocin-susceptible MRSA isolates. These profiles carried
115 one or two aminoglycoside resistance genes with or without the macrolide resistance *msr(A)*
116 gene. Profile 1: *msr(A) + aac(6′)-Ie-aph(2′′)-Ia + ant(4′)-Ia* (30.0% versus 0.9%; $p<0.001$),
117 Profile 2: *aac(6′)-Ie-aph(2′′)-Ia + ant(4′)-Ia* (25.0% versus 0%; $p<0.001$) and Profile 3:
118 *msr(A) + aac(6′)-Ie-aph(2′′)-Ia + ant(4′)-Ia + aph(3′)-IIIa* (12.5% versus 0%; $p=0.001$).
119 However, mupirocin-susceptible MRSA isolates were more related to isolates harboring the
120 combination of *msr(A) + ant(4′)-Ia* genes, only the *ant(4′)-Ia* gene or the susceptible phenotype.

121 All high-level resistant isolates carried the *ileS2* gene. No association between multidrug
122 resistance and HLMUPR-MRSA were found as described by Pérez-Roth *et al.*, (Pérez-Roth *et*
123 *al.*, 2013). These isolates showed higher gentamicin and tobramycin resistance rates than
124 mupirocin-susceptible MRSA isolates what could be related to the *aac(6′)-Ie-aph(2′′)-Ia*
125 presence. This fact has been previously demonstrated with the detection of the *aac(6′)-Ie-*
126 *aph(2′′)-Ia* and *ileS2* genes co-located on the same plasmid (McDougal *et al.*, 2010).

127 Four different *spa* types (t002, t067, t2220 and t2226) were observed among HLMUPR-MRSA
128 isolates. The *spa* type t067 was the predominant one (82%), although this *spa* type was also

129 frequent in mupirocin-susceptible group (52%) where 22 different *spa* types were found. *spa*
130 type t067 is the most frequent *spa* type identified in Spain (Argudín et al., 2009; González-
131 Domínguez et al., 2012; González-Domínguez et al., 2015; Lozano et al., 2013; Menegotto et
132 al., 2012), so this fact could be the reason for its high prevalence in both groups. Among the
133 HLMUPR-MRSA strains, 97.5% of them belonged to ST125 and 2.5% to ST5. Both ST are
134 included in CC5, widely distributed in hospital and community settings in Spain (Argudín et al.,
135 2009; González-Domínguez et al., 2012; González-Domínguez et al., 2015; Lozano et al.,
136 2013). An association between MRSA CC5/ST125/t067 and erythromycin resistance (encoded
137 by *msr(A)/msr(B)* genes) has been observed. This relation was previously described by other
138 authors (Daskalaki et al., 2009; González-Domínguez et al., 2015; Lozano et al., 2013; Pérez-
139 Roth et al., 2013). All HLMUPR-MRSA strains carried *SCCmec* type IVc and *agr* type II, with
140 the exception of one strain included in *SCCmec* type V that belonged to ST125. None of the
141 studied virulence genes were identified among HLMUPR-MRSA isolates. Pulsed field gel
142 electrophoresis grouped the HLMUPR-MRSA strains in three different PFGE pulsotypes (Table
143 1). The most prevalent was pulsotype A that included 65% of the strains. This pulsotype had 11
144 subtypes where subtype A9 was the most frequent. Thirty per cent of the strains were included
145 in pulsotype B, and subtype B1 was the most frequent. Finally, 5% of strains were included in
146 pulsotype C. These results showed that the strains were closely related.

147 Analyzing the HLMUPR-MRSA strains selected from vascular surgery we observed the
148 presence of different clones (genetically unrelated).

149 Considering clinical, microbiological and molecular data, seventy-five percent of HLMUPR-
150 MRSA strains were classified as healthcare-associated MRSA (HA-MRSA) clones (Table 1).
151 The remaining ones were community-onset MRSA (CO-MRSA) isolates related to HA-MRSA
152 clones (CO-HA-MRSA). HLMUPR-MRSA were not observed in community-associated MRSA
153 (CA-MRSA) clones.

154 In HLMUPR-MRSA isolates, distinct IS257-*ileS2* spacer arrangements (characterized by a
155 different location of direct repeated copies of *ileS2*-flanking IS257) allowed us to distinguish 9
156 IS257-*ileS2* amplification patterns, named p1 to p9. Transfer of *ileS2*-encoding plasmids is
157 important in the spread of HLMUPR-MRSA as evidenced by the recovery of distinct plasmid
158 configuration types in the same MRSA clone (Pérez-Roth et al., 2013). We know that the
159 IS257-*ileS2* PCR might have exceptional limitations (Pérez-Roth et al., 2006; Pérez-Roth et al.,
160 2010; Pérez-Roth et al., 2011; Pérez-Roth et al., 2013) but we used this PCR as a first and
161 simple approximation to address the molecular epidemiology of plasmid-mediated HLMUPR-
162 MRSA isolates in our clinical setting. Only plasmid sequencing could definitely clarify whether
163 *ileS2* plasmids are the same or not. The p3 pattern was the most frequent in our hospital and
164 community MRSA isolates (65%) (Table 1). All of them belonged to the same clonal lineage
165 ST125/t067 widely distributed in hospital and community settings in Spain. This suggests a
166 clonal spread in our environment which could explain the high prevalence of HLMUPR-MRSA
167 during the study period.

168 **Conclusions**

169 Most of HLMUPR-MRSA isolates that are circulating in our environment belonged to
170 ST125/t067. This specific lineage is predominant in our area and it is associated with resistance
171 to aminoglycosides, and to a lesser extent, to macrolides. The presence of the same IS257-*ileS2*
172 amplification pattern p3 in 65% of HLMUPR-MRSA, all of them ST125/t067, suggests a clonal
173 spread in our hospital and community environment which could explain the high prevalence of
174 HLMUPR-MRSA during the study period. An outbreak situation or an increase in mupirocin
175 consumption was not observed.

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185 **Conflict of interest**

186 None

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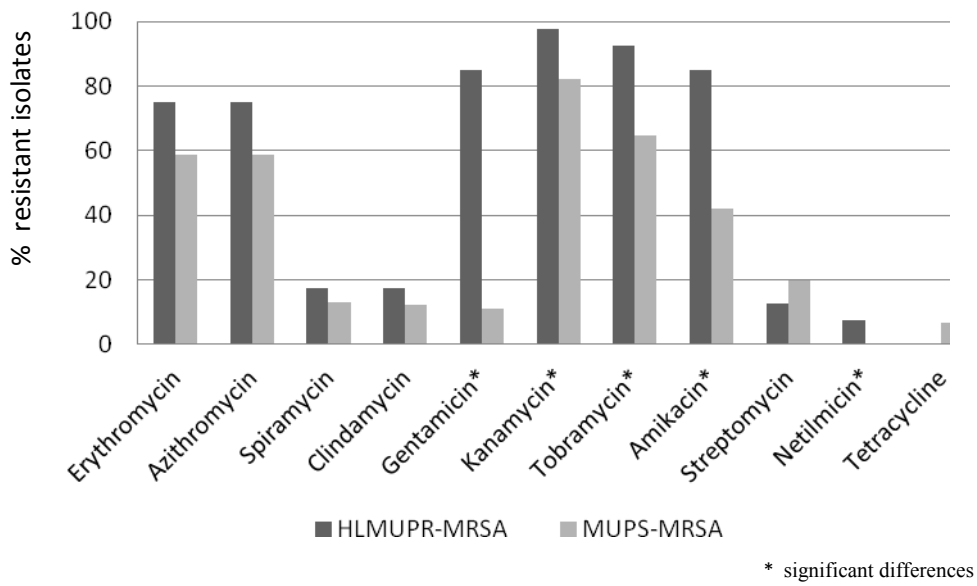


Figure 1.- Percentages of antibiotic resistance detected in high level mupirocin-resistant MRSA (HLMUPR-MRSA) and mupirocin-susceptible MRSA (MUPS-MRSA).

IS257-ileS2 amplification patterns	PFGE profile	ST	spa type	SCCmec	agr	Antibiotic resistance genes	Date of culture	Onset
p1	C	125	t067	IVc	II	<i>msr(A)</i> + <i>erm(C)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	24/07/2009	Hospital
p2	A ₄		t2226	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	28/07/2009	Hospital
	A ₅		t2226	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	10/11/2009	Hospital
	A ₅		t2226	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	24/12/2009	Hospital
	A ₆		t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	25/01/2010	Community
	A ₁₀		t2226	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	26/09/2009	Hospital
p3	A ₂		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	31/05/2010	Hospital
	A ₃		t067	IVc	II	<i>erm(C)</i> + <i>ant(4')</i> -Ia + <i>ileS2</i>	15/04/2010	Community
	A ₈		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	29/07/2009	Hospital
	A ₈		t067	IVc	II	<i>msr(A)</i> + <i>erm(C)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	30/07/2009	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	25/02/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	13/04/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	21/05/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	10/06/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	10/06/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	23/06/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	17/06/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>erm(C)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	02/03/2010	Hospital
	A ₉		t067	IVc	II	<i>msr(A)</i> + <i>erm(B)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	23/06/2010	Hospital
	A ₉		t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	28/06/2010	Hospital
	A ₁₀		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	11/05/2010	Hospital
	A ₁₁		t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	23/06/2010	Hospital
	B ₁		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	11/03/2010	Hospital
	B ₁		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	26/09/2009	Community
	B ₂		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	20/05/2010	Hospital
	B ₂		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	08/06/2010	Community
	B ₃		t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	31/05/2010	Hospital
	B ₄	t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ileS2</i>	05/07/2010	Hospital	
	B ₄	t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	19/04/2010	Hospital	
B ₄	t067	IVc	II	<i>msr(A)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>aph(3')</i> -IIIa + <i>ileS2</i>	21/04/2010	Hospital		
B ₆	t067	V	II	<i>msr(A)</i> + <i>ant(4')</i> -Ia + <i>ileS2</i>	03/11/2009	Community		
C	t067	IVc	II	<i>msr(A)</i> + <i>erm(C)</i> + <i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ileS2</i>	20/10/2009	Community		
p4	A ₇	t067	IVc	II	<i>erm(C)</i> + <i>ant(4')</i> -Ia + <i>ileS2</i>	20/01/2010	Hospital	
p5	B ₅	t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	03/05/2010	Hospital	
p6	A ₁₁	t2220	IVc	II	<i>msr(A)</i> + <i>aph(3')</i> -IIIa + <i>ileS2</i>	22/06/2010	Hospital	
	A ₁₁	t2220	IVc	II	<i>msr(A)</i> + <i>aph(3')</i> -IIIa + <i>ileS2</i>	25/01/2010	Community	
p7	B ₁	t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2'')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	08/10/2009	Hospital	
p8	A ₁	5	t002	IVc	II	<i>erm(C)</i> + <i>ileS2</i>	25/05/2010	Community

p9	A ₆	125	t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	31/05/2010	Community
	B ₁		t067	IVc	II	<i>aac(6')</i> -Ie- <i>aph(2')</i> -Ia + <i>ant(4')</i> -Ia + <i>ileS2</i>	12/06/2010	Community

Table 1.- Characteristics of 40 HLMUPR-MRSA isolates.

PFGE profile: Capital letters (A, B and C) mean strains with different PFGE profiles. The numbers in subscript ₍₁₋₁₁₎ indicate that those strains show closely related PFGE profiles.