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Clinical and molecular epidemiology of community-acquired, healthcare-associated and nosocomial methicillin-resistant *Staphylococcus aureus* in Spain

J. Rodríguez-Baño[1], M. Angeles Domínguez[2], A. Blas Millán[1], C. Borraz[2], M. Pau González[2], B. Almirante[3], E. Cercenado[4], B. Padilla[4], M. Pujol[5], on behalf of GEIH/GEMARA (SEIMC) and REIPI

1) *Sección de Enfermedades Infecciosas, Hospital Universitario Virgen Macarena, Sevilla,* 2) *Servicio de Microbiología, Hospital Universitario de Bellvitge, Barcelona,* 3) *Servicio de Enfermedades Infecciosas, Hospital Vall d'Hebrón, Barcelona,* 4) *Servicio de Microbiología, Hospital Gregorio Marañón, Madrid and* 5) *Servicio de Enfermedades Infecciosas, Hospital Universitario de Bellvitge, Barcelona, Spain*

Abstract

A prospective cohort study including all new cases of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization or infection in 64 Spanish hospitals during June 2003 was performed to investigate the epidemiology of MRSA in Spain. Only patients who yielded clinical MRSA-positive samples were included. Epidemiological and clinical data for a total of 370 cases were collected. Genotyping was performed using pulsed-field gel electrophoresis and multilocus sequence typing. Panton–Valentine leukocidin genes and the staphylococcal chromosomal cassette *mec* (SCC*mec*) were identified in representative isolates. MRSA was considered to be nosocomially acquired in 202 cases (55%), healthcare-associated (HCA) in 139 cases (38%), community-acquired (CA) in three cases, and of uncertain mode of acquisition in 26 (7%) cases. The pooled population-based rate was 2.31 cases/100 000 population/month, and the pooled nosocomial rate was 0.21 cases/1000 hospital stays (20.2% of *S. aureus*). Peripheral vascular disease, respiratory tract infections, catheter infections, bloodstream infections and crude mortality were more frequent among HCA cases, whereas neoplasia and urinary tract infections were more frequent among nosocomially acquired cases. Two clones related to the paediatric clone ST5-IV accounted for 71% of the isolates; EMRSA-16 has emerged in two different geographical areas. Only one isolate belonged to the formerly predominant Iberian clone. The three CA isolates were related to the USA300 clone. SCC*mec* type IV was the most frequent type in nosocomial and HCA isolates. The epidemiology of MRSA has changed in Spain; outpatients with previous healthcare contact represent a very important reservoir of MRSA, and community isolates are emerging.

Keywords: Healthcare-associated infections, methicillin-resistant *Staphylococcus aureus*, molecular epidemiology, multicentre study, nosocomial infections

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Corresponding author and reprint requests: J. Rodríguez-Baño, Sección de Enfermedades Infecciosas, Avda. Dr Fedriani 3, 41071 Seville, Spain

E-mail: jesusrodriguez@medynet.com

Introduction

Methicillin-resistant *Staphyloccocus aureus* (MRSA) is one of the most important healthcare-associated (HCA) pathogens worldwide [1,2]. The proportion of nosocomial infections caused by MRSA continues to increase in most countries, although with substantial variations [3–5].

Some significant changes in the epidemiology of MRSA have occurred during the last decade. Although the number of infections due to HCA MRSA in non-hospitalized patients is increasing [6], there are scarce data on the real burden of HCA MRSA in outpatients. Also, a shift in the predominance of HCA MRSA clones has been noted in some areas [7]. However, most studies have been performed in specific units or hospitals, or lack information about the patients. Finally, MRSA has recently emerged as a relevant community pathogen in several countries [8], but only anecdotal cases have been reported in Spain so far [9,10].

The objectives of this study were to investigate the clinical and molecular epidemiology of MRSA in a large sample of Spanish hospitals, to provide comprehensive information on

the burden of MRSA as a pathogen in non-hospitalized patients, and to analyse the clinical features of nosocomial and HCA MRSA infections.

Materials and Methods

Site

This study is part of the GEIH/GEMARA/REIPI MRSA 2003 project, which was aimed at investigating the epidemiological, microbiological, clinical and prognostic features of MRSA in Spain. The project also included a survey regarding the control measures for MRSA in Spanish hospitals, which has been published elsewhere [11]. Sixty-six hospitals, providing healthcare to >16 million people, participated in the study. Among them, 16 (24%) have <200 beds, 23 (35%) have 200–499 beds, and 27 (41%) have >500 beds; 36 (54%) are tertiary centres, 23 (35%) are community centres, and seven (11%) are private hospitals.

Design

A prospective cohort study, including all new patients from whom MRSA was isolated from any clinical sample during the month of June 2003 in the participating hospitals, was performed. The microbiology records were reviewed to avoid the inclusion of patients previously colonized or infected with MRSA; patients from whom MRSA was isolated during the previous 2 years were excluded. As surveillance policies differ from hospital to hospital, patients who yielded only MRSA-positive surveillance samples were excluded.

Variables and definitions

For each patient, the following data were recorded: age, gender, hospitalization, previous healthcare contact, ward, type and severity of underlying diseases (according to the McCabe classification) [12], invasive procedures, and antimicrobial use during the preceding 2 months. Also, the presence of infection due to MRSA and the type of infection (both assessed according to CDC criteria) [13], the presence of sepsis, severe sepsis, or septic shock [14], antimicrobial treatment and outcome were prospectively assessed. Patients were followed for 30 days, or until discharge or death if it occurred within 30 days.

MRSA acquisition was initially classified according to epidemiological criteria. Thus, MRSA was considered to be nosocomially acquired if isolated >48 h after admission of the patient. In all other cases, MRSA was considered to be HCA, i.e. if during the previous year any of the following applied: admission for >2 days to a hospital, nursing home, or other healthcare facility, surgery, dialysis, specialized home care, visit at day hospitals, or permanent indwelling catheters. Also, MRSA in healthcare workers was considered to be HCA. If none of the above applied, MRSA was considered to be community-acquired (CA) [15]. Epidemiological criteria were further assessed by analysing the microbiological features of the isolates [15]. The project was approved by the local ethic committees.

Microbiological studies

The first isolate from each patient was sent to Hospital Universitario de Bellvitge, Barcelona, where identification was confirmed by standard methods, and the presence of the *mec*A gene was determined by PCR [16]. Susceptibility testing was performed using the disk diffusion method according to the CLSI criteria [17]. Inducible resistance to clindamycin was detected by placing erythromycin and clindamycin disks 15–20 mm apart (D-zone test). MICs of mupirocin for resistant strains were determined by Etest (AB biodisk, Solna, Sweden). Heteroresistance to glycopeptides was screened for on brain–heart infusion agar plates containing vancomycin (6 mg/L) [18], using ATCC 700699 (Mu50) as a control strain [19]. All MRSA isolates were classified according to their resistance patterns (RPs), considering their resistance to non-β-lactam antibiotics.

Genotyping was performed by macrorestriction analysis of *Sma*I-digested DNA using pulsed-field gel electrophoresis (PFGE). PFGE was carried out for 23 h at 6 V/cm and 14°C, with pulses ranging from 1 to 30 s. PFGE patterns were interpreted according to the criteria of Tenover *et al.* [20]. They were compared with those of the pandemic clones EMRSA-15, EMRSA-16 [21], ATCC BAA-44 (Iberian clone) [22], and ATCC BAA-42 (paediatric clone) [23]. Panton–Valentine leukocidin genes were screened for with PCR [24]. Multilocus sequence typing and characterization of the staphylococcal chromosomal cassette *mec* (SCC*mec*) were performed for representative strains [25,26].

Statistical analysis

Nosocomial rates were calculated as the number of new nosocomial MRSA cases per 100 admissions and 1000 inpatient-days. The participating hospitals were required to indicate the number of all new patients from whom *S. aureus* had been isolated from clinical samples during the study period, in order to calculate the proportion of MRSA. Population-based incidence rates were determined only in basic health areas in which all hospitals participated in the study; they were calculated as the number of new cases per 100 000 population-months. The assigned population for each area during 2003 was used for the denominators. Qualitative variables were compared using the chi-squared test,

and quantitative variables using Student's *t*-test or the Mann–Whitney *U*-test, as appropriate. The correlation between continuous variables was evaluated by linear regression analysis.

Results

Rates and acquisition

During the study period, there were 370 new cases of colonization or infection with MRSA in 59 of the 64 participating hospitals/areas (92%); the five hospitals without MRSA cases were community hospitals with <200 beds. The first samples yielding MRSA from the included patients were wound/ulcer exudates in 179 cases (48%), respiratory tract samples in 75 cases (20%), blood culture samples in 50 cases(14%), urine in 31 cases (8%), catheter-tip samples in ten cases(3%), joint fluid in four cases(1%), and others in 21 cases(6%).

MRSA was considered to be nosocomially acquired in 202 patients (55%), HCA in 139 patients (38%), and CA in three patients (<1%); the mode of acquisition was uncertain in 26 outpatients (7%), because the previous healthcare contact could not be adequately assessed. The range in the number of cases per hospital was 0–23. Population-based rates could be calculated for 39 basic health areas. The pooled incidence rate of MRSA colonization or infection was 2.31 cases/100 000 population-months, and that of MRSA bacteraemia was 0.23. Nosocomial rates are shown in Table 1. Nosocomial rates would have been approximately two-fold higher if all HCA MRSA cases had been included in the numerators (0.34 cases per 100 admissions or 0.47 per 1000 patient-days). The pooled percentage of patients with nosocomial MRSA among patients with nosocomial *S. aureus* was 20.2%, and the median percentage was 20.0% (interquartile range: 4.1–28.0%). We found no significant differences between hospitals with 200–499 beds and those with ≥500 beds in terms of nosocomial incidence rates (median number of cases/1000 hospital-days, 0.15 vs. 0.21, p 0.1) or percentage of MRSA (median, 19% vs. 20%, p 0.4).

TABLE 1. Rates of nosocomial colonization/infection due to methicillin-resistant *Staphylococcus aureus* (MRSA) in Spanish hospitals

	Pooled		Median (interquartile range)	
	Cases/100 admissions	Cases/1000 patient-days	Cases/100 admissions	Cases/1000 patient-days
Global	0.15	0.21	0.11 (0.04–0.20)	0.16 (0.06–0.27)
Medical wards	0.18	0.19	0.19 (0–0.30)	0.15 (0–0.30)
Surgical wards	0.10	0.17	0 (0–0.18)	0 (0–0.24)
Intensive-care units	0.65	1.18	0.06 (0–2.25)	0 (0–4.22)

Patients with healthcare-associated MRSA were not included in the numerators.

Epidemiology and clinical features

Owing to insufficient data, we excluded the 26 cases with uncertainty about the MRSA acquisition; thus, the analysis includes 344 patients. Among the 202 nosocomially acquired cases, 40 (20%) were admitted to an intensive-care unit. The median previous hospital stay of nosocomial cases was 10 days (range, 3–330). Among the 139 patients with HCA MRSA, 40 (29%) were in nursing homes or long-term-care facilities, 63 (45%) had been previously admitted (one was a nursing home resident), 39 (28%) received care in specialized home-care programmes or day hospitals, six (4%) received haemodialysis, and one was a healthcare worker. The predisposing features of the 341 patients with nosocomial MRSA and HCA MRSA are shown in Table 2. Among the three community-acquired cases, two patients were <30 years old and had no comorbidities.

MRSA was considered to be causing an infection in 138 (68%) patients with nosocomial MRSA and in 90 (65%)

TABLE 2. Predisposing factors of patients with nosocomial and healthcare-associated methicillin-resistant *Staphylococcus aureus*

	Total (*n* = 341)	Nosocomial cases (*n* = 202)	Healthcare-associated cases (*n* = 139)	p[a]
Male gender	199 (58)	131 (65)	75 (54)	0.04
Median age in years (range)	71 (11–100)	65 (11–99)	70 (32–100)	0.001[b]
Chronic underlying disease				0.2
Non-fatal	215 (63)	125 (62)	90 (65)	
Ultimately fatal	96 (28)	41 (20)	41 (29)	
Rapidly fatal	30 (9)	22 (11)	8 (6)	
Diabetes mellitus	100 (29)	61 (30)	39 (28)	0.6
Neoplasia	75 (22)	52 (26)	23 (16)	0.04
Chronic pulmonary disease	71 (21)	41 (20)	29 (21)	0.9
Peripheral vascular disease	59 (17)	25 (12)	34 (24)	0.004
Chronic renal insufficiency	36 (11)	19 (9)	17 (12)	0.4
Liver cirrhosis	19 (6)	14 (7)	5 (4)	0.1
Pressure ulcer	43 (13)	12 (6)	11 (8)	0.4
Intravascular catheter	192 (56)	140 (69)	52 (37)	<0.001
Urinary catheter	101 (30)	72 (36)	29 (21)	0.003
Mechanical ventilation	37 (11)	34 (17)	0	<0.001
Surgery	107 (31)	82 (41)	25 (18)	<0.001
Previous antimicrobials	241 (71)	157 (78)	86 (62)	0.001
Penicillins	109 (32)	97 (48)	61 (44)	0.4
Cephalosporins	70 (21)	69 (34)	29 (21)	0.007
Fluoroquinolones	110 (32)	93 (46)	68 (49)	0.6

Data are expressed as no. of cases (%), except where indicated.
[a]Chi-squared test except where indicated.
[b]Mann–Whitney *U*-test.

patients with HCA MRSA (p 0.5). Clinical and prognostic features of patients with infection due to nosocomial and HCA MRSA are shown in Table 3. There were no differences with respect to frequency or type of infection, bacteraemia or mortality among isolates from the most frequent clonal groups. The three patients with CA MRSA had cellulitis and were cured.

Microbiological results

All 370 isolates were analysed. Susceptibility data are shown in Table 4. The macrolide–lincosamide–streptogramin B (MLS_B) resistance phenotype was found in 46% of the strains, whereas selective resistance to macrolides and streptogramin B (MS_B phenotype) was found in 29%. Among the 72 mupirocin-resistant strains, 17 were isolated in a single hospital, 50 showed MICs >512 mg/L, and ten were clonally related. No resistance or heteroresistance to glycopeptides was detected in any strain. Although 26 RPs were found, 247 isolates (67%) belonged to one of the four predominant RPs (Table 5). The three CA isolates were susceptible to all non-β-lactam antibiotics.

PFGE analysis of nosocomial and HCA MRSA revealed 33 different types. There were two predominant clonal groups, present in 45 hospitals: clonal group Q (138 isolates, 37%), and clonal group P (125 isolates, 34%). Both clonal groups were present at similar frequencies in nosocomial and HCA isolates, or in those of uncertain origin (Table 4). The 12 selected strains from subtypes of clones Q and P studied by multilocus sequence typing belonged to clonal complex 5: ST5 (one strain) or their single-locus variants ST125 (eight strains) and ST146 (three strains). Thirty-five clonally related strains (clone BA, 10%) from 13 hospitals belonged to the EMRSA-16 (ST36) clone; 31 of these isolates came from hospitals in Galicia (22 isolates) and the Canary Islands (nine). Also, seven strains (six from a single hospital in Majorca) belonged to the EMRSA-15 (ST22) clone. Only one strain belonged to the formerly predominant Iberian clone (ST247). There were no differences in predisposing factors, clinical features or prognosis among patients with the most frequent clones.

The SCC*mec* type was studied in 80 isolates, including 22 clone P isolates, 22 clone Q isolates, six clone BA isolates, and one representative of every other PFGE type. In two isolates, the SCC*mec* type could not be determined; 18/22 and 17/22 clone P and clone Q isolates (82% and 77%, respectively) carried SCC*mec* type IV, and five or six clone BA (ST36) isolates carried SCC*mec* type II. The SCC*mec* types as related to the mode of MRSA acquisition are shown in Table 4.

PVL gene detection was performed in 104 selected cases, including CA cases, cases of uncertain mode of acquisition, cases of soft tissue infections, and most susceptible isolates; three of them (two CA MRSA and one HCA MRSA) were PVL gene-positive. The PVL gene-positive strains were isolated in two hospitals in Barcelona, belonged to ST8 (which was not found in other isolates), and harboured SCC*mec* type IV.

TABLE 3. Clinical features and outcome of patients with infection due to nosocomial and healthcare-associated methicillin-resistant *Staphylococcus aureus* (patients without criteria for infection were excluded)

	Nosocomial infections (*n* = 138)	Healthcare-associated infections (*n* = 90)	p[a]
Type of infection[b]			
Skin and soft tissue	61 (44)	43 (48)	0.5
Respiratory tract	31 (22)	11 (12)	0.05
Urinary tract	7 (5)	13 (14)	0.01
Bone and joints	6 (4)	7 (8)	0.2
Catheter-related infection	19 (14)	3 (3)	0.009
Primary bacteraemia	7 (5)	4 (4)	0.8
Intra-abdominal	5 (4)	2 (2)	0.5
Miscellaneous	2 (1)	6 (7)	0.06
Bloodstream infection (primary or secondary)	42 (30)	15 (17)	0.01
Surgical site infections	46 (33)	11 (12)	<0.001
Crude mortality[c]	33 (24)	11 (12)	0.02
Infection-related mortality[d]	17 (12)	9 (10)	0.5

Data are expressed as no. of cases (%) except where indicated.
[a]Chi-squared test.
[b]Incisional surgical infections are classified as skin and soft tissue infections. Organ/space surgical site infections are classified according to the organ affected.
[c]Includes all deaths that occurred during follow-up.
[d]Includes all deaths that occurred as a direct consequence of MRSA infection without another plausible cause, as determined by the investigators.

Discussion

In this prospective multicentre study performed in Spain, some significant changes in MRSA epidemiology were observed with regard to older data. MRSA was found to be nosocomially acquired in only 55% of the cases, and the rest of the MRSA acquisition occurred in outpatients, in whom MRSA was considered to be HCA, with the exception of three cases of CA MRSA. Approximately 70% of the isolates belonged to two PFGE groups, and SCC*mec* type IV was predominant.

To our knowledge, this is the first multicentre study to integrate multiple aspects of MRSA epidemiology. The study was performed in 2003; no significant changes in the percentage of MRSA [6] or circulating MRSA clones seem to have occurred in Spain since then (47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 2007, Abstract C2-148). The duration of the study period (1 month) is the main limitation for the estimation of inci-

TABLE 4. **Resistance to selected antimicrobial agents, distribution of molecular types (pulsed-field gel electrophoresis (PFGE)), SCC*mec* types, and presence of Panton–Valentine leukocidin (PVL) genes among methicillin-resistant *Staphylococcus aureus* isolates according to mode of acquisition**

	All isolates (*n* = 370)	Nosocomial isolates (*n* = 202)	HCA isolates (*n* = 139)	CA isolates (*n* = 3)	Uncertain mode of acquisition (*n* = 26)
Susceptibility					
Erythromycin	278 (75)	153 (76)	111 (79)	0	15 (58)
Clindamycin	169 (46)	106 (53)	60 (43)	0	2 (8)
Gentamicin	90 (24)	56 (28)	30 (22)	0	3 (12)
Tobramycin	320 (87)	178 (88)	120 (86)	0	21 (81)
Rifampin	5 (1)	1 (<1)	4 (3)	0	0
Ciprofloxacin	363 (98)	199 (99)	138 (99)	0	26 (100)
Trimethoprim–sulphamethoxazole	6 (2)	2 (1)	3 (2)	0	1 (4)
Tetracycline	3 (1)	1 (<1)	2 (1)	0	0
Chloramphenicol	39 (11)	22 (11)	14 (10)	0	3 (12)
Mupirocin	72 (19)	38 (19)	20 (14)	0	5 (19)
Vancomycin	0	0	0	0	0
Quinupristin–dalfopristin	0	0	0	0	0
Linezolid	0	0	0	0	0
PFGE					
Clone Q (CC5)	138 (37)	75 (37)	55 (39)	0	8 (31)
Clone P (CC5)	125 (34)	65 (32)	47 (34)	0	13 (50)
Clone BA (ST36)	35 (9)	23 (12)	11 (8)	0	0
Other clones	73 (20)	39 (19)	26 (19)	3 (100)	5 (19)
SCC*mec* type	*n* = 78	*n* = 40	*n* = 26	*n* = 3	*n* = 9
I	18 (23)	11 (28)	5 (19)	1 (33)	1 (11)
II	10 (13)	8 (20)	2 (8)	0	0
III	1 (1)	1 (2)	0	0	0
IV	49 (63)	20 (50)	19 (73)	2 (66)	8 (89)
PVL genes	*n* = 104	*n* = 20	*n* = 55	*n* = 3	*n* = 26
Positive	3	0	1	2	0

HCA, healthcare-associated; CA, community-acquired.
SCC*mec* type and presence of PVL genes were determined in representative isolates (see text). Data are expressed as no. of isolates (percentage).

TABLE 5. **Distribution of resistance patterns of methicillin-resistant *Staphylococcus aureus* strains according to genotypes**

Resistance patterns	Clone Q	Clone P	EMRSA-16	Other clones	Total
Er, Cl, Gen, Tob, Cip	14 (10)	17 (14)	6 (17)	18 (25)	55 (15)
Er, Cl, Tob, Cip	16 (12)	33 (26)	20 (57)	9 (13)	78 (21)
Er, Tob, Cip	43 (31)	13 (10)	1 (3)	8 (11)	65 (18)
Tob, Cip	13 (9)	30 (24)	0	6 (8)	49 (13)
Other patterns	52 (38)	32 (25)	8 (23)	31 (43)	123 (33)
Total	138 (100)	125 (100)	35 (100)	72 (100)	370 (100)

Data are expressed as no. of isolates (%).
Er, erythromycin; Cl, clindamycin; Gen, gentamicin; Tob, tobramycin; Cip, ciprofloxacin.

dence rates, which should be considered here to be minimum rates. However, a longer study period would have made the inclusion of precise epidemiological and clinical data very difficult.

The percentage of MRSA found in this study (20%) is lower than those found in some recent prevalence studies performed in Spain (29–41%) [6,27]. Apart from the fact that one of these studies included patients identified as MRSA-positive by means of active surveillance [6], prevalence studies may overestimate the real percentage of MRSA, as patients colonized or infected by this organism have a longer hospital stay and are more frequently tested than patients with methicillin-susceptible *S. aureus* [2], thus increasing the probability of their being included in prevalence studies. In fact, the present data are similar to those of other incidence studies performed in Spain (with MRSA rates ranging from 22% to 28%) [28,29]. With regard to population-based MRSA rates, data are scarce. Morgan *et al.* found 92.4 cases of MRSA colonization or infection (including cases detected by active surveillance) and 5.2 cases of MRSA bacteraemia per 100 000 population/year during 1996 in Wales [30]. The extrapolated population-based rates per year of the present study would be 27.7 and 2.7, respectively. This is in agreement with the fact that percentages of MRSA among *S. aureus* are much lower in Spain than in the UK [5]. One of the main findings of this study is that only 55% of the MRSA cases were nosocomially acquired, and that almost all the rest were outpatients with previous healthcare contact. This emphasizes the importance of chronic colonization after MRSA acquisition in healthcare facilities. As characterizing the epidemiology of MRSA in outpatients is difficult, previous healthcare contact could not be adequately assessed in 7% of the cases; however, isolates from outpatients were clonally related to HCA and nosocomial isolates. Charberny *et al.* [31] found a similar proportion of non-nosocomial cases in Germany. Thus, approximately half of the HCA MRSA clini-

cal cases are now being diagnosed in outpatients. These data show that the determination of nosocomial rates is insufficient to provide an idea of the global epidemiology of HCA MRSA and of the enormous reservoir of HCA MRSA in outpatients, and they support the need for targeted active surveillance of patients at admission [32]. Regarding the nosocomial rates, this study also shows that if all HCA cases are included in the numerators, nosocomial rates may be considerably overestimated. Thus, a consensus for a precise definition of MRSA rates is necessary.

There are only scarce data in the literature concerning the predisposing factors and clinical features of outpatients with HCA MRSA. We found few differences in intrinsic features between patients with nosocomial and HCA MRSA: the latter were older and suffered more frequently from peripheral vascular disease. Crude mortality was higher among patients with nosocomial infection, but infection-related mortality was similar in both groups.

With regard to the antimicrobial susceptibility data, no correlation was found between PFGE genotypes and RPs, as previously described [29]. The susceptibility data and molecular typing results confirm a shift from some multidrug-resistant clones (particularly the Iberian clone) to more susceptible clones [7,29,33,34]. More than 70% of the isolates analysed here belonged to two clonal groups; these dominant genotypes belong to a common clonal complex (CC5) that may have evolved from the paediatric clone ST5-IV [26]. Isolates of this clonal complex emerged in Spain in 1996 [34] and are now predominant. Other international clones detected in this study were EMRSA-16 (ST36) and EMRSA-15 (ST22); EMRSA-16 had been rare in Spain [34], except in the Canary Islands [7], but our study documents its spread also in Galicia. EMRSA-15 was found almost exclusively in one hospital in Majorca. It is of interest that SCC*mec* type IV is not a good marker for community isolates in Spain, as it was the most frequent type among HCA and nosocomial isolates. Another interesting finding was the remarkably low number of PVL gene-positive strains in this study; their features suggest the initial spread of a clone with genetic traits related to those of the USA300 clone (ST8-IV). Infections due to CA MRSA have been recently described in Madrid [9] and Barcelona [10].

In conclusion, the vast reservoir of a few predominant HCA MRSA clones in outpatients (mainly patients with previous hospital admission and residents in long-term-care facilities) in Spain represents a challenge for infection control. Also, continuous surveillance and action are needed to control the dissemination of some emergent international HCA and CA clones. In this context, a consensus document concerning MRSA control has recently been published [35].

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Transparency Declaration

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Appendix

Participants in the MRSA 2003 GEIH/GEMARA/REIPI project:

J. Álvarez, M. Alcalde, C. Zamorano (Hosp. de Cieza, Murcia);

G. Sauca, L. Force, E. Martínez (Hosp. de Mataró, Barcelona);

E. Hidalgo, E. Mata, J. S. Rubio (Hosp. de Jove, Gijón, Asturias);

A. Bordes, M. A. Figuerola, B. Lafarga (Hosp. Dr Negrín, Las Palmas, Gran Canaria)

A. Leturia (Hosp. Nal. de Parapléjicos, Toledo);

F. Calvo, J. Pérez, J. L. Sánchez (Hosp. de Santa Marina, Bilbao);

J. Blázquez, C. Domínguez, C. Mirete, M. Gea, M. Bermúdez (Hosp. de S. Vicente, Alicante);

T. Alarcón, C. Cuevas, N. Carrasco, J. Jimeno (Hosp. Univ. Princesa, Madrid);

A. Canut, A. Labora, J. L. Novales, I. Orio (Hosp. Santiago Apóstol, Vitoria);

J. Serralta, D. Arzua (Clínica Benidorm, Alicante);

G. Esteban, B. Fernández, M. D. Díaz (Hosp. Sta. María Mai, Ourense);

R. Carranza, D. Tena, J. R. Barberá (Hosp. Mancha Centro, Alcázar de San Juan, Toledo);

A. Asensio, J. Lobera, T. Marco, A. Ramos (Hosp. Pta. de Hierro, Madrid);

M. L. García, M. D. Miguel (Hosp. Cabueñes, Asturias);

F. Chaves, F. Jaén, M. Lizasoain, M. I. Sanz (Hosp. 12 de Octubre, Madrid);

E. Cercenado, B. Padilla, F. Grande (Hosp. Gregorio Marañón, Madrid);

M. D. Rodríguez, J. F. García, M. V. Lorenzo (Hosp. P. Novoa Santos, Ferrol, A Coruña)

A. Yagüe, A. Cabrera, J. Gregori, J. Salinas (Hosp. Vega Baja, Orihuela, Alicante);

M. Elía, J. M. Ramos, A. M. Haro (Hosp. de Elche, Alicante);

M. T. Bastida, R. Porrón, F. Nonell (Hosp. Esperit Sant, Santa Coloma de Gramanet, Barcelona);

T. Delgado, M. de Frutos, I. Montesinos (Hosp. Univ. de Canarias, Tenerife);

M. Xercavins, N. Juan, M. Riera, N. Freixas (Hosp. M. de Terrassa, Barcelona);

M. A. Sepúlveda, C. Gómez (Hosp. V. de la Salud, Toledo);

P. Álvarez, R. Ojeu, F. Vazquez, J. Paz (Hosp. de Pontevedra);

J. A. Jiménez, E. Laviñeta (Policlínica Guipúzcoa, San Sebastián);

R. M. Ferreruela, A. Viñuales, J. M. Tenias (Hosp. L. Alcanyís, Xátiva, Valencia);

A. Zapata, J. L. de Francisco (Hosp. de Jerez de la Frontera);

A. Moreno, M. C. Durán, N. Batista (Hosp. Candelaria, Santa Cruz, Tenerife);

C. Martínez, M. J. Gallego, C. Amador (Hosp. Marina Baixa, Villajoyosa, Alicante);

F. J. Noguera, D. Panizo, E. Melian (Hosp. Gral. de Lanzarote);

A. Fleites, A. Moreno (Hosp. Gral. de Asturias, Oviedo);

F. Pardo, M. L. Pérez, E. Carballo (Hosp. de Santiago de Compostela);

M. A. Gasos, A. Pérez, M. Vidal (Hosp. S. Joan de Déu, Martorell, Barcelona);

J. R. Hernández, L. García (Hosp. Macarena, Sevilla);

A. Coloma, F. Navarro, J. Barrio, P. Coll (Hosp. S. Pau, Barcelona);

M. Bolaños, E. Álvarez, M. Hernández, J. Molina (Hosp. Insular, Gran Canaria);

P. García, B. R. Blazquez, M. Cal (Hosp. de Getafe, Madrid);

S. Giner, P. García, N. Tolosa (Hosp. Dr Moliner, Serra, Valencia);

A. Calderón (Hosp. de Badalona);

C. Arana (Hosp. V. Victoria, Málaga);

F. Ulloa, E. Pazos, M. Maresclot (Hosp. N. Peña, Vigo);

J. E. Corzo, J. L. García (Hosp. Valme, Sevilla);

J. L. Hernández, K. Aguirrebengoa, J. M. Hernández (Hosp. Cruces, Vizcaya);

M. Motjé, J. de Batlle, D. García, L. Gavaldá (Hosp. Dr J. Trueta, Gerona);

M. Álvarez, C. Potel (Hosp. Cies, Vigo);

R. Escoms, J. Prat, C. González, M. Pinazo (Hosp. de Sagunto, Valencia);

M. Canals, D. Mariscal, N. García (Hosp. Parc Tauli, Sabadell, Barcelona);

A. Andreu, M. Campins, N. Larrosa (Hosp. Vall d'Hebron, Barcelona);

J. M. Cisneros, A. C. Llanos (Hosp. V. Rocío, Sevilla);

J. L. Pérez, A. Oliver, M. Peñaranda, C. Juan, E. Ruiz (Hosp. Son Dureta, Palma de Mallorca);

V. Pintado, R. Cantón, J. Oliva, L. García, F. Grill (Hosp. Ramón y Cajal, Madrid);

J. Díaz, V. Cárcava (Hosp. Oriente, Arriendas, Asturias);

C. García, J. Cucurull (Hosp. Figueres, Gerona);

D. Sousa, G. Bou (Hosp. J. Canalejo, La Coruña);

E. Limón (Hosp. Bellvitge, Barcelona);

J. Cuquet, C. Martí, M. D. Navarro (Hosp. de Granollers, Barcelona);

P. Berdonces, J. Mayo (Hosp. de Galdakao, Vizkaya);

M. Sierra, M. Vaque, M. Calsina (Hosp. SCIAS, Barcelona);

S. Illescas, A. López (Hosp. V. Altagracia, Manzanares, Ciudad Real);

A. Hernández, M. L. Abellán, A. García (Hosp. S. Jaume, Calella, Barcelona);

A. García, C. Gallés (Hosp. de la Selva, Blanes, Gerona);

M. Salvadó (Hosp. del Mar, Barcelona);

L. Matas, N. Sopena, M. Caraballo (Hosp. G. Trias i Pujol, Badalona);

A. Coira, J. Varela (Hosp. Calde, Lugo);

M. Cuenca, A. Guerrero (Hosp. de la Ribera, Alcira);

J. P. Horcajada, F. Marco, M. Zaragoza, M. Piazuelo, J. A. Martínez (Hosp. Clinic, Barcelona).