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Molecular epidemiology of MRSA in 13 ICUs from eight European countries

D. J. Hetem[1]*, L. P. G. Derde[2], J. Empel[3], A. Mroczkowska[3], M. Orczykowska-Kotyna[3], A. Kozińska[3], W. Hryniewicz[3], H. Goossens[4] and M. J. M. Bonten[1,5] on behalf of the MOSAR WP3 Study Group†

[1]Department of Clinical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, The Netherlands; [3]Department of Epidemiology and Clinical Microbiology, National Medicines Institute, Warsaw, Poland; [4]Department of Medical Microbiology, Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium; [5]Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands

*Corresponding author. Tel: +31-88-7558108; E-mail: d.j.hetem@umcutrecht.nl
†Members of the MOSAR WP3 Study Group are listed in the Acknowledgements section.

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Objectives: The European epidemiology of MRSA is changing with the emergence of community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). In this study, we investigated the molecular epidemiology of MRSA during 2 years in 13 ICUs in France, Greece, Italy, Latvia, Luxemburg, Portugal, Slovenia and Spain.

Methods: Surveillance cultures for MRSA from nose and wounds were obtained on admission and twice weekly from all patients admitted to an ICU for ≥3 days. The first MRSA isolate per patient was genotyped in a central laboratory by MLST, *spa* typing, *agr* typing and SCC*mec* (sub)typing. Risk factors for patients with an unknown history of MRSA colonization were identified.

Results: Overall, 14 390 ICU patients were screened, of whom 8519 stayed in an ICU for ≥3 days. Overall MRSA admission prevalence was 3.9% and ranged from 1.0% to 7.0% for individual ICUs. Overall MRSA acquisition rate was 2.5/1000 patient days at risk and ranged from 0.2 to 8/1000 patient days at risk per ICU. In total, 557 putative MRSA isolates were submitted to the central laboratory for typing, of which 511 (92%) were confirmed as MRSA. Each country had a distinct epidemiology, with ST8-IVc (UK-EMRSA-2/-6, USA500) being most prevalent, especially in France and Spain, and detected in ICUs in five of eight countries. Seventeen (3%) and three (<1%) isolates were categorized as CA-MRSA and LA-MRSA, respectively. Risk factors for MRSA carriage on ICU admission were age >70 years and hospitalization within 1 year prior to ICU admission.

Conclusions: The molecular epidemiology of MRSA in 13 European ICUs in eight countries was homogeneous within, but heterogeneous between, countries. CA-MRSA and LA-MRSA genotypes and Panton–Valentine leucocidin-producing isolates were detected sporadically.

Introduction

MRSA can colonize and infect hospitalized and non-hospitalized humans. It is the leading nosocomial pathogen and hospital-acquired MRSA (HA-MRSA) infections are associated with high morbidity and mortality and increased healthcare spending.[1,2]

The global epidemiology of MRSA has changed with the emergence of community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). Ten years ago, MRSA was regarded as a sole nosocomial pathogen, mainly affecting patients with healthcare exposure, invasive medical devices, high age and undergoing surgical procedures. Since then, we have witnessed a rapid increase of MRSA infections occurring in previously healthy non-hospitalized persons, so-called CA-MRSA.[3] The risk factors for developing CA-MRSA infections differ from the traditional healthcare-related risk factors for HA-MRSA infections and include crowding, lack of cleanliness and participation in contact sports. In the USA, CA-MRSA (predominantly USA300) became the most important pathogen for community-acquired skin and soft tissue infections and has replaced traditional healthcare-associated strains in being the most common strain causing nosocomial MRSA bacteraemia.[4] In Europe, most nosocomial MRSA infections are still caused by HA-MRSA genotypes.[5,6] Yet, in Europe, animals in the agricultural industry have become a large reservoir of LA-MRSA (predominantly ST398), currently accounting for 40% of all MRSA colonization and infections in the Netherlands, a country with traditionally low prevalence of MRSA.[7]

It is unknown to what extent the global changes in MRSA epidemiology affect ICU populations in Europe. We therefore determined prevalence, acquisition rates and molecular epidemiology

of MRSA in 13 ICUs in eight European countries that participated in a prospective trial to control transmission of antibiotic resistance in ICUs.[8]

Methods

We performed a *post hoc* analysis from the ICU trial within the Mastering Hospital Antimicrobial Resistance in Europe (MOSAR) project (http://www.clinicaltrials.gov number NCT00976638), which evaluated different interventions to reduce transmission of antibiotic-resistant nosocomial pathogens including MRSA, VRE and highly resistant Enterobacteriaceae in ICUs. The study was conducted in 13 ICUs from eight European countries: France (3 ICUs), Greece (2 ICUs), Portugal (2 ICUs), Slovenia (2 ICUs), Italy (1 ICU), Latvia (1 ICU), Luxembourg (1 ICU) and Spain (1 ICU). Written approval of the study protocol was obtained from each institution's review board or national ethics committee when required.

Design and data collection

Data were obtained during the MOSAR trial between May 2008 and April 2011 in three phases: (i) a 6 month baseline period (phase I); (ii) a 6 month period with implementation of a hand hygiene improvement programme (largely based on the WHO 'five moments' programme), and feedback of compliance to personnel, as well as universal chlorhexidine body washings (phase II); (iii) followed by a 12–15 month cluster-randomized intervention phase (phase III) as described in detail elsewhere.[8]

In phase III, six ICUs were randomized to chromogenic agar-based screening for MRSA and seven were randomized to PCR-based screening for MRSA, both including feedback of screening results (from either cultures on chromogenic media or molecular tests) to personnel and the use of contact precautions for identified carriers.

Nasal and wound swabs were obtained from all patients admitted to an ICU for ≥3 days and within 48 h of ICU admission and twice weekly thereafter. Culture frequency was reduced to once weekly after 21 days of ICU admission.

MRSA colonization was considered ICU acquired if detected on or after the third day of ICU admission, in the absence of colonization on admission. The MRSA admission prevalence and MRSA acquisition risk per 1000 patient days at risk were calculated for all ICUs and countries. Risk factor analysis for MRSA colonization on ICU admission was performed for patients with no known history of MRSA carriage or infection. Aggregated data of 13 ICUs were used for the identification of risk factors.

Microbiology

Nasal and wound swabs were tested in local laboratories for the presence of MRSA by chromogenic agar for MRSA detection (BBL CHROMagar MRSA II; Becton Dickinson, Franklin Lakes, NJ, USA) in all study phases, including both arms of phase III, throughout the trial. ICUs randomized to rapid MRSA detection by PCR additionally used a GeneXpert real-time PCR system (Cepheid, Sunnyvale, CA, USA) in phase III on the admission swabs. During phases I and II surveillance cultures were stored in cryopreservative fluid for ≥2 months before analysis, to prevent feedback of results to clinicians. All participating laboratories were required to perform proficiency panels for MRSA detection.[9]

The first MRSA isolate of each patient was sent to a central laboratory (National Medicines Institute, Warsaw, Poland) for confirmation on both the phenotypic and genotypic level. Here, all isolates were reidentified using routine microbiological methods, including slide agglutination (Prolex Staph Xtra Latex Kit; PRO-LAB Diagnostics, Richmond Hill, Ontario, Canada) in combination with coagulase. The presence of *mecA* and *lukS/lukF* genes, indicative of the presence of Panton–Valentine leucocidin (PVL), were determined by PCR as described elsewhere.[10,11] *mecA*-negative isolates were additionally screened for the *mecC* gene as described in Cuny *et al*.[12] All MRSA isolates were characterized by *spa* typing, using Ridom® StaphType software on the *spa* server (http://spaserver.ridom.de) to allocate *spa* types.[13] Accessory gene regulator (*agr*) allotypes were determined according to Gilot *et al*.[14] Staphylococcal cassette chromosome *mec* (SCC*mec*) typing and subtyping were performed as previously described.[15,16] In case of equivocal results, additional typing was applied.[17] All SCC*mec* elements of type VI were confirmed by the methods described by Kondo *et al*.[18] SCC*mec* was classified according to guidelines proposed by the International Working Group on the Classification of SCC Elements (2009).[19] For each isolate, the ST was inferred from either the *spa* type in the *spa* server or from the literature or, if it was infeasible, the ST was determined by MLST according to Enright *et al*.[20] MRSA clones were defined by ST and SCC*mec* type as previously proposed by Enright *et al*.[20] and additionally by PVL and the arginine catabolic mobile element (ACME).[21] MLST was performed on at least one isolate from each *spa* type detected in each MRSA clone identified in each ICU.[20] We called isolates with the same MLST type and SCC*mec* (sub)type identical, i.e. the same clone. STs were assigned through the *Staphylococcus aureus* MLST database (http://saureus.mlst.net). In case of USA300 clone ($n=1$), presence of ACME was confirmed by duplex PCR with primers AIPS.29 and AIPS.28 (locus *arc*) and AIPS.45 and AIPS.46 (locus *opp3*).[22]

Two clones (ST130-XI and ST398-IVa) were considered LA-MRSA.[12,23] We used a molecular genotypic definition for the identification of CA-MRSA and considered the following clones to be CA-MRSA: ST1-IVa (USA400); *pvl* and ACME positive ST8-IVa (USA300); ST30-IVc (the South-West Pacific-related clone); *pvl* positive ST80-IVc (the European clone); ST88-IVa and *pvl* positive ST152-V (Balkan clone).[3,24] Simpson's index of diversity was calculated as previously described using Ridom's EpiCompare software (version 1.0).[25,26]

Statistical analysis

Univariate analysis was performed using the χ^2 test or Student's *t*-test where appropriate. Risk factors with a *P* value <0.1 in the univariate analysis were subsequently analysed by backward stepwise regression analysis to calculate ORs and 95% CIs. Two-sided significance was assessed for all variables, applying a cut-off value of $P<0.05$. Data analysis was performed with SPSS v20.0 (IBM, Armonk, NY, USA).

Results

Admission prevalence and acquisition

During the study period of 24–27 months, 14390 patients were screened upon admission to the ICU, of whom 8976 were admitted for ≥3 days. From 8519 of 8976 (95%) patients, at least one nasal swab was taken during ICU admission and these patients were therefore subsequently analysed. From 931 (10%) of these 8976 patients, additional wound swabs were obtained. A total of 631 MRSA-colonized patients were detected in the local laboratories, of whom 335 (53%) were colonized on admission (Table 1). MRSA prevalence on ICU admission was 3.9% (335 of 8519 patients) across all ICUs during the study period and was 4.3%, 4.2% and 3.7% during phases I, II and III, respectively. The highest admission prevalence (7.0%) was observed in one of the Greek ICUs, followed by a Portuguese (6.4%) and a French ICU (5.4%). Admission prevalence in other ICUs ranged from 1.0% to 5.0% (Table 1). MRSA colonization on admission was identified in 2.9%, 4.4% and 8.0% of patients admitted to the ICU directly from home, a hospital ward or a long-term care facility, respectively.

The overall MRSA acquisition rate was 2.5 per 1000 patient days at risk during the 2 year study period and 3.5, 3.1 and 2.0 per 1000 patient days at risk during phases I, II and III, respectively. Acquisition rates of MRSA for individual ICUs ranged from 0.2

Table 1. MRSA admission prevalence and MRSA acquisition rates across countries and ICUs

Country/ICU	Patients screened, $n=8519$	MRSA colonization on admission, $n=335$	MRSA colonization through acquisition, $n=296$	Acquisition of MRSA/1000 patient days at risk
France				
ICU 1	1419	77 (5.4%)	22 (1.6%)	1.4
ICU 2	666	33 (5.0%)	23 (3.5%)	2.3
ICU 3	502	14 (2.8%)	5 (1.0%)	0.8
Latvia	921	40 (4.3%)	90 (9.8%)	8.0
Portugal				
ICU 1	408	26 (6.4%)	28 (6.9%)	6.8
ICU 2	615	19 (3.1%)	34 (5.5%)	4.5
Italy	534	20 (3.7%)	13 (2.4%)	1.9
Greece				
ICU 1	704	49 (7.0%)	52 (7.4%)	3.8
ICU 2	268	6 (2.2%)	1 (0.4%)	0.2
Slovenia				
ICU 1	422	17 (4.0%)	12 (2.8%)	1.7
ICU 2	505	5 (1.0%)	4 (0.8%)	0.6
Spain	638	17 (2.7%)	3 (0.5%)	0.5
Luxembourg	917	12 (1.3%)	9 (1.0%)	0.7
Total	8519	335	296	2.5

to 8.0 per 1000 patient days, being highest in Latvia and lowest in one ICU in Greece (Table 1).

Fifty patients (0.6%) had MRSA bacteraemia: 17 on admission, 2 before admission and 31 acquired bacteraemia during ICU stay. Twenty-three (74%) of 31 patients with ICU-acquired MRSA bacteraemia were colonized on admission ($n=7$) or acquired MRSA colonization during their ICU stay before bacteraemia ($n=16$).

Risk factors

Risk factors for MRSA colonization on admission were analysed for 8196 patients who had no known history of MRSA carriage or infection. Univariate analysis on the aggregated data of all 13 ICUs identified age >70 years, haemodialysis, chronic hepatic failure, recent hospitalization (<1 year), recent surgery (<1 year) and admission to the ICU from a long-term care facility as significant risk factors (Table 2). Subsequent multivariate analysis identified age >70 years and recent hospitalization as risk factors for colonization on ICU admission (Table 3). Risk factor analysis for the 13 individual ICUs is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

Genotyping

Overall, 631 patients were colonized with MRSA according to local test results and 557 putative isolates (88%) were submitted to the central laboratory, of which 511 (92%) were confirmed as MRSA. Thirty-four (6%) were MSSA, seven (1%) were CoNS and five (<1%) appeared to be a non-staphylococcal species. One patient had two different MRSA genotypes, isolated during different admissions. From 11 patients, identical MRSA isolates were submitted from different ICU admissions, which were excluded for the subsequent analysis.

Of the 500 MRSA isolates, 480 (96%) were categorized as HA-MRSA, 17 (3%) as CA-MRSA and 3 (<1%) as LA-MRSA. The Brazilian/Hungarian clone ST368-III ($n=99$) was most prevalent and was solely found in the Latvian ICU [99 of 105 (94%) isolates] (Table 4). ST8-IVc (UK-EMRSA-2/-6, USA500) was the most prevalent clone in ICUs in France and Spain [France: 85/157 (54%); Spain: 8/18 (44%)] and was detected in seven ICUs in five of eight countries. ST22-IVh (UK-EMRSA-15) was detected in seven ICUs in five countries. All participating ICUs had a distinct molecular epidemiology and there was little homogeneity in isolated genotypes between countries. Only ICUs from two sets of countries (France and Spain, and Italy and Luxembourg) shared dominant clones. A high level of homogeneity in STs existed in ICUs within the same country (Table 4). The three most commonly found *spa* types across countries and ICUs are shown in Table 4. In two isolates, the *spa* type was non-typeable. All the individual *spa* types found in different ICUs are available as Supplementary data.

In eBURST analysis, MRSA isolates were grouped into 11 clonal complexes, with 253 isolates (51%) belonging to CC8/239. Other prevalent clonal complexes were CC5 ($n=129$, 26%) and CC22 ($n=79$, 16%). All clonal complexes identified during the study period in each country can be found in Figure 1.

SCC*mec* typing revealed 211 (42%) SCC*mec* type IV, 141 (28%) SCC*mec* type III, 69 (14%) SCC*mec* type II, 40 (8%) SCC*mec* type I, 34 (7%) SCC*mec* type VI, 4 (1%) SCC*mec* type V and 1 (<1%) SCC*mec* type XI (Table 5).

In seven (1.4%) isolates, obtained in five countries, *pvl* was detected, of which six were detected in CA-MRSA clones (Table 6). Typing of *agr* revealed type 1 in 334 (67%) isolates. In three ICUs

Table 2. Univariate analysis of risk factors for previously unknown MRSA colonization on ICU admission from all ICUs combined

	MRSA colonization upon ICU admission			
	MRSA colonized, $n=223$	no MRSA colonization, $n=7973$	P	OR (95% CI)
Female, *n* (%)	82 (37)	3176 (40)	0.357	
Age (years), median	65	62	0.005	
Age >70 years, *n* (%)	98 (44)	2960 (37)	0.038	1.33 (1.02–1.74)
Comorbidities, *n* (%)				
haemodialysis	10 (5)	197 (3)	0.059	1.85 (0.97–3.55)
peritoneal dialysis	0	43 (0.5)	0.272	
solid tumour	29 (13)	1109 (14)	0.700	
haematological malignancy	7 (3)	333 (4)	0.443	
stem cell or bone marrow transplant	2 (1)	60 (1)	0.806	
solid organ transplant	4 (2)	139 (2)	0.955	
chronic hepatic failure	17 (8)	345 (4)	0.018	1.83 (1.10–3.03)
HIV	3 (1)	120 (2)	0.847	
Recent hospitalization (<1 year)[a], *n* (%)	125 (57)	3858 (49)	0.016	1.34 (1.01–1.83)
Recent surgery (<1 year)[a], *n* (%)	55 (25)	1539 (19)	0.038	1.39 (1.02–1.89)
Emergency surgery prior to admission, *n* (%)	32 (15)	1293 (16)	0.492	
Location prior to ICU admission, *n* (%)				
home or private residence	74 (33)	3030 (38)	1.000[b]	
non-ICU ward, non-surgical	70 (31)	2513 (32)	0.436	
non-ICU ward, surgical	38 (17)	1166 (15)	0.153	
other ICU	24 (11)	858 (11)	0.568	
long-term care facility	8 (4)	120 (2)	0.006	2.73 (1.29–5.79)

[a]Hospitalization >24 h.
[b]Reference category.

Table 3. Multivariate analysis of risk factors for previously unknown MRSA carriage on ICU admission on aggregated data

Risk factor	OR	95% CI	P
Age >70 years	1.57	1.01–2.45	0.046
Recent hospitalization (<1 year)[a]	1.85	1.19–2.89	0.007
Location prior to ICU: long-term care facility	2.02	0.94–4.37	0.074

[a]Hospitalization >24 h.

from three countries (Italy, Slovenia and Luxembourg), *agr* type 2 was most prevalent. In two isolates, *agr* was non-typeable.

CA-MRSA and LA-MRSA

Seventeen (3%) isolates were, according to the molecular epidemiological definitions used, CA-MRSA clones, of which nine (53%) were from the Greek ICUs. Six patients carried *pvl* negative ST1-IVa (USA400), four *pvl* positive ST80-IVc (the European clone), four *pvl* negative ST30-IVc (the South-West Pacific-related clone) and three patients with either USA300 (*pvl* and ACME positive ST8-IVa), ST88-IVa and ST152-V, respectively. Eight of 17 (47%) patients colonized with CA-MRSA were >70 years of age, 71% were male and 8 of 17 (47%) had been admitted to a hospital in the previous year. Three patients carried LA-MRSA, two from Luxembourg and one from Greece (two ST398-IVa and one ST130-XI).

Genetic diversity

Simpson's index for genetic diversity was 0.88 (95% CI 0.87–0.89) for all ICUs combined, without significant differences between the three study phases (phase I: 0.89, 95% CI 0.87–0.91; phase II: 0.90, 95% CI 0.88–0.92; and phase III: 0.85, 95% CI 0.83–0.88). The index for individual ICUs ranged from 0.11 to 0.84 (Table 4) and genetic diversity was inversely correlated to acquisition rate ($\beta=-0.06$, 95% CI -0.10 to -0.03, $P=0.003$) (Figure 2). Diversity was lowest in the ICU from Latvia (0.11, 95% CI 0.03–0.19) and in one ICU from Portugal (0.32, 95% CI 0.15–0.48). No significant differences were found between ICUs within the same country.

Discussion

This descriptive *post hoc* analysis of a multinational prospective study in 13 European ICUs across eight countries reveals that the molecular epidemiology of MRSA was homogeneous within, but heterogeneous between, countries and that CA-MRSA and LA-MRSA genotypes and PVL-producing isolates were detected sporadically. In the MOSAR trial, the implementation of universal chlorhexidine body washing together with improving hand hygiene (phase II, 6 months) was associated with a statistically significant

Table 4. Molecular epidemiology of MRSA strains across countries and ICUs

Country/ICU	Typed MRSA isolates, $n=500$	Number of different clones	Most common isolated clones (*n*, %)			Most common isolated *spa* types (*n*, %)			Index of diversity (95% CI)
			1st	2nd	3rd	1st	2nd	3rd	
France									
ICU 1	91	13	ST8-IVc (52, 57%)	ST5-VI (17, 19%)	ST247-I (6, 7%)	t008 (37, 41%)	t777 (18, 20%)	t052 (6, 7%)	0.64 (0.54–0.74)
ICU 2	50	6	ST8-IVc (27, 54%)	ST5-VI (13, 26%)	ST247-I (5, 10%)	t008 (21, 42%)	t777 (13, 26%)	t024 (5, 10%)	0.64 (0.53–0.75)
ICU 3	16	8	ST8-IVc (6, 38%)	ST5-VI (3, 19%)	ST247-I (2, 13%)	t008 (4, 25%)	t777 (3, 19%)	t002 (2, 13%)	0.84 (0.71–0.98)
Latvia	105	4	ST368-III (99, 94%)	ST22-IVa (4, 4%)	ST5-IVa (1, 1%)	t425 (78, 74%)	t3563 (19, 18%)	t4326 (4, 4%)	0.11 (0.03–0.19)
Portugal									
ICU 1	51	5	ST22-IVh (42, 82%)	ST36-II (3, 6%)	ST105-II (3, 6%)	t747 (26, 51%)	t032 (8, 16%)	t002 (5, 10%)	0.32 (0.15–0.48)
ICU 2	42	5	ST22-IVh (27, 64%)	ST36-II (10, 24%)	ST239-III (3, 7%)	t747 (16, 38%)	t018 (10, 24%)	t032 (8, 19%)	0.54 (0.40–0.68)
Italy	18	6	ST228-I (8, 44%)	ST8-IVc (3, 17%)	ST36-II (3, 17%)	t041 (5, 28%)	t008 (3, 17%)	t018 (3, 17%)	0.77 (0.62–0.92)
Greece									
ICU 1	60	7	ST239-III (32, 53%)	ST225-II (14, 23%)	ST5-II (5, 8%)	t037 (32, 53%)	t003 (13, 22%)	t002 (3, 5%)	0.66 (0.55–0.76)
ICU 2	5	2	ST239-III (4, 80%)	ST30-IVc (1, 20%)	—	t037 (3, 60%)	t018 (1, 20%)	t030 (1, 20%)	0.4 (0.0–0.83)
Slovenia									
ICU 1	19	5	ST111-I (7, 37%)	ST225-II (7, 37%)	ST228-I (2, 11%)	t041 (7, 37%)	t003 (6, 32%)	t001 (2, 11%)	0.74 (0.63–0.85)
ICU 2	7	2	ST111-I (4, 57%)	ST225-II (3, 43%)	—	t003 (3, 43%)	t041 (3, 43%)	t9393 (1, 14%)	0.57 (0.47–0.68)
Spain	18	8	ST8-IVc (7, 39%)	ST5-IVc (2, 11%)	ST22-IVh (2, 11%)	t008 (6, 33%)	t018 (2, 11%)	t032 (2, 11%)	0.84 (0.70–0.97)
Luxembourg	18	7	ST225-II (8, 44%)	ST710-II (4, 22%)	ST8-IVc (2, 11%)	t003 (12, 67%)	t008 (2, 11%)	t002 (1, 6%)	0.77 (0.62–0.93)
Total	500	31	ST368-III (99, 20%)	ST8-IVc (98, 20%)	ST22IVh (75, 15%)	t0425 (75, 15%)	t008 (74, 15%)	t747 (44, 9%)	0.88 (0.87–0.89)

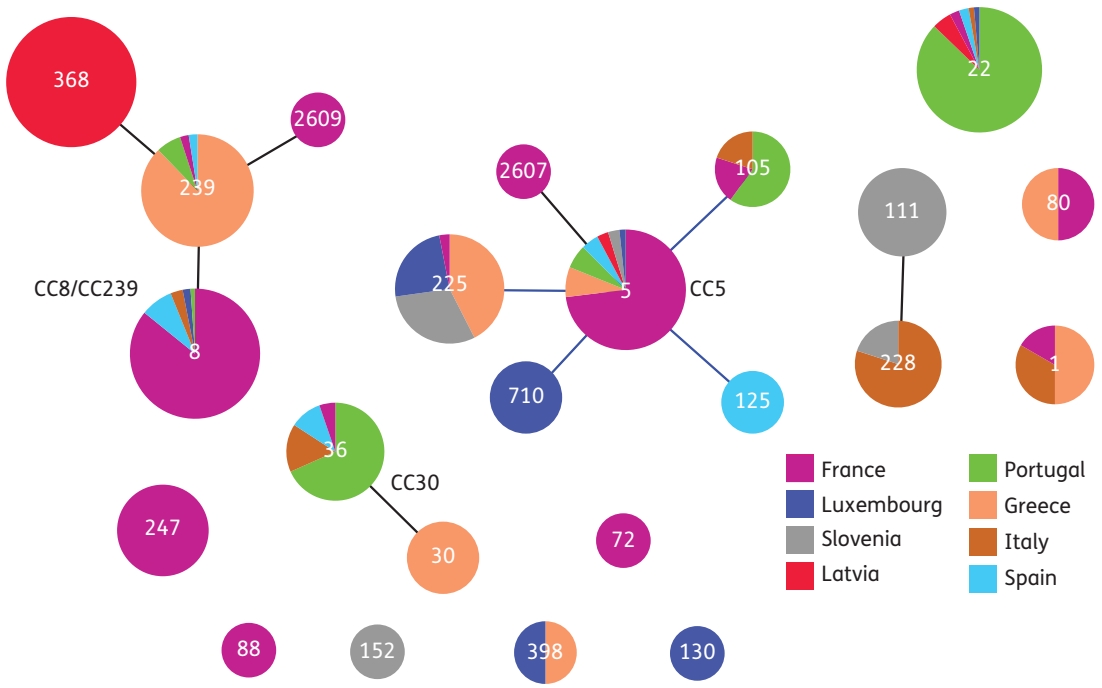

Figure 1. Minimum spanning tree analysis based on the STs of 500 typed *S. aureus* strains. Each circle represents an ST, with the ST number given inside the circle. Circle size reflects the number of isolates. Black lines connect single-locus variants. The coloured pie charts indicate the geographical distribution of STs among different countries.

Table 5. SCC*mec* (sub)types identified in MRSA isolates across all countries and ICUs ($n=500$)

SCC*mec* type	*n* (%)
I	40 (8)
II	69 (14)
III	141 (28)
IV	211 (42)
IVa	21 (4)
IVc	113 (23)
IVg	1 (<1)
IVh	75 (15)
IV-NT	1 (<1)
V	4 (1)
VI	34 (7)
XI	1 (<1)

NT, non-typeable.

Table 6. *spa* typing results of the most commonly isolated MRSA clones across all ICUs and countries and PVL positivity in HA-MRSA, CA-MRSA and LA-MRSA clones

Clone	*n* (%) ($n=500$)	Top three *spa* types isolated per clone (% of MRSA clone)	*pvl*, *n* (%)
HA-MRSA	480 (96)		
ST368-III	99 (20)	t425 (78), t3563 (19), t4410 (2)	0
ST8-IVc	98 (20)	t008 (76), t304 (6), t024 (4)	0
ST22-IVh	75 (15)	t747 (59), t032 (25), t020 (3)	0
ST239-III	41 (8)	t037 (95), t030 (2), t945 (2)	0
ST5-VI	34 (7)	t777 (97), t179 (3)	0
ST225-II	33 (7)	t003 (94), t045 (3), t4336 (3)	0
ST36-II	19 (4)	t018 (95), t012 (5)	0
ST247-I	13 (3)	t052 (69), t024 (23), t844 (8)	0
ST111-I	11 (2)	t041 (91), t9393 (9)	0
ST228-I	10 (2)	T041 (50), t001 (40), t1628 (10)	0
ST5-II	8 (2)	t002 (63), t895 (25), t688 (13)	0
other clones	39 (8)		1[a]
CA-MRSA	17 (3)	t127 (35), t044 (24), t018 (18)	6 (35%)[b]
LA-MRSA	3 (<1)	t011, t899, t1736	0

[a]*pvl*-positive isolate: ST5-IVc.
[b]*pvl*-positive isolates: four ST80-IVc, one ST8-IVa and one ST152-V.

reduction of MRSA-acquisition, which persisted but did not further reduce in phase III (12–15 months), in which admission screening and isolation of carriers was added as a control measure.[8]

The homogeneity of STs between ICUs within the same country may result from the geospatial distribution of ICUs. Participating ICUs from France were situated in the region of Île-de-France, both hospitals from Greece were in Athens and in Slovenia both ICUs were in or close to Ljubljana. Only the two ICUs in Portugal were 100 km apart. Our findings confirm previously reported regional distribution of MRSA in Europe,[27] but extrapolation of our results from 13 individual ICUs in eight different countries is not possible.

During the 2 year study period, 17 patients with CA-MRSA and 7 patients with *pvl*-positive genotypes were identified among

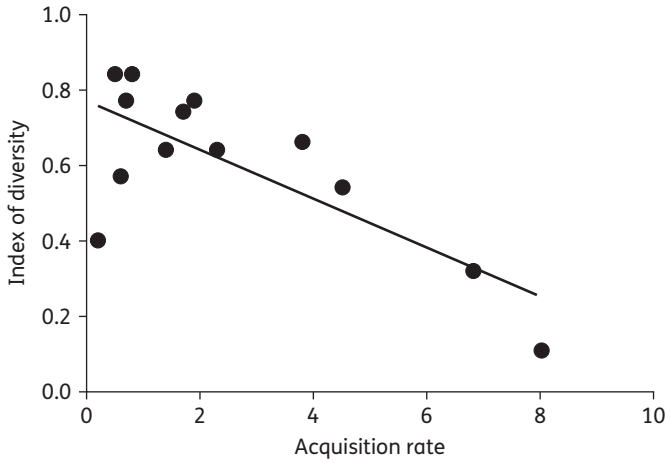

Figure 2. Relation between diversity and acquisition rate. On the *y*-axis is the index of diversity as a measure of the diversity of the MRSA population within ICUs. On the *x*-axis is the MRSA acquisition risk per 1000 patient days at risk.

8519 patients. In 11 patients, carriage of CA-MRSA or *pvl*-positive genotypes was detected during the first 48 h of ICU admission. A study evaluating the molecular epidemiology of MRSA in an Italian ICU reported that of 62 MRSA samples typed from clinical cultures, 8 (13%) were CA-MRSA. For comparison, in 18 ICUs in the USA, in which a similar surveillance strategy as used in our study was applied in 2006, 626 of 5512 (11.3%) patients were colonized with MRSA at ICU admission, of which 30 of 210 (14%) typed isolates were considered CA-MRSA. The majority of these isolates were USA300.[28] A meta-analysis concluded that MRSA colonization at ICU admission shows global variability with 5.8%–8.3% of patients being colonized.[29] Reported colonization rates on ICU admission in Europe ranged from 4.4% (95% CI 3.4%–5.4%) in Northern and Central Europe, to 3.5% (95% CI 1.4%–6.7%) in Southern Europe. More recently, CA-MRSA, and particularly USA300, has become the dominant clone in some US hospitals with evidence of replacement of HA-MRSA.[4,30] The reason for the lower admission rate of CA-MRSA in these European ICUs, as compared with ICUs in the USA, is not well understood and more detailed analyses are warranted. Furthermore, little is known about the transmission capacity of different MRSA genotypes in ICUs and the hospital setting. There is evidence of lower transmissibility of CA-MRSA strains in Danish hospital settings[31] and of LA-MRSA in Dutch hospitals.[32,33] Only three LA-MRSA were detected in the current study, which might result from the localization in urbanized areas of all participating ICUs. LA-MRSA is predominantly found in rural areas with a high density of pigs and pig farmers, mainly in subjects with professional exposure to these animals.[34]

Risk factor analysis was performed on aggregated data from all 13 ICUs, potentially leading to a failure to detect some risk factors for individual ICUs or individual countries. Only age >70 years and hospitalization within 1 year prior to ICU admission were independent risk factors for MRSA colonization on admission, reflecting the dominance of HA-MRSA genotypes. ICU-specific risk factors for MRSA colonization on admission were found, which were not detected in the aggregated data from all ICUs. For example, haemodialysis was a significant risk factor in two participating ICUs.

Strengths of this study include the rigorous screening using standardized methods in 13 ICUs and their local microbiology laboratories and the centralized genotyping. The approach of surveillance of carriage, rather than investigating clinical isolates, yields a comprehensive and more complete representation of MRSA prevalence and epidemiology in the participating ICUs. We used the results of the local laboratories to calculate the MRSA prevalence on admission and acquisition rates of MRSA. Eight percent of all isolates sent to the central laboratory were misclassified as MRSA by the local laboratories: 55% of these misclassified isolates were from swabs taken at admission and 73% came from two ICUs. Therefore, admission rates and acquisition rates as reported might be slightly overestimated. However, as only one MRSA isolate per patient was sent to the central laboratory, the percentage of misclassified isolates may actually be lower. The surveillance schedule applied, including nasal and wound swabs only, may also have induced some underestimation of MRSA carriage, as screening of additional body sites, e.g. perineum and/or throat, may increase sensitivity of MRSA detection.[35]

In conclusion, the molecular epidemiology of MRSA in 13 European ICUs in eight countries appeared diverse and both CA-MRSA and LA-MRSA genotypes were rarely identified.

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Transparency declarations

None to declare.

Supplementary data

Risk factor analysis for the 13 individual ICUs and all the individual *spa* types found in different ICUs are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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