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Diffusion of meticillin-resistant *Staphylococcus aureus* USA300 strains in central Italy

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ABSTRACT

Meticillin-resistant *Staphylococcus aureus* (MRSA) is an outstanding, clonally evolving pathogen that in the last years, under the selective pressure of antibiotics, has acquired the crucial ability to infect people outside of hospitals. MRSA USA300 has progressively become synonymous with severe community-associated staphylococcal disease worldwide. While spreading worldwide, these clones have progressively acquired resistance to several antibiotics and have gained the ability to cause infections in hospital settings. Recently, USA300-related strains showing resistance to several antibiotics have been isolated from community-acquired infections in Italy. This paper reports the high frequency of isolation of USA300-related strains both from community- and hospital-acquired infections in central Italy as well as their genotypic characteristics and antibiotic susceptibility. Analysis of these characteristics by partial least squares discriminant analysis enabled it to be demonstrated that while moving from the community to the hospital setting these isolates underwent an adaptive process that generated clones showing distinctive characteristics. These observations further support the hypothesis that the threatening generation of strains combining both resistance and virulence is becoming a reality, and stress the necessity of constant molecular epidemiological surveillance of MRSA.

1. Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is an outstanding, clonally evolving, nosocomial pathogen, but in the last decades it has become with increasing frequency the cause of severe community-acquired infections worldwide [1].

The emergence and spread of MRSA clones is generally regarded as an accelerated evolutive process during which clones are generated and propagate globally through the selective pressure of large amounts of antimicrobial agents [2]. The role of the selective pressure of antibiotics in promoting the genetic evolution of MRSA is undisputable, but recent research suggests that the geographical spread of MRSA clones over long distances is a rare event compared with the frequency with which the staphylococcal cassette chromosome (SCC) island has been imported along with the meticillin-resistant phenotype from cohabitant donors [3].

Community-acquired (CA)-MRSA were once assumed to be derived from the dissemination of strains circulating in hospitals [4], but evidence from molecular epidemiological studies indicated that CA-MRSA and hospital-acquired (HA)-MRSA isolates show distinct phenotypic and genotypic features [5,6]. In fact, CA-MRSA generally produce a larger variety of pathogenicity and virulence factors, harbour resistance to meticillin within smaller genetic cassettes, are typically susceptible to a number of antibiotics other than β -lactams [7] and cause different serious infections, including acute, invasive and necrotizing syndromes. The absolute distinction between HA-MRSA and CA-MRSA is sometimes difficult to make, and CA-MRSA clones have been shown to cause infections in hospitalised patients whilst HA-MRSA clones have caused infections in the general community [8,9]. The reasons that MRSA, after 40 years of inability to colonise

people outside of the hospital setting, apparently quite suddenly acquired this crucial ability remain obscure [10]. Results of recent studies revealed that the selective pressure operated by antibiotics not only accelerated genetic evolution of MRSA but also eventually broke interlineage barriers, allowing the frequent acquisition and/or loss of virulence genes conveyed by mobile genetic elements, enhancing the ability of *S. aureus* to adapt to changing environments [11].

The alarming results of a recent study aiming to define the prevalence of MRSA among outpatients in the Ligurian area of Italy and to characterise the molecular profile of the strains isolated from these subjects showed that the majority of strains belonged to two well known international clones [epidemic MRSA type 15 (EMRSA-15) and USA300] and their variations and that the most represented clonal group (USA300) can acquire multiresistance [12].

In recent years, MRSA USA300 has become synonymous with severe community-associated staphylococcal disease, at first especially in the USA and subsequently and progressively worldwide [13]. The epithet 'USA300' is used to indicate one of the eight initial MRSA 'USA' strain types (USA100–USA800) [14]. USA300 isolates did not have a clearly identifiable European counterpart, carried the genes encoding the Panton–Valentine leukocidin (PVL), contained the *msrA* erythromycin resistance gene, carried SCC*mec* type IV and showed several closely related pulsed-field gel electrophoresis (PFGE) banding patterns [15], indicating that they did not belong to a single clone but rather to a family of isolates with related PFGE patterns. Although at first apparently restrictedly diffused to the USA alone, infections caused by USA300 isolates have been recognised almost worldwide, including Italy [12], probably as a consequence of significant

adaptive and pathogenic potential. While spreading worldwide, USA300 isolates have progressively acquired resistance to several antibiotics [15].

We recently provided validity evidence of a simple algorithm for the classification of HA- and CA-MRSA by studying a sample of MRSA isolated both from outpatients and inpatients in the area of Viterbo in central Italy over a 5-year period [16]. While involved in analysing molecular epidemiological studies on MRSA isolates in the same area, we noticed a significant modification of the characteristics of isolates compared with the previous situation. This study reports on the high frequency of isolation of MRSA strains with PFGE restriction profiles (RPs) compatible with the USA300 MRSA clone and its variations that occurred recently in the studied area both from hospitalised patients and outpatients as well as on the antimicrobial resistance and genotypic characteristics of these isolates.

2. Methods

This study was conducted during the period December 2008 to December 2009 on different clinical samples submitted for routine analyses to the microbiology laboratory of Hospital 'BelColle' in Viterbo, Italy, where isolation, identification and antimicrobial susceptibility testing were performed. MRSA isolates were characterised by molecular methods at the Department of Public Health and Infectious Diseases of the University of Rome 'La Sapienza' (Rome, Italy). Differentiation of HA-MRSA and CA-MRSA was performed according to previously published criteria [16], as follows: an infection was considered to be HA if it developed within 48 h after hospital admission, up to 3 days after discharge from hospital or up to 30 days following surgery.

2.1. Microbiological analyses

Clinical samples were obtained from patients attending the hospital itself, withdrawal centres, clinics, day hospitals and nursing services in Viterbo and district and were processed by standard methods [17]. Identification and antimicrobial susceptibility of *S. aureus* was performed by the Vitek2 automated system (bioMérieux Italy, Florence, Italy). Interpretation of antimicrobial susceptibility tests was performed according to breakpoints of the automated system. Isolates were classified as MRSA on the basis of resistance to oxacillin (≥ 4 $\mu\text{g/mL}$) and positivity of the penicillin-binding protein (PBP2') latex agglutination test (Oxoid S.p.A, Rodano, Milan, Italy).

2.2. Molecular characterisation of MRSA

Molecular characterisation of MRSA isolates included polymerase chain reaction (PCR) assays to detect genes for toxins, adhesins and *agr* groups I to IV, *SCCmec* typing and PFGE profiling. PCR assays were performed as described previously [16,18]. *SCCmec* PCR typing was based on three separate multiplex PCR assays and a specific single PCR reaction according to published methods [19,20].

PCR amplifications were performed in a T personal thermal cycler (Biometra GmbH, Göttingen, Germany) with DreamTaqTM DNA polymerase (Fermentas Life Sciences, Vilnius, Lithuania). Genomic DNA (100 ng) was added to each PCR mix containing a 10 mM concentration of the deoxynucleoside triphosphates (dNTPs), the appropriate concentration of MgCl_2 and of each forward and reverse primer, and *Taq* polymerase. PCR products were analysed by 2% agarose gel electrophoresis.

2.3. Pulsed-field gel electrophoresis

RPs following digestion of chromosomal DNA with *Sma*I endonuclease (Fermentas) and separation of the DNA fragments by PFGE were determined using a CHEF-DRII apparatus (Bio-Rad Laboratories, Segrate, Milan, Italy) as described previously [14,21,22]. Reference strain USA300-0114 was included in the analysis. Banding patterns were interpreted visually following published guidelines [23] and by means of TotalLabTM 1D software (Nonlinear Dynamics Ltd., Newcastle upon Tyne, UK) in order to construct a dendrogram of the relatedness of strains by the unweighted pair group method with arithmetic mean (UPGMA) algorithm.

2.4. Partial least squares discriminant analysis (PLS-DA)

To evaluate whether the presence/absence of specific characteristics (both phenotypic and genotypic) correlated with the classification of isolates as HA-MRSA and CA-MRSA, or as USA300-related (U3R) and USA300-unrelated (U3U), or with their classification based on RPs obtained by PFGE typing, three 1/0 matrices were constructed based on the results of PCR assays and antibiotic susceptibility tests. Moreover, to evaluate whether the presence/absence of specific bands in RPs obtained by PFGE correlated with the classification of isolates as HA-MRSA and CA-MRSA, a 1/0 matrix was constructed based on the results of PFGE typing.

Logistic PLS-DA, included in SIMCA-P+ software (Umetrics AB, Umeå/Malmö, Sweden), was performed to assess the model predictability and characteristic importance in classifying strains. Data were automatically mean centred and unit variance scaled by the statistical software. Each characteristic was hierarchically classified based on a software-assigned variable importance (VIP) value. Variables with a VIP value >1 were chosen as

discriminatory for each strain belonging to a class (i.e. 1 = HA-MRSA, 0 = CA-MRSA, or 1 = U3R, 0 = U3U, or 1 = presence of the band in the RP, 0 = absence of the band in RP).

Data were presented as a dendrogram in which the proximity of each gene variable to class centroid (1,0) indicated the relevance for inclusion into that class.

Evaluation of the significance of differences for positivity of PCR assays of single genes or of susceptibility to antibiotics were performed by χ^2 test, with Yates' correction for continuity, at significance levels of $P \leq 0.05$ and $P \leq 0.01$.

3. Results

3.1. Microbiological analyses

During the course of the study, 34 MRSA strains were isolated. According to previously published criteria [16], 15 (44.1%) of these were HA-MRSA whilst the remaining 19 (55.9%) were CA-MRSA. Moreover, 15 (44.1%) of the 34 MRSA (6 HA-MRSA and 9 CA-MRSA) were isolated from wounds, 11 (32.4%) (6 HA-MRSA and 5 CA-MRSA) were from blood samples and 8 (23.5%) (3 HA-MRSA and 5 CA-MRSA) were from urine. Nine MRSA (26.5%) came from the day hospital services, seven (20.6%) each came from both the infectious diseases and oncology services, five (14.7%) came from the services of general medicine and from the services of surgery, four (11.8%) from homecare services and two (5.9%) from the first aid service and the Intensive Care Unit.

3.2. Susceptibility to antimicrobials

Results of antimicrobial susceptibility tests performed on the 34 MRSA strains isolated during the study showed that no strain was resistant to teicoplanin, vancomycin, linezolid,

dalfopristin, sulfamethoxazole/trimethoprim (SXT) or fusidic acid. Moreover, no isolate was resistant to β -lactams alone. Patterns of resistance to the other tested antibiotics both for HA-MRSA and CA-MRSA are reported in Table 1. Surprisingly, resistance to antibiotics other than oxacillin was extremely diffused in both classes of isolates, with only minor differences. In fact, the 34 MRSA isolates were resistant to 4.65 ± 2.6 antibiotics; the 15 HA-MRSA were resistant to 5.0 ± 2.6 antibiotics and the 19 CA-MRSA were resistant to 4.4 ± 2.2 antibiotics. Results of statistical analyses showed that none of the tested antibiotics showed significant differences in percentage of resistant strains. Differences in percentage of gentamicin-resistant strains between HA-MRSA and CA-MRSA were probably not statistically significant only as a consequence of the limited number of strains analysed.

3.3. *agr* typing of MRSA strains

Among the 34 MRSA strains isolated during the study, *agr* I (52.9%) and *agr* II (32.3%) were the most prevalent types (Table 1), whilst *agr* III and *agr* IV isolates were less represented (11.8% and 2.9%, respectively). The prevalence of *agr* I isolates was higher among CA-MRSA; 13 (68.4%) of 19 CA-MRSA strains were *agr* I compared with 5 (33.3%) of 15 HA-MRSA, although the limited number of strains analysed probably made the differences not statistically significant (Table 1).

3.4. Presence of putative virulence determinants in MRSA strains

The distribution of virulence genes in the 34 MRSA strains is reported in Table 1. Similar to previously reported data from the same area [16], several genes, including *sdrC*, *sdrD*, *sdrE*, *clfA*, *lukS-F-PV* and *sec*, showed positivity in >50% of the strains, whilst *cna*-positive strains represented only a minority. In contrast to previous reports, *seb*- and *tst*-positive

strains were more frequent, whilst *etb*-positive strains were less frequent. Comparative analysis of the distribution of these genes between HA-MRSA and CA-MRSA (Table 1) showed that the percentage of *tst*-positive strains was significantly higher among HA-MRSA ($P = 0.016$); other differences, although not statistically significant owing to the limited number of isolates, were observed for *etb* and *clfA* ($P = 0.096$ in both cases).

3.5. *SCCmec* typing of MRSA strains

The distribution of *SCCmec* types in the 34 MRSA strains is reported in Table 1; 22 isolates (64.7%) were *SCCmec* type IVc, whilst of the remaining 12 isolates 7 (20.6%) were *SCCmec* type I and 5 (14.7%) were *SCCmec* type II. No significant differences in the distribution of *SCCmec* types between HA-MRSA and CA-MRSA were evident.

3.6. PFGE typing of MRSA strains

Several different RPs were obtained by PFGE from the 34 isolates (Fig. 1). Overall, 27 different bands were identified from the RPs of the 34 isolates and were numbered progressively from b1 to b27 according to decreasing molecular weight. Comparative analysis of these RPs according to previously published criteria [23] showed that 12 (35.3%) of the 34 isolates were closely related to the USA300-0114 reference strain, 9 (26.5%) were possibly related to the same strain, whilst the remaining 13 isolates (38.2%) were unrelated. Isolates showing RPs closely related to USA300 mostly belonged to the CA-MRSA group (11/12; 91.7%), whilst those showing RPs possibly related to USA300 mainly belonged to the HA-MRSA group (7/9; 77.8%). When analysis of RPs was performed using the TotalLab 1D software, 19 (55.9%) of the 34 isolates were grouped in a cluster related to the USA300-0114 reference strain (Fig. 1). This cluster included all the

12 closely related isolates identified above and 7 of the 9 possibly related isolates (with the exclusion of isolates 385 and 621 that were clearly separated from the remaining isolates).

3.7. PLS-DA analysis

Significant differences in the patterns of selected characteristics of MRSA isolates classified as HA-MRSA and CA-MRSA according to simple temporal criteria were shown by PLS-DA, resulting in an overall predictability of 100%, with a Fisher probability of 3.1×10^{-10} (Fig. 2A). The PLS-DA model did not require the re-allocation of any isolate into the other class. Construction of a dendrogram plotting the hierarchical classification of characteristics according to VIP in discriminating HA-MRSA from CA-MRSA showed that no character resulted in a VIP value >1 , and that only by reducing the limit to 0.5 did the presence of resistance to gentamicin and PCR positivity for *tst* and *SCCmec I* become discriminatory for HA-MRSA, whilst PCR positivity for genes *etb* and *agr I* were discriminatory for CA-MRSA (Fig. 2B).

To evaluate whether the presence/absence of specific bands in RPs correlated with the classification of isolates as HA-MRSA and CA-MRSA, the PLS-DA model was performed and gave an overall predictability of 91.4%, with a Fisher probability of 7.1×10^{-7} (Fig. 3A). The PLS-DA model required the re-allocation of 2 (10.5%) of the 19 CA-MRSA isolates into the HA-MRSA class (i.e. isolates 271 and 916 considered to be 57% HA-MRSA) and 1 (6.7%) of the 15 HA-MRSA isolates into the CA-MRSA class (isolate 817 considered to be 84% CA-MRSA). Construction of a loading plot representing the hierarchical classification of bands according to their variable importance in discriminating HA-MRSA from CA-MRSA isolates (Fig. 3B) showed that bands b4 and b12 were highly discriminatory for HA-

MRSA isolates, whilst bands b2, b8 and b15 were highly discriminatory for CA-MRSA isolates.

All of the 21 isolates that were classified as ‘closely related’ or ‘possibly related’ to the USA300-0114 reference strain were considered as U3R isolates and were compared for the previously determined characteristics with the remaining 13 U3U isolates (Table 1). Comparison of antibiotic resistance profiles showed that the U3R isolates were significantly less resistant than U3U isolates (Table 1). Resistance to ciprofloxacin and moxifloxacin was extremely frequent in both groups.

No statistically significant difference was evident in the distribution of *agr* types between the two groups. Moreover, the U3U group showed a higher prevalence of isolates positive for *eta* and for the *SCCmec* I cassette, whilst the U3R group included mostly isolates with the *SCCmec* IVc cassette.

The existence of significant differences in the patterns of selected characteristics of MRSA isolates classified as U3R and U3U according RP analysis was confirmed by PLS-DA, resulting in an overall predictability of 97.1%, with a Fisher probability of 1.6×10^{-8} (Fig. 4A). The PLS-DA model was generated with an overall variance of 77.5% and required the re-allocation of 1 (7.7%) of the 13 U3U isolates into the U3R class (i.e. isolate 197 considered to be 51.3% U3R). Construction of a dendrogram plotting the hierarchical classification of characteristics according to VIP in discriminating U3R from U3U showed that positivity for *SCCmec* type IV cassette, *agr* I, *cna*, *sea* and *sdrD* were discriminatory for U3R, whilst positivity for *eta* and *SCCmec* type I cassette along with resistance to clindamycin, clarithromycin, azithromycin and erythromycin were discriminatory for U3U (Fig. 4B).

3.8. Resistance to antimicrobials and genotypic characterisation of U3R isolates

To assess whether any difference in genotypic and phenotypic characteristics existed between U3R strains isolated from CA infections and U3R strains isolated from HA infections, the 21 U3R isolates were classified as HA-MRSA and CA-MRSA according to previously proposed criteria [16] and were compared for the studied characteristics (Table 1).

The limited number of isolates available for this comparison evidently affected the possibility to detect significant differences between the two groups. Nevertheless, *P*-values obtained for some characteristics (i.e. resistance to clindamycin, macrolides and gentamicin) suggest that if a more consistent number of isolates were studied differences would have been significant.

These observations were confirmed by PLS-DA analysis. In fact, significant differences in the patterns of selected characteristics of U3R isolates classified as HA-MRSA and CA-MRSA were shown by PLS-DA, resulting in an overall predictability of 90.9%, with a Fisher probability of 3.5×10^{-4} (Fig. 5A). The PLS-DA model required the re-allocation of 1 (12.5%) of the 8 HA isolates into the CA class (i.e. isolate 552 considered to be 69.3% CA-MRSA) and of 1 (7.7%) of the 13 CA isolates into the HA class (i.e. isolate 25 considered to be 53.9% HA-MRSA). Construction of a dendrogram plotting the hierarchical classification of characteristics according to VIP in discriminating U3R HA isolates from U3R CA isolates showed that resistance to gentamicin was discriminatory for HA-MRSA isolates, whilst resistance to clindamycin and macrolides and positivity for *eta* were discriminatory for CA-MRSA isolates (Fig. 5B).

4. Discussion

To our knowledge, this is the first report on the isolation of U3R MRSA strains from hospitalised patients in Italy. Previous reports on the isolation of USA300 MRSA in Italy are a single case report of a CA-MRSA causing severe pneumonia and identified as USA300 on the basis of some characteristics (ST8, *spa* type 008, *SCCmec* type IV and *agr* group I) but not PFGE [24], as well as data from a recent study aiming to define the prevalence of MRSA among outpatients in the Ligurian area of Italy [12]. Data in this second study showed an alarming figure in which a consistent proportion of isolates were USA300 and its variations and showed extended drug resistance profiles.

This study was originally planned to prolong a former study dedicated to the molecular epidemiological surveillance of MRSA isolated both from CA and HA infections, classified according to a new and simple algorithm [16]. In the period December 2008 to December 2009, 34 MRSA were isolated from various clinical samples and were characterised for susceptibility to a panel of 17 antibiotics, including oxacillin. In accordance with previous data, no isolate was resistant to linezolid, teicoplanin or vancomycin; moreover, no MRSA isolate was resistant to dalfopristin (2.1% resistance in the previous set) or SXT (10% resistance in the previous set), or to fusidic acid (not tested previously).

However, the number of multidrug-resistant CA-MRSA was significantly higher than previously: resistance to clindamycin increased from 10.2% to 36.8% ($P = 0.026$), resistance to erythromycin from 28.6% to 53.6% ($P = 0.06$), resistance to gentamicin from 6.1% to 26.3% ($P = 0.06$), resistance to tetracycline from 2% to 10.5% ($P = 0.38$),

resistance to rifampicin from 4.1% to 31.6% ($P = 0.006$) and resistance to ciprofloxacin from 12.2% to 100% ($P = 0.0$).

These modifications suggested the possible diffusion of HA-MRSA in the community and prompted us to evaluate the relatedness of the 34 MRSA isolates by PFGE analysis of *Sma*I RPs. PFGE analysis showed that the majority of the isolates, both CA-MRSA and HA-MRSA, were related to the USA300 epidemic clone, although with significant differences between the two groups. Unfortunately, PFGE was not performed on MRSA strains isolated during the previous 5 years and consequently it is not possible to acquire evidence about the period of initial diffusion of U3R strains in the studied area and to know whether these strains arrived in the area with the actual resistance profiles or whether they acquired them later. Data presented in this paper confirm previous observations on the diffusion in Italy of U3R isolates showing resistance to several antibiotics [12] and demonstrate that, in the studied area, these strains are at present diffusing to hospital settings. Diffusion of U3R isolates and their aptitude to exchange mobile genetic elements could possibly explain the high percentage of strains harbouring genes encoding the PVL among all MRSA (including U3U) and particularly among isolates from HA infections.

MRSA USA300 is certainly characterised by a significant adaptive potential [25]. In fact, it was originally associated with severe community-acquired infections alone, but while diffusing worldwide [12,13] it significantly modified its phenotypic and genotypic characteristics [15] under the selective pressure of the environment. The present data, despite the limited number of isolates, confirm these observations. In fact, U3R isolates could be distinguished by PLS-DA into two groups, according to isolation from HA or CA infections. The validity of the previously proposed temporal algorithm [16] to separate HA-

MRSA from CA-MRSA is confirmed with this set of isolates and its discriminatory power appeared comparable with that of PFGE.

These data confirm that local studies are constantly needed to evaluate the incidence and distribution of epidemic MRSA both in the hospital and community settings. The spread of U3R isolates in both settings, owing to the peculiar adaptive and evolutive capacities shown by these isolates, is making the threat of strains combining both resistance and virulence a reality.

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Competing interests

None declared.

Ethical approval

Not required.

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Fig. 1. Dendrogram of *Sma*I restriction profiles of 34 meticillin-resistant *Staphylococcus aureus* (MRSA) isolates and of the USA300-0114 reference strain. A scheme summarising all 27 detected bands in the 35 profiles is reported at the bottom.

Fig. 2. (A) Three-dimensional plot of the distribution of 34 meticillin-resistant *Staphylococcus aureus* (MRSA) strains and of the USA300-0114 reference strain according to the presence of specific characteristics, and correlation with the classification of isolates as hospital-acquired (HA)-MRSA (○) or community-acquired (CA)-MRSA (●) according to simple temporal criteria, calculated by partial least squares discriminant analysis (PLS-DA) analysis. (B) Dendrogram of the hierarchical classification of characteristics for discriminating between groups. *SCC_{mec}*, staphylococcal cassette chromosome *mec*. GEN, TET, CLA, RIF, FOS, CLI, AZT, ERM, CIP and MOX indicate resistance to gentamicin, tetracycline, clarithromycin, rifampicin, fosfomycin, clindamycin, aztreonam, erythromycin, ciprofloxacin and moxifloxacin, respectively.

Fig. 3. (A) Three-dimensional plot of the distribution of 34 meticillin-resistant *Staphylococcus aureus* (MRSA) strains and of the USA300-0114 reference strain according to the presence of bands on *Sma*I restriction profiles, and correlation with the classification of isolates as hospital-acquired (HA)-MRSA (○) or community-acquired (CA)-MRSA (●) according to simple temporal criteria, calculated by partial least squares discriminant analysis (PLS-DA) analysis. (B) Loading plot representing the hierarchical classification of bands according to their variable importance in discriminating between groups.

Fig. 4. (A) Three-dimensional plot of the distribution of 34 meticillin-resistant *Staphylococcus aureus* (MRSA) strains and of the USA300-0114 reference strain

according to the presence of specific characteristics, and correlation with the classification of isolates as USA300-related (U3R) (○) or USA300-unrelated (U3U) (●) according to results of pulsed-field gel electrophoresis (PFGE), calculated by partial least squares discriminant analysis (PLS-DA) analysis. (B) Dendrogram of the hierarchical classification of characteristics for discriminating between groups. *SCC_{mec}*, staphylococcal cassette chromosome *mec*. RIF, GEN, FOS, CLI, CLA, AZT, ERM, CIP, MOX and TET indicate resistance to rifampicin, gentamicin, fosfomycin, clindamycin, clarithromycin, aztreonam, erythromycin, ciprofloxacin, moxifloxacin and tetracycline, respectively.

Fig. 5. (A) Three-dimensional plot of the distribution of the 21 USA300-related (U3R) strains and of the USA300-0114 reference strain according to the presence of specific characteristics, and correlation with the classification of isolates as hospital-acquired (HA)-MRSA (●) or community-acquired (CA)-MRSA (○) according to simple temporal criteria, calculated by partial least squares discriminant analysis (PLS-DA) analysis. (B) Dendrogram of the hierarchical classification of characteristics for discriminating between groups. *SCC_{mec}*, staphylococcal cassette chromosome *mec*. GEN, CLA, CLI, AZT, ERM, RIF, FOSF, TET, CIP and MOX and indicate resistance to gentamicin, clarithromycin, clindamycin, aztreonam, erythromycin, rifampicin, fosfomycin, tetracycline, ciprofloxacin and moxifloxacin, respectively.

Table 1

Antibiotic resistance patterns, *agr* typing, frequency of putative virulence genes and SCC*mec* types among 34 meticillin-resistant *Staphylococcus aureus* (MRSA) isolates divided according to origin into hospital-acquired (HA) and community-acquired (CA), and according to pulsed-field gel electrophoresis (PFGE) profiling into USA300-related (U3R) and USA300-unrelated (U3U)

Antibiotic	Overall (<i>n</i> = 34)	HA (<i>n</i> = 15)	CA (<i>n</i> = 19)	<i>P</i> -value ^{a,b}	U3U (<i>n</i> = 13)	U3R			<i>P</i> -value ^{a,c}	<i>P</i> -value ^{a,d}
						Overall (<i>n</i> = 21)	HA (<i>n</i> = 8)	CA (<i>n</i> = 13)		
	% of resistant strains/class (<i>n</i>)					% of resistant strains/class (<i>n</i>)				
Clindamycin	44.1 (15)	53.3 (8)	36.8 (7)	0.539	76.9 (10)	23.8 (5)	12.5 (1)	46.2 (6)	0.007	<u>0.266</u>
Clarithromycin	41.2 (14)	46.7 (7)	36.8 (7)	0.82	84.6 (11)	14.3 (3)	0 (0)	46.2 (6)	0.0002	<u>0.076</u>
Azithromycin	41.2 (14)	53.3 (8)	31.6 (6)	0.353	92.3 (12)	9.5 (2)	12.5 (1)	46.2 (6)	0.00001	<u>0.266</u>
Erythromycin	52.9 (18)	53.3 (8)	53.6 (10)	0.76	92.3 (12)	28.6 (6)	12.5 (1)	46.2 (6)	0.001	<u>0.266</u>
Gentamicin	41.2 (14)	60.0 (9)	26.3 (5)	<u>0.103</u>	69.2(9)	23.8 (5)	50.0 (4)	7.7 (1)	0.024	<u>0.092</u>
Tetracycline	5.9 (2)	0.0 (0)	10.5 (2)	0.575	7.7 (1)	4.8 (1)	0.0 (0)	7.7 (1)	0.691	0.802
Rifampicin	32.4 (11)	33.3 (5)	31.6 (6)	0.794	61.5 (8)	14.3 (3)	12.5 (1)	15.4(2)	0.013	0.647
Ciprofloxacin	97.1 (33)	93.3 (14)	100 (19)	0.906	100 (13)	95.2 (20)	87.5 (7)	100 (13)	0.806	0.802
Moxifloxacin	97.1 (33)	93.3 (14)	100 (19)	0.906	100 (13)	95.2 (20)	87.5 (7)	100 (13)	0.806	0.802
Fosfomycin	11.8 (4)	13.3 (2)	10.5 (2)	0.776	30.8 (4)	0.0 (0)	0 (0)	0 (0)	0.031	
<i>agr</i> type	% of PCR-positive strains/class (<i>n</i>)					% of PCR-positive strains/class (<i>n</i>)				
I	52.9 (18)	33.3 (5)	68.4 (13)	<u>0.091</u>	46.2 (6)	57.1 (12)	62.5 (5)	53.8 (7)	0.787	0.95

II	32.3 (11)	40.0 (6)	26.3 (5)	0.633	30.8 (4)	33.3 (7)	25.9 (2)	38.5 (5)	0.825	0.874
III	11.8 (14)	20.0 (3)	5.3 (1)	0.431	23.1 (3)	4.8 (1)	0 (0)	7.7 (1)	0.288	0.802
IV	2.9 (1)	6.7 (1)	0.0 (0)	0.906	0.0 (0)	4.8 (1)	12.5 (1)	0 (0)	0.806	0.802
Gene	% of PCR-positive strains/class (n)				% of PCR-positive strains/class (n)					
<i>sea</i>	35.3 (12)	33.3 (5)	36.8 (7)	0.882	23.1 (3)	42.9 (9)	37.5 (3)	46.2 (6)	0.421	0.95
<i>seb</i>	32.3 (11)	40.0 (6)	26.3 (5)	0.633	38.5 (5)	28.6 (6)	37.5 (3)	23.1 (3)	0.825	0.832
<i>sec</i>	58.8 (20)	60.0 (9)	57.9 (11)	0.82	61.5 (8)	57.1 (12)	50.0 (4)	61.5 (8)	0.916	0.95
<i>tst</i>	23.5 (8)	46.7 (7)	5.3 (1)	0.016	30.8 (4)	19.0 (4)	37.5 (3)	7.7 (1)	0.713	<u>0.264</u>
<i>eta</i>	41.2 (14)	46.7 (7)	36.8 (7)	0.82	76.9 (10)	19.0 (4)	0 (0)	30.8 (4)	0.003	<u>0.241</u>
<i>etb</i>	14.7 (5)	0.0 (0)	26.3 (5)	<u>0.096</u>	30.8 (4)	4.8 (1)	0 (0)	7.7 (1)	<u>0.113</u>	0.802
<i>lukS-F-PV</i>	70.6 (24)	80.0 (12)	63.2 (12)	0.489	69.2 (9)	71.4 (15)	87.5 (7)	61.5 (8)	0.802	0.434
<i>clfA</i>	85.3 (29)	100 (15)	73.7 (14)	<u>0.096</u>	92.3 (12)	80.9 (17)	87.5 (7)	69.2 (9)	0.682	0.67
<i>cna</i>	5.9 (2)	0.0 (0)	10.5 (2)	0.623	0.0 (0)	9.5 (2)	0 (0)	15.4 (2)	0.691	0.688
<i>eap</i>	41.2 (14)	33.3 (5)	47.4 (9)	0.635	30.8 (4)	47.6 (10)	62.5 (5)	38.5 (5)	0.541	0.534
<i>sdrC</i>	97.1 (33)	100 (15)	94.7 (18)	0.906	100 (13)	95.2 (20)	87.5 (7)	92.3 (12)	0.806	0.688
<i>sdrD</i>	88.2 (30)	86.7 (13)	89.5 (17)	0.776	84.6 (11)	90.5 (19)	87.5 (7)	92.3 (12)	0.975	0.688
<i>sdrE</i>	47.1 (16)	46.7 (7)	47.4 (9)	0.76	53.8 (7)	42.9 (9)	37.5 (3)	46.2 (6)	0.787	0.95
SCC <i>mec</i> I	20.6 (7)	33.3 (5)	10.5 (2)	0.228	53.8 (7)	0.0 (0)	0 (0)	0 (0)	0.0008	
SCC <i>mec</i> II	14.7 (5)	13.3 (2)	15.8 (3)	0.775	23.1 (3)	9.5 (2)	0 (0)	15.4 (2)	0.622	0.688
SCC <i>mec</i> IVc	64.7 (22)	53.3 (8)	73.7 (14)	0.383	23.1 (3)	90.5 (19)	87.5 (7)	76.9 (10)	0.0003	0.975

SCC*mec*, staphylococcal cassette chromosome *mec*; PCR, polymerase chain reaction.

^a A two-tailed χ^2 test with Yates' correction for continuity was applied. Bold indicates statistically significant differences ($P < 0.05$); underlined indicates differences that are not statistically significant owing to the limited number of isolates.

^b P -values obtained comparing all studied isolates divided into HA and CA.

^c P -values obtained comparing all studied isolates divided into U3R and U3U.

^d P -values obtained comparing U3R isolates divided into HA and CA.

A

B