Original article

Molecular epidemiology and virulence factors of methicillin-resistant *Staphylococcus aureus* strains in a Romanian university hospital

Epidemiologia moleculară şi factori de virulenţă ai tulpinilor de *Staphylococcus aureus* meticilino-rezistente izolate dintr-un spital clinic din România

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Abstract

The aim of the present study was to analyze the clonal relations and virulence profile of clinically relevant MRSA strains collected during one year from patients admitted to clinical wards with high risk for nosocomial infections in a university hospital. One hundred and twenty two MRSA strains isolated from patients admitted to surgical and intensive care units during 2010 were analyzed. Molecular characterization was performed using PFGE (pulsed-field gel electrophoresis), determining spa polymorphism and SCCmec type. The presence of genes encoding for virulence factors was assessed. We have identified 25 distinct pulsotypes clustering into 4 major clonal groups. Group A comprised 82% of MRSA strains (n=100), corresponding to spa type t030. These strains harboured SCCmec type III and were positive for enterotoxin A genes. Fifteen percent of the strains (n=18) belonged to group B representing spa types t127, t015 and t321, carrying SCCmec type IV and genes encoding for diverse enterotoxins. Groups C and D were represented by one strain each, belonging to spa type t044 and t582, respectively. These strains were positive for genes encoding for Panton-Valentine leukocidin (PVL), enterotoxin G and toxic shock syndrome toxin. Two strains were non-typeable by PFGE, these belonged to spa type t034, characteristic to livestock-associated MRSA (LA-MRSA) strains. The majority of MRSA strains were clonally related. Supported by epidemiological data, our findings point to the intrahospital origin of these strains. Community-associated MRSA strains (SCCmec type IV) were sporadically involved in infections. Strains with multiple virulence factors and new, emerging strains (LA-MRSA) were detected.

Keywords: *molecular typing, PFGE, nosocomial infections, community-onset infections*

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Rezumat

Scopul acestui studiu a fost analiza relațiilor clonale şi a profilului de toxine ale tulpinilor de S. aureus meticilino-rezistente (MRSA) izolate de la pacienți internați la secții cu risc crescut pentru infecții nosocomiale dintr-un spital clinic. Am analizat 122 de tulpini MRSA izolate în cursul anului 2010 de la pacienți spitalizați la secțiile de terapie intensivă şi chirurgicale. Tipizarea moleculară s-a efectuat prin metoda PFGE (pulsed-field gel electrophoresis), prin studiul polimorfismului genei spa şi determinarea tipului de casetă SCCmec. Totodată s-a urmărit prezența genelor ce codifică factori de virulență. S-au diferențiat 25 de pulsotipuri distincte care s-au încadrat în patru grupe clonale. Grupul clonal A a cuprins 82% (n=100) dintre tulpini, reprezentând tipul spa t030 cu casetă SCCmec tip III şi fiind pozitive pentru gena ce codifică enterotoxina A. Din grupul clonal B au făcut parte 15% (n=18) dintre tulpini, corespunzând cu tipurile spa t127, t015 şi t321 şi prezentând caseta SCCmec tip IV, fiind pozitive pentru diverse gene de enterotoxine. Din grupurile clonale C şi D au făcut parte câte o tulpină, de tip spa t044, respectiv t582, purtând caseta SCCmec de tip IV, pozitive pentru genele ce codifică leucocidina Panton-Valentine (PVL), enterotoxina G şi toxina şocului toxic TSST. Două tulpini au fost netipabile prin PFGE, acestea aparținând tipului spa t034, cunoscut ca fiind asociat animalelor de fermă (LA-MRSA). Majoritatea tulpinilor MRSA au aparținut aceluiaşi grup clonal, ceea ce corelat cu datele epidemiologice dovedeşte originea intraspitalicească ale acestora. Sporadic au apărut infecții cauzate de tulpini MRSA de tip comunitar (SCCmec tip IV). Se remarcă prezența unor tulpini cu multipli factori de virulență şi apariția unor tipuri noi de MRSA şi anume LA-MRSA.

Cuvinte cheie: *tipizare moleculară, PFGE, infecții nosocomiale, infecții de origine comunitară*

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Introduction

The worldwide emergence and spread of new and diverse methicillin-resistant *Staphylococcus aureus* (MRSA) strains led to major changes in the epidemiology of infections caused by them. A decade ago strains descending from only five major clones were considered to be responsible of all MRSA infections and their vast majority was hospital or health-care associated (HA-MRSA), whereas infections occurring outside the hospital setting were uncommon. The emergent strains presented different genetic background and showed distinct features compared to earlier MRSA strains: faster growth rate, susceptibility to several non-ϑ-lactam antibiotics, high clonal diversity, production of different virulence factors, such as Panton-Valentine leukocidin (PVL), etc. (1-3). These new strains were labeled community-associated or community-acquired MRSA (CA-MRSA), although these terms led to confusion as in time CA-MRSA strains crossed the boundaries between the hospital and community settings and might be involved in nosocomial infections as well (4) Hence, the term CA-MRSA refers to strains with particular genetic background rather to the origin of the infections they are involved in. For the reliable identification of the strains molecular tests that can determine the actual type of SCC*mec* cassette they harbour are required, types IV-VIII being the landmark of CA-MRSA (5). Since their emergence, a huge amount of variability has been witnessed, unseen before in relation to the classical HA-MRSA strains. A new turn in MRSA epidemiology was the emergence in humans of a particular CA-MRSA lineage associated with livestock, especially with pigs and cattle (6). Several reports have described human colonization and infections with LA-MRSA (livestock-associated MRSA) strains (7 – 9).

In spite of changes in the epidemiology of MRSA strains, they remain one of the most important causes of nosocomial infections (10, 11). Several reports have been published about the molecular epidemiology of MRSA in different geographical regions and online databases (www.mlst.net, www.spaserver.ridom.de) allow tracking their distribution. It is clear that some strains are geographically restrained while others

are found worldwide and some strains are more successful than others in causing outbreaks. It is also well-known that epidemiology of MRSA can differ from one hospital to another, even in if they are from the same town. Based on these facts it seems to be important for each hospital, especially for those facing high MRSA burden, to assess their own epidemiological situation in order to implement proper infection control measures, to set realistic objectives for fighting MRSA strains and to monitor their evolution.

Although there are several data from Romania regarding MRSA, few hospitals have undertaken large scale studies correlating molecular, clinical and epidemiological data. The aim of the present study was to analyze the clonal relations and virulence profile of clinically relevant MRSA strains collected during one year from patients admitted to clinical wards with high risk for nosocomial infections in a Romanian university hospital. We have also performed a retrospective descriptive analysis of epidemiological and clinical data associated with MRSA infection.

Materials and methods

Setting

The study was performed during 2010 in Tîrgu-Mureş Clinical Emergency Hospital, a tertiary care university hospital with 1084 beds. The general intensive care unit had 37 beds for adults and 10 pediatric beds. Surgical units were of different specialty, including general, thoracic, orthopedic, ear-nose-throat, gynecology-obstetric, pediatric, plastic, vascular and neurosurgery, involving 488 beds overall.

Bacterial isolates

Non-duplicate methicillin-resistant *S. aureus* strains isolated during routine diagnosis from patients hospitalized in surgical or intensive care units were analyzed. Only strains recovered from clinically significant specimens were considered, e.g. blood, specimens from other normally sterile sites (joint, pleural, peritoneal, cerebrospinal fluid), purulent secretions and lower respiratory tract specimens. Strains originating from sites associated with colonization, such as nasal or pharyngeal mucosa, were excluded from analysis.

Corresponding clinical and epidemiological data, such as underlying diseases, site of infection, date of MRSA isolation, admission date, ward stay, and previous hospitalizations were collected for each bacterial strain. MRSA was considered nosocomial when it was isolated from samples collected after 72 hours of hospitalization.

Susceptibility tests

Methicillin-resistance was detected by disk-diffusion method, according to CLSI guidelines (2010), using 30 ɡg cefoxitin disks. Confirmation was performed by the detection of PBP2a using latex agglutination tests (MRSA slidex, bioMérieux).

Susceptibility against erythromycin, clindamycin, trimethoprim-sulfamethoxazol, gentamicin, ciprofloxacin, tetracycline, linezolid and rifampicin was tested by disk diffusion method applying the same standard. In case of glycopeptides we determined the minimum inhibitory concentrations using E-tests (AB Biodisk, Solna).

Molecular characterization

MRSA strains were typed by pulsed field gel electrophoresis (PFGE), as described elsewhere (12). Briefly, cultures of *S. aureus* strains were included in agarose and digested with *Sma*I restriction enzyme. Resulting fragments were separated in agarose gel in 0.5xTBE buffer using a CHEF-DR III electrophoresis cell (Bio-Rad), under the following conditions: initial switch time 5 s, final switch time 35 s, voltage at 6 V/cm, 11°C, running time 20 h. The gel was stained with ethidium bromide for 30 minutes, destained with distilled water for 30 minutes and visualized using a UV transilluminator. Pulsotypes were examined using Fingerprinting II. Informatix (version 3.0) software at the National Center for Epidemiology from Budapest. Strains with a genomic similarity index of more than 80% were considered belonging to the same clonal group.

Table 1. Primers used in the triplex PCR and for exotoxin detection

Gene	Primer name	Primer sequence (5'→3')	Fragment size (bp)	Reference
nuc	Nuc F Nuc R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	290	16
lukS/F-PV	PVL R PVL F	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAGC	433	17
mecA	MECA P4 MECA P7	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	167	14
sea	SEA-1 SEA-2	GAAAAAAGTCTGAATTGCAGGGAACA CAAATAAATCGTAATTAACCGAAGGTTC	560	15
seb	SEB-1 SEB-2	ATTCTATTAAGGACACTAAGTTAGGGA ATCCCGTTTCATAAGGCGAGT	404	
sec	SEC-1 SEC-2	GCATAAAAGCTAGGAATTT AAATCGGATTAACATTATCC	257	
sed	SED-1 SED-2	GAATTAAGTAGTACCGCGCTAAATAATATG GCTGTATTTTTCCTCCGAGAGT	492	
see	SEE-1 SEE-2	CAAAGAAATGCTTTAAGCAATCTTAGGC CACCTTACCGCCAAAGCTG	482	
seg	SEG-1 SEG-2	AATTATGTGAATGCTCAACCCGATC AAACTTATATGGAACAAAAGGTACTAGTTC	642	
seh	SEH-1 SEH-2	CAATCACATCATATGCGAAAGCAG CATCTACCCAAACATTAGCACC	376	
sei	SEI-1 SEI-2	CTCAAGGTGATATTGGTGTAGG AAAAAACTTACAGGCAGTCCATCTC	576	
tst	TST-1 TST-2	TTCACTATTTGTAAAAGTGTCAGACCCACT TACTAATGAATTTTTTTATCGTAAGCCCTT	180	
eta	mpETA-1 mpETA-3	ACTGTAGGAGCTAGTGCATTTGT TGGATACTTTTGTCTATCTTTTTCATCAAC	190	
etb	mpETB-1 mpETB-2	CAGATAAAGAGCTTTATACACACATTAC AGTGAACTTATCTTTCTATTGAAAAACACTC	612	

S. aureus NCTC 8325 was used as control strain.

Bacterial DNA for PCR based methods was extracted using InstaGene Matrix (BioRad) extraction kit, following the manufacturer's instructions. Amplifications were carried out in GeneAmp PCR System 9700 (Applied Biosystems) thermocycler. PCR products were separated in agarose gel, stained with ethidium bromide and visualized in UV transilluminator.

Amplification of the *S. aureus* protein A (*spa*) repeat region was performed according to the SeqNet protocol (13). DNA sequencing was performed commercially (Macrogen, Amsterdam) and *spa* types were assigned using Ridom StaphType software.

Multiplex PCR reactions were carried out for SCC*mec* as described by Milheiricio et al (14).

Strains were tested for the presence of the *lukS/F*-PVL, *nuc* and *mec*A genes by a triplex PCR method. PCR mix was prepared in 25 µl final volume containing *mecA*, *lukS/F*-PVL and *nuc* primers at concentrations of 0.6, 0.5 and 0.2 µM, re-

Table 2. Origins of *Staphylococcus aureus* strains according to hospital wards

Wards	*S. aureus* (No.)	MRSA (No.)	% methicillin resistant
Intensive care unit	112	83	74
Surgical wards	109	38	35
Medical wards	55	14	25
Pediatric wards	17	3	18
Total	293	138	47

spectively. The thermocycling profile was set as follows: 95°C for 5 min, 30 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 60s, and 72°C for 10 min.

Strains were further studied for the presence of exotoxin encoding genes (toxic shock syndrome toxin-1, exfoliative toxins A and B, staphylococcal enterotoxins A-E, G-I) as described elsewhere (15).

Primer sets used for the triplex PCR and genes encoding for exotoxins are presented in *Table 1*.

Control strains used for PCR reactions were previously characterized clinical isolates (strains DJ31 positive for *sea* and *sed*, DJ32 positive for *seh* and SV15 positive for *sei* and *seg* were characterized at the molecular epidemiology laboratory of the National Institute for Research and Development for Microbiology and Immunology Cantacuzino, Bucharest; *S. aureus* strains 11-50008-TX positive for *sea* and *tst*, 08-50044-TX positive for *seb*, 08-50036-TX positive for *sec*, 08-50045-TX positive for *eta* and *etb* were characterized at the National Center for Epidemiology, Budapest).

Statistical analysis

Statistical analysis was performed using GraphPad InStat software, version 3.05.

Results

A number of 293 patients presented infection with *S. aureus*, documented by bacteriological analysis. Out of these 138 (47%) had infection with methicillin-resistant strains, corresponding to an incidence rate of 42/100,000 hospital bed-days. The distribution of strains according to clinical wards is

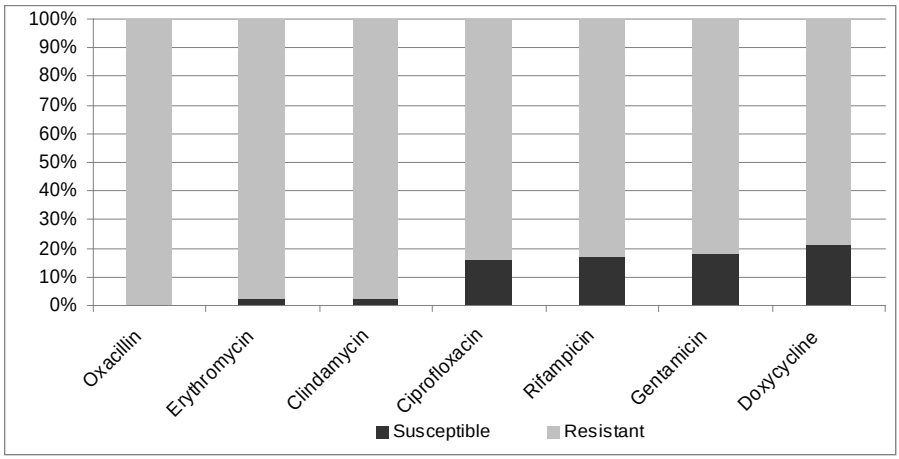

Figure 1. Antimicrobial susceptibility of MRSA strains isolated during 2010 from intensive care and surgical wards (n=122)

Table 3. Demographic and clinical data of patients with MRSA infection

Gender	No:
Female	36
Male	86
Age	Years:
Mean	54
Range	1-85
Median	58
Underlying health conditions (several per patient may apply)	No. of patients:
Cardiovascular disease	24
Abdominal pathology	22
Malignancies	21
Trauma	21
Chronic respiratory tract disease	9
Diabetes	9
Postsurgical status	7
Osteoarticular lesions	7
Renal impairment	6
Chronic hepatopathy	6
Influenza A H1N1 infection	3
Others	2
Without	4
Diagnosis of infection (several per patient may apply)	No. of patients
Pneumonia (ventilation associated)	52
Sepsis	30
Surgical site infection	32
Abscess	12
Others	4
Mean time from patient admittance to isolation of MRSA strains:	No. of days: 16 (13-19, 95% CI)

presented in *Table 2*. The highest rates of MRSA were registered in ICU wards ($p<0.05$, χ^2 test). MRSA rate was the lowest in case of pediatric infections (3 strains out of 17; 18%). During 2010 no MRSA infections were recorded in neonatal wards.

For further analysis, we have selected MRSA strains isolated from patients treated at intensive care or surgical units, 122 non-duplicate isolates being available for molecular tests.

The strains were recovered from blood cultures (n=17), lower respiratory tract specimens (n=53), normally sterile sites (n=8) and purulent secretions (n=44).

Clinical and demographic data are presented in *Table 3*.

All MRSA strains were susceptible *in vitro* to vancomycin, teicoplanin, trimethoprim/sulfamethoxazol and linezolid. Antibiotic susceptibility of MRSA strains is presented in *Figure 1*.

PFGE revealed 25 distinct pulsotypes that clustered into 4 major clonal groups designated A-D. Dendrogram of representative MRSA pulsotypes is presented in *Figure 2*. Two strains were not typeable by PFGE, being resistant to digestion by *Sma*I. The largest clonal group (groupA) included 100 (82%) strains and 15 subtypes. Subtype A3 was the most frequently encountered pulsotype that accounted for 42% (n=51) of the typed strains, followed by A1 (n=11; 9%), A4 (n=10; 8%), A2 (n=8; 7%) and others. Group B was represented by 18

Table 4. Molecular characteristics of the MRSA strains

PFGE pulsotype	No. of strains	*spa* type	SCC*mec*	PVL	Genes encoding for exotoxins
A	98	t030	III	negative	*sea*
B	18	t127 (n=16) t015 (n=1) t321 (n=1)	IV*	negative	*seh* *seg, sei, sea* *seh*
C	1	t044	IV	positive	*tst-1*
D	1	t582	IV	positive	*tst-1*
Nontypeable	2	t034	V	negative	negative

* *dcs* locus negative subtypes were present among *spa* type t127 (12 strains out of 16)
sea, *seg*, *seh*, *sei*: genes encoding for staphylococcal enterotoxin A, G, H, I;
tst-1: gene encoding for toxic shock syndrome toxin 1
lukS/F-PV: genes encoding for Panton-Valentine leukocidin

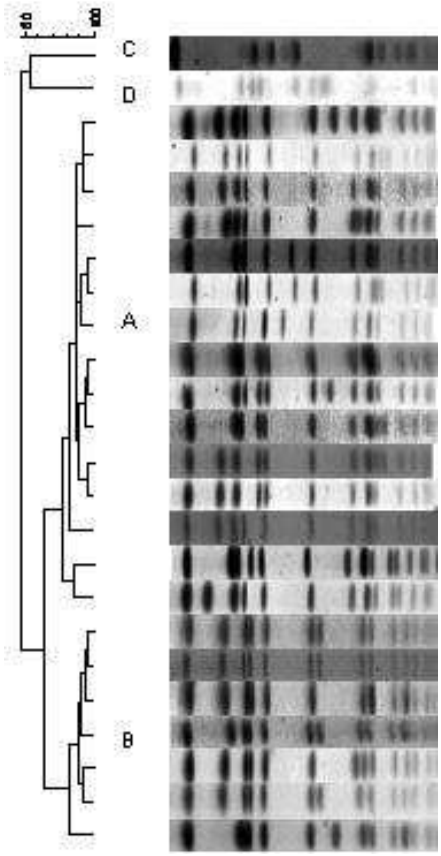

Figure 2. Dendrogram constructed from the main PFGE types and subtypes of MRSA strains, according to the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm, based on Dice coefficient with a band position tolerance of 1%.

strains, of these subtype B4 was the most frequent (n=4; 5%) followed by B2 (n=3; 2.5%) and others. Groups C and D included one strain each.

The presence of exotoxins was studied in all strains belonging to clonal groups other than A. In case of clonal group A only representatives of the different subtypes were analyzed. Molecular characteristics of the strains are presented in *Table 4.*

Analyzing epidemiological data, such as date of admission, date of MRSA isolation, ward stay, previous hospitalizations and molecular data, the followings were remarked: (1) overall 92 MRSA strains were recovered after 72 hours from admission, suggesting intrahospital acquisition of MRSA; (2) cross-transmission between patients was supported by molecular data in 27 cases - transmission of A3 subtype in 14 cases, A2 subtype in 2 cases, A1 subtype in 4 cases and A4 subtype in 7 cases; (3) no cross-transmission was documented in case of strains belonging to pulsotype B.

Intrahospital distribution of different MRSA subtypes is presented in *Table 5*.

Isolation of MRSA strains belonging to clonal group A most frequently occurred after 72 hours from patient admission (86 cases out of 100), suggesting intrahospital acquisition. Although there were 14 cases in which group A MRSA strains were isolated from samples collected during the first 72 hours of hospitalization, in

Table 5. Intrahospital distribution of the most frequent MRSA subtypes belonging to clonal group A

MRSA subtype	No. of strains
Subtype A1:	
Intensive care unit	9
General surgery 2	1
Ear, nose and throat service	1
Subtype A2:	
Intensive care unit	5
General surgery 1	3
Subtype A3:	
Intensive care unit	40
Orthopedics	1
Plastic surgery	1
General surgery 1	6
General surgery 2	2
Vascular surgery	1
Subtype A4:	
Intensive care unit	9
Neurosurgery	1

12 cases previous contact with a hospital setting was documented.

Intrahospital acquisition of MRSA strains belonging to clonal groups other than A was registered in only 6 out of 16 infections significantly less frequently than in case of group A MRSA infections ($p<0.05$, Fisher test).

The mean number of days required from admittance to isolation of group A MRSA strain was 16 (13-18, 95% CI), significantly higher than in case of MRSA strains belonging to a non-A clonal group - 6 days, (2-11, 95% CI).

Discussion

MRSA has been endemic in our hospital for several years, with an average incidence rate of 44 strains reported to 100,000 hospital bed-days (19). In 2010 the mean incidence rate was slightly below the multiannual average.

The ratio of methicillin-resistant strains was high (47%) compared to data published from Romania. In a multidisciplinary hospital in Brasov the ratio of MRSA was 38.4% (20), while in an infectious diseases hospital in Iaşi 45.7% of *S. aureus* strains were found methicillin-resistant (21). Twenty percent of *S. aureus* strains collected from Clinical Hospital of Emergencies for Children in Iaşi were MRSA (22) These values are among the highest reported from Europe (23).

MRSA infection was complicating different underlying diseases of hospitalized patients, most often for cardiovascular or abdominal pathology, malignancies or trauma. The study period included in part the influenza A H1N1 epidemic of 2009/2010, during which three patients with influenza admitted to the ICU acquired MRSA infection. Several reports have underscored the vulnerability of these patients to infection with MRSA, condition that is associated with high mortality rate (24, 25).

It is noteworthy that MRSA was usually isolated after two weeks of hospitalization, im-

plying that disease onset had been preceded by colonization, a succession of events typical for hospital-acquired MRSA infections (26 - 28).

The most frequently encountered MRSA *spa* type among our strains was t030, probably the most widespread MRSA in Romanian hospitals, being reported from several hospitals (29). It was a typical multidrug resistant HA-MRSA with SCC*mec* type III, PVL negative and enterotoxin A producing strain, a staphylococcal exotoxin with potential emetic and superantigenic activity (30). Its presence for several years in our hospital (19) led to high diversification proven by the several PFGE subtypes clustering in group A. This MRSA type remained the predominant clone in our hospital suggesting adaptation to hospital environment and successful transmission strategies.

The second most frequent *spa* type was t127, a CA-MRSA also widely distributed in Romania. Strains belonging to this type harboured SCC*mec* type IV cassettes. Some of the strains lacked the *dcs* locus, indicating heterogeneity of this cassette among strains belonging to type t127. The typing protocol used by us did not permit to differentiate these subtypes. Most of the infections caused by t127 MRSA strains were acquired outside the hospital setting and no cross-transmission was documented.

Franco *et al.* described t127 strains associated with cows, nevertheless animal strains showed distinct PFGE features compared to human t127 MRSA strains (31).

MRSA t034 was not typeable by PFGE, being resistant to restriction by *SmaI*. This *spa* type is usually associated with sequence type 398, known as LA-MRSA, distributed in Europe (32). Although it was found mainly in persons having close contact with farm animals (slaughterhouse workers, farmers), most of them being only carriers of MRSA (33), human infections were also reported by some authors (34, 35). Recent reports have shown that retail meat may also be contaminated by MRSA (including *spa* type t034) (36). Nevertheless, currently there is no evidence of increased risk of human colonization or infection associated with meat consumption (37).

The two isolates detected by us are the first such strains reported from Romania. Both were isolated from community onset infections, one from severe sepsis, and the other from an abscess. Contact of the patients or their household members with farm animals is not known. To our knowledge there are no published studies evaluating MRSA colonization of livestock in Romania.

These isolates were unique in our collection also because they were the only strains that harboured SCC*mec* type V and did not produce any of the exotoxins we have searched for. As it is typical to animal related MRSA isolates, these strains were resistant to tetracyclines, an antibiotic class frequently used in farm animals. This is a constant finding in several studies (38, 39), nevertheless it cannot be used as a phenotypic marker of LA-MRSA in our region, because other CA-MRSA strains (especially *spa* type t127 strains) can be resistant to tetracyclines as well.

MRSA *spa* types t015 and t321 were not encountered in our previous studies.

There were only 2 MRSA strains positive for PVL and TSST-1. One of these strains was the common European CA-MRSA type t044, and the other t587, both detected previously sporadically in our laboratory and were reported also from other regions in Romania (29).

In most studies reported from Romania the frequency of PVL producing strains is similarly reduced (20, 29). This can be explained by the predominance of t127 and t030 *spa* types, known as PVL-negative strains. In a study performed by Vremera *et al* a strikingly high rate (52%) of PVL positive strains was noted (40). Because no typing was performed, it is not known, whether this was due to clonally diverse MRSA strains or there was a horizontally disseminated MRSA strain responsible of infections.

The predominance of isolates belonging to clonal group A suggests an intrahospital reser-

voir of these strains. As we analyzed only strains involved in infections, we have no insight into the magnitude of dissemination of these strains as colonizers of patients and/or health-care workers. Although cross-transmission was documented only in 27 patients, we assume that this phenomenon was more extended, the source of infection being asymptomatic carriers.

Large variations of MRSA ratio were recorded according to wards, with the highest percentages in the ICU. Certain pulsotypes clustered mainly within the ICU (pulsotypes A1-4), but the recovery of such strains also from other wards suggests intrahospital spread of these strains.

MRSA strains belonging to clonal group A showed a large variety of subtypes, all of which belonged to *spa* type t030. Although *spa* typing was proposed as a rapid method to investigate hospital outbreaks (41), in our study this had no discriminatory power in case of clonal group A. On the other hand, *spa* typing revealed differences in case of strains that were belonging to clonal group B, different *spa* types sharing identical pulsotypes. These data highlight the utility of combining different typing methods in order to achieve high degree molecular characterization, fact supported also by other authors (42).

The cumulative susceptibility pattern of MRSA strains was greatly influenced by the predominance of clonal group A, isolates belonging to this group being multidrug resistant. This explains the very low rate of susceptibility to macrolides, fluoroquinolones and aminoglycosides. Notably all MRSA strains remained susceptible to glycopeptides and linezolid, antibiotics used in the treatment of severe infections. A particular trait of our strains is the preserved susceptibility to trimethoprim-sulphamethoxazol. Although this antibiotic is less efficient in the treatment of severe infections, it can be used for mild MRSA infections.

A major limitation of our study was the retrospective analysis of data. Furthermore, the results of molecular typing were not available for solving daily epidemiological issues.

Conclusions

Despite its limitations, our study provides an understanding of the epidemiology of MRSA in one of the largest tertiary care university hospitals in Romania, where complex medical cases are referred to. We have shown the predominance of a successful HA-MRSA clone, *spa* type t030. Distinct CA-MRSA strains were involved in sporadic infections, typically with community onset. LA-MRSA strains previously unreported from Romania have been characterized.

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The authors declare that they have no conflict of interest.

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