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Original paper

Molecular Analysis of Community-Acquired Staphylococcus aureus strains isolated from Skin and Soft-Tissue Infections in Romania

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Abstract

The aim of this study was to characterise S. aureus strains from community onset Skin and Soft Tissues Infections (SSTIs) in two locations: Cantacuzino Institute (A strains) and Elias University Emergency Hospital (B strains), in the January 2014 – August 2015 interval. All strains from the A location, and three strains from the B location have been isolated from recurrent staphylococcal infections.

Materials. Seventy-one *S. aureus* strains (A - 42; B - 29) have been collected from different types of SSTIs. Methods: PCR was used to identify virulence factors and AMR genes, disc diffusion and broth microdilution for AST, SCCmec and spa typing.

Results and Discussions. MRSA rate of 59.52% and 17.24% among A and B strains, respectively. Twenty of A strains and one of B strains were positive for lukS/F-PV genes; two A strains and four B strains were positive for tst1 gene. Panton-Valentine Leukocidin was present in all t008 and t044 strains. Four strains from A location and seven strains from B location were positive for *Staphylococcal Enterotoxins*. Three new spa-types were discovered. Conclusions. The most prevalent *S. aureus* clone in community onset SSTIs was spa type t127, followed by spa types t044, t008. Molecular characterisation of *S. aureus* strains may predict the tendency to recurrence of stapylococcal SSTIs.

Keywords CA-*Staphylococcus aureus*, methicillin-resistant, Panton-Valentine Leukocidin, *tst*1 gene, SEs genes.

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Introduction

S. aureus can cause a wide spectrum of diseases attributable to the range of virulence determinants it is able to express: the production of specialized binding proteins, immune evasion molecules and immune cell targeting toxins (FOSTER [1]). The human skin, nose, throat or infected blood and the environment (surfaces, foods) can be reservoirs of susceptible or resistant to antibiotics and high virulent *S. aureus* strains.

Skin and soft tissue infections (SSTIs) are the most frequent forms of the disease. *S. aureus* is the quasi universal cause of furuncles, carbuncles and skin abscesses. Through gene acquisition and alterations in regulatory networks, *S. aureus* is able to counter the physical and immunological barriers of the skin. *S. aureus* SSTIs frequently begin as minor infectious diseases, such as boils or abscesses and may progress to severe infections involving muscle or bone and may disseminate via bloodstream to the lungs or heart valves.

S. aureus strains resistant to penicillinase-resistant β-lactam antimicrobial drugs, termed methicillin-resistant *S. aureus* (MRSA), were recognized from the 1960s until the 1990s as healthcare associated pathogens. Community associated strains of MRSA appeared late, in the early 1990s (GORDON and LOWY [2]). Currently, MRSA is considered the most common bacterial pathogen isolated in many parts of the world and according to the sources and ways of transmission it is divided into several categories: healthcare-associated MRSA (HCA-MRSA) is a term covering not only hospital-acquired MRSA (HA-MRSA) (GRUNDMANN & al [3]), but also any other type of infection associated with medical care giving, while community-associated MRSA or community-acquired MRSA (CA-MRSA) is considered when there is not known evidence of host contact with a health-care institution. On the other hand, livestock-associated MRSA (LA-MRSA) may colonize people coming into close contact with animal farms (SMITH [4]). MRSA have become a significant public health problem with economic consequences worldwide (GOETGHEBEUR & al [5]).

Epidemiologically, the definition of CA-MRSA is problematic. Most studies have used a timebased definition (e.g., infections recognized within 24-72 h after hospital admission) (SALGADO & al [6]). CA-MRSA may occur when an individual with a skin lesion is in contact with others who are carrying the bacteria. This can occur with direct skin contact with infected individuals (MORAN & al [7]), colonized subjects (ELLIS & al [8]), sharing infected devices (such as razors) and public places that are not cleaned properly (BEGIER & al [9]), such as the gyms.

HA-MRSA can be transmitted during surgery, by medical devices (such as catheters) and direct skin contact without proper hand hygiene from hospital staff or visitors. However, *S. aureus* can persist as a colonizer for months or years (SCANVIC & al [10]), leading to misclassification of the source. Some community-acquired infections may in fact be caused by hospital-acquired strains and vice versa (SALGADO & al [6]; SCANVIC & al [10]), CA-MRSA are often isolated from hospital environments (GONZALEZ & al [11]). Thus, the distinction between CA-MRSA, HCA-MRSA and HA-MRSA is still unclear (WEBER [12]; CAMPBELL & al [13]).

Many of the community-acquired strains produce exotoxins and are epidemiologically distinct from healthcare-acquired strains (DRYDEN [14]). The presence of type IV SCC*mec* and Panton-Valentine Leukocidin (PVL) have been useful molecular markers of CA-MRSA strains (CARLETON & al [15]).

In the United States, a single clone of CA-MRSA (ST8/USA300) has become the most prevalent cause of staphylococcal SSTIs acquired in the community (TENOVER & al [16]), which then proved to be able to disseminate on other continents (LARSEN & al [17]) and has moved into other settings, causing not only SSTIs, but also invasive diseases (GONZALEZ & al [11]; KLEVENS & al [18]).

Among multiple virulence determinants, some *S. aureus* strains secrete several exotoxins directly associated with particular disease symptoms. These include Panton-Valentine Leukocidin (PVL), Toxic Shock Syndrome Toxin 1 (TSST1), Staphylococcal Enterotoxins (SEs), Exfoliative Toxins (ETs). Antibiotics resistance, particularly resistance to methicillin, can be also a predictive factor for the outcome of the disease (CAMERON & al [19]).

PVL is one of the four pore forming bi-component toxins that may be expressed by *S. aureus* strains. It can lyse human cells expressing C5a receptors, including neutrophils (polymorphonuclear cells) of the host (SPAAN & al [20]). PVL has been mainly associated with CA-MRSA strains (TRISTAN & al [21]; VANDENESCH & al [22]), but some studies suggest a more common association with community-acquired methicillin-sensitive *S. aureus* (CA-MSSA) strains (SHALLCROSS & al [23]). PVL-producing CA-MRSA caused suppurative SSTIs, severe necrotising community-acquired pneumonia (CAP) (LINA & al [24]; KREIENBUEHL & al [25]), bone and joint infections. The effects of antimicrobial agents on *S. aureus* PVL-producing strains has been studied by Dumitrescu & al ([26]) who demonstrated an augmentation of toxin production, especially in the presence of β-lactam antibiotics. The discovery of PVL, encoded by *luk*S/F-PV genes, was reported in the 1930s (PANTON and VALENTINE [27]). PVL has not been identified in typical epidemic or endemic HA-MRSA strains (LINDSAY and HOLDEN [28]).

Aproximately 5-25% of *S. aureus* strains are TSST1 producing. TSST1, encoded by *tst*1 gene, is a superantigen that acts on host lymph cells triggering vascular reaction with inflammation, fever and shock (SHANDS & al [29]). TSST1 is a bacterial exotoxin found in patients who have developed toxic shock syndrome (TSS). In general, the toxin is not produced by bacteria growing in the blood, rather it is produced at the local site of an infection, i.e. mucosal (vaginal, nasopharyngeal, tracheal) or sequestered (empyema, abcess) sites, and then enters the blood stream (BERGDOLL & al [30]).

Some *S. aureus* strains are able to produce Staphylococcal Enterotoxins (SEs) (BERGDOLL & al [31]) and are the causative agents of Staphylococcal Food Poisonings (SFPs). Several SEs have been identified, but the most common cause of SFPs worldwide is SEA, a highly heat-stable SE (KADARIYA & al [32]). SEs found in food and drink can trigger molecular signals at the intestinal wall level and cause local or general disease. The symptoms of SFPs are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone). The onset of the symptoms is rapid (from 30 min. to 8 h after SEs ingestion) and usually spontaneous remission is observed after 24 h. Genes encoding SEs have different genetic supports, most of which are mobile genetic elements. For example, *se*A is carried by a family of temperate phages (BETLEY and MEKALANOS [33]), *se*B is chromosomally located in some clinical isolates (SHAFER and IANDOLO [34]), whereas it has been found in a 750-kb plasmid in other *S. aureus* strains (SHALITA & al [35]), *se*C_{bovine} is encoded by a gene located on a pathogenicity island (FITZGERALD & al [36]), *se*D is carried by a plasmid (plB485) (BAYLES and IANDOLO [37]) and *se*E is carried by a defective phage (COUCH & al [38]).

Exfoliative toxins (ETs) are serine proteases with high substrate specificity responsible for staphylococcal scalded skin syndrome (SSSS), a disease predominantly affecting infants and characterized by the loss of superficial skin layers, dehydration and secondary infections (BUKOWSKI & al [39]).

It is important for clinicians to be familiar with the incidence of CA-MRSA in their communities, as prevalence can vary from region to region (MCDONALD & al [40]).

In this article we aimed to investigate the virulence markers and resistance profiles to antibiotics of *S. aureus* strains implicated in SSTIs, isolated from two different locations in Bucharest, during one year and a half (A strains and B strains) and to evaluate strains by comparing SCC*mec* and *spa* molecular types.

All strains from the A location, and only 3 strains from the B location have been isolated from recurrent staphylococcal infections.

Materials and Methods

Patients and Bacterial isolates

A total of 71 patients with acute and chronic SSTIs who were reffered for medical care and/or laboratory investigations to locations A and B (A – 42; B – 29) were enrolled in this study. All 71 *S. aureus* isolates were collected from these patients during one year and a half in the January 2014 – August 2015 interval, from different SSTIs: superficial pustular dermatitis, acne, folliculitis, furunculosis, abscess, cellulitis, paronychia, hidradenitis or open wound. All patients from the A location have been reffered for recurrent staphylococcal infections, while from B location 8 patients were hospitalized within 1-9 months before harvest sample. All the patients were outpatients by the time of SSTIs sampling.

All isolates selected in this study have been identified by a combination of phenotypic and genotypic tests: bound and free coagulase, PCR for the *nuc* gene detection. Community-acquired *S. aureus* (CA-SA) was defined as a *S. aureus* strain isolated from an outpatient or within 2 days since patient's hospital admission.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles of *S. aureus* isolates were determined by the Kirby-Bauer disk diffusion method, according to EUCAST 2014 ([41]) and 2015 guidelines ([42]). Antibiotics tested included benzylpenicillin (P, 1 unit), cefoxitin (FOX, 30 μg), erythromycin (E, 15 μg), clindamycin (DA, 2 μg), kanamycin (K, 30 μg), tobramycin (TOB, 10 μg), gentamicin (CN, 10 μg), ciprofloxacin (CIP, 5 μg), tetracycline (TE, 30 μg), rifampicin (RD, 5 μg), sulfamethoxazole-trimethoprim (SXT, 25 μg), quinupristin-dalfopristin (QD, 15 μg), linezolid (LZD, 10 μg), mupirocin (MUP, 200 μg) and fusidic acid (FD, 10 μg). The minimum inhibitory concentration (MIC) of vancomycin was detected by microdilutions in Mueller-Hinton broth. Inducible clindamycin resistance was demonstrated by the D-test. *S. aureus* ATCC 29213 was used as quality control for both the disk diffusion test and MIC detection.

DNA extraction

Total genomic DNA was obtained by use of the thermal and enzymatic lysis method (lysostaphin 1 U/μL, proteinase K 2 mg/mL, 5 min. at 100°C).

Detection of virulence and resistance genes by PCR

A variety of significant genes in respect of their implication in *Staphylococcus aureus* pathogenicity were identified by PCR, including *nuc* (encoding thermonuclease); *luk*S/F-PV (encoding PVL) and *mec*A (encoding

resistance to methicillin) detected simultaneously by a triplex PCR protocol optimized in our laboratory (DRĂGULESCU & al [43]); *tst*1 (encoding toxic shock syndrome toxin 1); *eta* and *etb* (encoding exfoliative toxin A and B); *se*A-E (encoding enterotoxins A-E implicated in food-borne diseases).

For toxic shock syndrome toxin 1 (*tst*1 gene), the exfoliative toxins (*eta*, *etb* genes) and enterotoxins A-E (*se*A-E genes), simplex PCRs were performed, with 0.4 μM of each specific primer (Invitrogen) and 1.25U of Taq DNA polymerase (Promega Corporation, Madison, WI, USA). The primers used are listed in Table 1 and the amplification programs are listed in Table 2.

Detection of antimicrobial resistance genes by PCR

Besides *mec*A (see previous chapter), antimicrobial resistance genes detected by PCR were *bla*Z (encoding benzylpenicillin resistance) (MARTINEAU & al [44]); *erm*(A) and *erm*(C) (encoding erythromycin and clindamycin resistance); *aac*A-*aph*D (encoding gentamicin resistance); *tet*(K) and *tet*(M) (encoding tetracycline resistance) (STROMMENGER & al [45]).

For each simplex PCR reactions, we used 0.4 μM of each specific primer (Invitrogen) and 1.25U of Taq DNA polymerase (Promega Corporation, Madison, WI, USA). The list of the primers is presented in Table 1 and the amplification programs are listed in Table 2.

Table 1. PCR primers used in triplex and simplex PCRs

Triplex / simplex PCR	Primer name	Primer Sequence (5'-3')	Target gene	Product (bp)	References
Triplex PCR	nucF	GCGATTGATGGTGATACGGTT	*nuc*	279	BRAKSTAD & al. [46]
	nucR	AGCCAAGCCTTGACGAACTAAAGC			
	luk-PV-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	*luk*S/F-PV	433	LINA & al. [24]
	luk-PV-2	GCATCAA(CG)TGTATTGGATAGCAAAAGC			
	mecA1	AAAATCGATGGTAAAGGTTGGC	*mec*A	532	STROMMENGER & al. [45]
	mecA2	AGTTCTGCAGTACCGGATTTGC			
Simplex PCRs	GETAR-1	GCAGGTGTTGATTTAGCATT	*eta*	93	MEHROTRA & al [47]
	GETAR-2	AGATGTCCCTATTTTTGCTG			
	GETBR-1	ACAAGCAAAAGAATACAGCG	*etb*	226	
	GETBR-2	GTTTTTGGCTGCTTCTCTTG			
	TSST1	ATGGCAGCATCAGCTTGATA	*tst*1	350	JOHNSON & al [48]
	TSST2	TTTCCAATAACCACCCGTTT			
	SEA1	TTGGAAACGGTTAAAACGAA	*se*A	120	
	SEA2	GAACCTTCCCATCAAAAACA			
	SEB1	TCGCATCAAACTGACAAACG	*se*B	478	
	SEB2	GCAGGTACTCTATAAGTGCC			
	SEC1	GACATAAAAGCTAGGAATTT	*se*C	257	
	SEC2	AAATCGGATTAACATTATCC			
	SED1	CTAGTTTGGTAATATCTCCT	*se*D	317	
	SED2	TAATGCTATATCTTATAGGG			
	SEE1	TAGATAAAGTTAAAACAAGC	*se*E	170	
	SEE2	TAACTTACCGTGGACCCTTC			
	blaZ1	ACTTCAACACCTGCTGCTTTC	*bla*Z	173	MARTINEAU & al [44]
	blaZ2	TGACCACTTTTATCAGCAACC			
	ermA1	AAGCGGTAAACCCCTCTGA	*erm*(A)	190	STROMMENGER & al [45]
	ermA2	TTCGCAAATCCCTTCTCAAC			
	ermC1	AATCGTCAATTCCTGCATGT	*erm*(C)	299	
	ermC2	TAATCGTGGAATACGGGTTTG			
	aacA-aphD1	TAATCCAAGAGCAATAAGGGC	*aac*A-*aph*D	227	
	aacA-aphD2	GCCACACTATCATAACCACTA			
	tetK1	GTAGCGACAATAGGTAATAGT	*tet*(K)	360	
	tetK2	GTAGTGACAATAAACCTCCTA			
	tetM1	AGTGGAGCGATTACAGAA	*tet*(M)	158	
	tetM2	CATATGTCCTGGCGTGTCTA			

Table 2. Cycling conditions for triplex and simplex PCRs

PCR method	Cycling conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
Triplex PCR	95°C 4 min	94°C 1 min	55°C 1 min	72°C 1 min 30 sec	72°C 4 min
			Repeated for 30 cycles		
Simplex PCR *tst*1	94°C 5 min	94°C 2 min	55°C 2 min	72°C 1 min	72°C 7 min
			Repeated for 35 cycles		
Simplex PCRs *eta*/*etb*	94°C 5 min	94°C 2 min	57°C 2 min	72°C 1 min	72°C 7 min
			Repeated for 35 cycles		
Simplex PCRs *se*A-E	95°C 5 min	94°C 1 min	55°C 1 min	72°C 1 min 30 sec	72°C 10 min
			Repeated for 30 cycles		
Simplex PCR *blaZ*	95°C 3 min	95°C 30 sec	54°C 30 sec	72°C 30 sec	72°C 4 min
			Repeated for 30 cycles		
Simplex PCRs *erm*(A)/ *erm*(C)/ *aac*A-*aph*D/ *tet*(K)/ *tet*(M)	94°C 3 min	94°C 30 sec	55°C 30 sec	72°C 30 sec	72°C 4 min
			Repeated for 30 cycles		

The following reference and Laboratory collection strains were used as controls: 33/2009 strain (Nosocomial Infections Laboratory collection) for *nuc*, *luk*S/F-PV, *mec*A genes, IC13456 strain (for *eta* gene), IC13455 strain (for *etb* gene), IC13454 strain (for *tst*1 gene), *S. aureus* National Collection of Type Cultures, U.K. (NCTC) 10652 (*se*A positive), *S. aureus* NCTC 10654 (*se*B positive), *S. aureus* NCTC 10655 (*se*C positive), *S. aureus* NCTC 10656 (*se*D positive), *S. aureus* Food Research Institute, U.S.A. (FRI) 326 (*se*E positive), 5681/2010 strain (Nosocomial Infections Laboratory collection) for *blaZ*, *erm*(C), *aac*A-*aph*D, *tet*(K) genes, 39/2008 strain (Nosocomial Infections Laboratory collection) for *erm*(A), *tet*(M) genes.

The PCR products were resolved by agarose (1,5% or 2%) gel electrophoresis, stained with ethidium bromide and visualized in an UV transilluminator.

SCCmec typing was performed by multiplex PCR using a scheme proposed by MILHEIRICO & al ([49]). The following reference strains were used as controls: COL (SCC*mec* type I), BK2464 (SCC*mec* type II), ANS46 (SCC*mec* type III), MW2 (SCC*mec* type IVa), WIS (SCC*mec* type V), HDE288 (SCC*mec* type VI).

Spa-typing

Spa-typing is a molecular typing method used to identify clones assets. Detection of polymorphism in the X region of the staphylococcal protein A (*spa*) gene was carried out as described by HARMSEN & al ([50]) using the forward primer spa-1113f: 5'TAAAGACGATCCTTCGGTGAGC3' and the reverse primer spa-1514r: 5'CAGCAGTAGTGC CGTTTGCTT3'. PCR products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA). PCR products of the *spa* gene were sequenced using the same PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) as recommended by manufacturer, on an Applied BioSystems ABI PRISM 3130 Genetic Analyzer. *Spa* sequences were analyzed using Ridom StaphType software version 2.2.1 (Ridom GmbH, Wurzburg, Germany).

All the PCR reactions were performed either in an Applied Biosystems or in a Corbett Research Thermocyclers.

Results and Discussions

The median age of patients from the A location was 32.5 years and from the B location was 50 years (range: A: 4-76 years; B: 4 month – 88 years), while the sex distribution (male / female) was 26 / 16 (61.90% / 38.10%) for A location patients and 10 / 19 (34.48% / 65.52%) for B location patients.

Distribution of antimicrobial resistance phenotypes of A and B strains are illustrated in Figures 1 and 2.

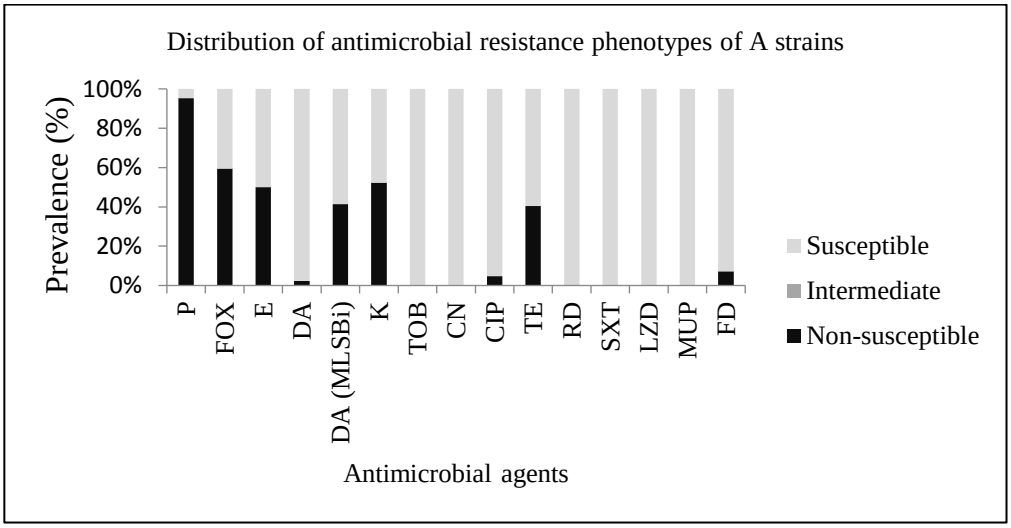

Figure 1. Distribution of antimicrobial resistance phenotypes of A strains.

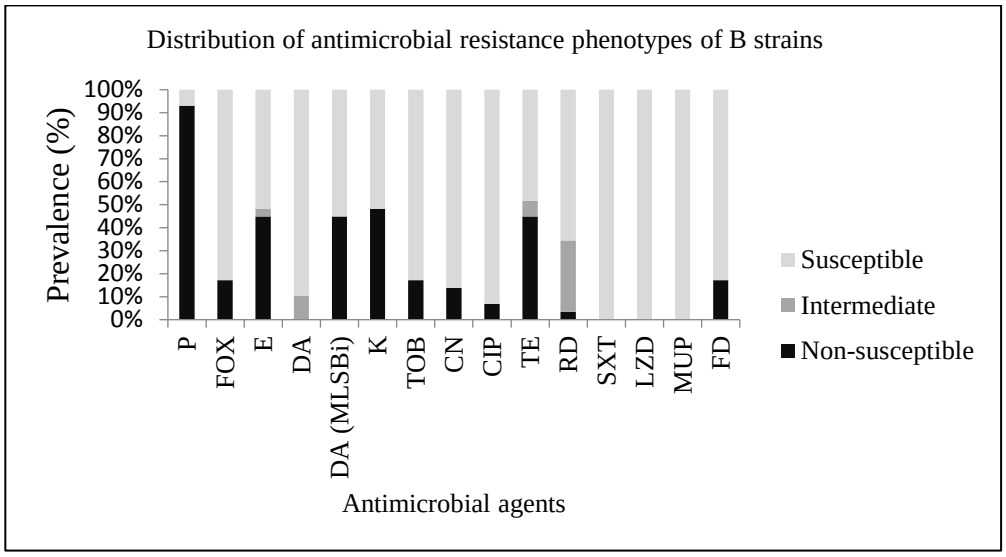

Figure 2. Distribution of antimicrobial resistance phenotypes of B strains.

59.52% (n = 25) of the A strains were found to be MRSA, while only 17.24% (n = 5) B strains were MRSA. Most of the A (95.24%) and B (93.10%) strains were resistant to P. The MLSBi phenotype was detected in 40.48% (n = 17) of A strains and 44.83% (n = 13) of B strains. Strains isolated in the A location were also resistant to: E (21-50%), DA (17-40.48%) – inducible resistance and DA (1-2.38%) – constitutive resistance, K (22-52.38%), TE (17-40.48%), QD (17-40.48%) – reported resistant according to the MLSBi phenotype, CIP (2-4.76%), FD (3-7.14%). Antimicrobial susceptibility testing of B strains revealed the following resistance rates: E (13- 44.83%) and E intermediate (1-3.45%), DA (13-44.83%) – inducible resistance and DA intermediate (3-10.34%) – constitutive resistance, K (14-48.28%), TOB (5-17.24%), CN (4-13.79%), TE (13-44.83%) and TE intermediate (2 – 6.90%), CIP (2-6.90%), RD (1-3.45%) and RD intermediate (9-31.03%), QD (13-44.83%) – reported resistant according to the

MLSBi phenotype and QD intermediate (3-10.34%), FD (5-17.24%). No Vancomycin resistant isolates have been found.

The genetic analyses confirmed the presence of *mec*A gene in MRSA strains, included in SCC*mec* cassettes type IV (9 A strains) or type IVE (15 A strains and 5 B strains). A strain from the A location could not be included in any SCC*mec* cassette type by the using the Milheirico method. The presence of the *bla*Z gene was detected in all P resistant strains from the A and B locations. 40.48% (n = 17) of A strains with phenotypic resistance to TE presented the *tet*(K) gene, 41.38% (n = 12) of the B strains were positive for the *tet*(K) gene and one B strain (3.45%) was positive for the *tet*(M) gene. Two B strains (6.90%) with intermediate resistance to TE did not express neither the *tet*(K) or the *tet*(M) genes. The presence of the *erm*(C) gene in the case of certain strains was positively correlated with the E and MLSBi resistance phenotype in both A and B strains. Three A strains (7.14%) with phenotypic resistance to E and one strain (2.38%) with phenotypic resistance to E and DA were negative for *erm*(A)/ *erm*(C) genes. One B strain (3.45%) with intermediate phenotypic resistance to E and three strains (10.34%) with intermediate phenotypic resistance to DA were negative for *erm*(A)/ *erm*(C) genes. Two strains (6.90%) from B location, with phenotypic resistance to CN and TOB, were positive for *aac*A-*aph*D genes and 2 strains (6.90%) were PCR negative, probably due to a different resistance mechanisms.

Figures 3 and 4 show the distribution of virulence factors genes in the A and B strains. All the A and B strains were positive for the *nuc* gene. The *luk*S/F-PV genes were found in 47.62% (n = 20) isolates from the A location and only in 3.45% (n = 1) isolates from the B location; 4.76% (n = 2) strains from the A location and 13.79% (n = 4) strains from the B location were positive for *tst*1 gene. From the A location 2.38% of the strains (n = 1) were positive for *se*B gene and 7.14% (n = 3) were positive for *se*C gene. From the B location 10.34% (n = 3) were positive for *se*A gene and 13.79% (n = 4) were positive for *se*C gene.

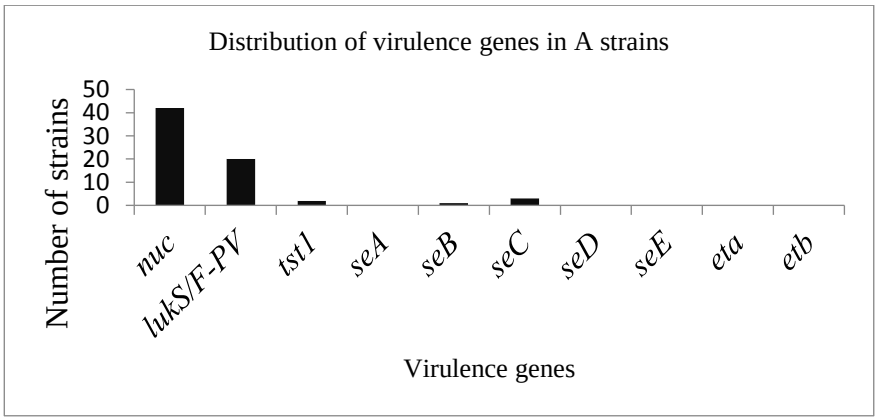

Figure 3. Distribution of virulence genes in A strains.

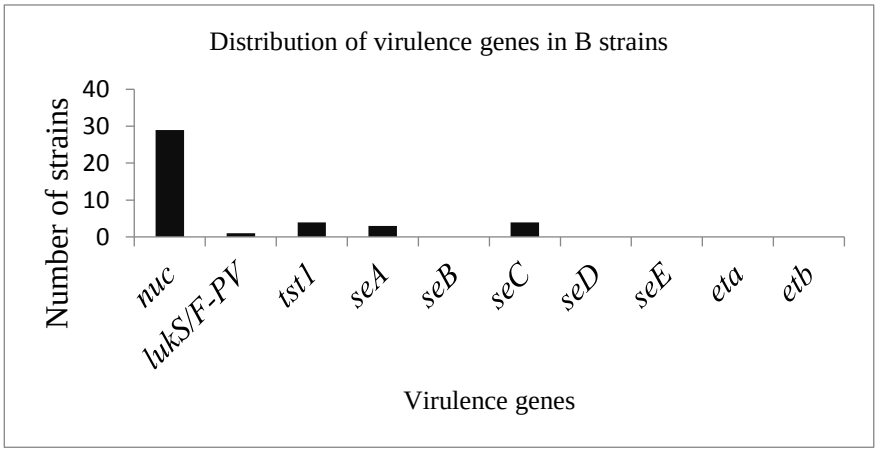

Figure 4. Distribution of virulence genes in B strains.

Spa-typing

The prevalent *spa* type identified in strains from the A location was t127 (30.95%, n = 13), followed by t044 (11.90%, n = 5), t008 (7.14%, n = 3), t284 (7.14%, n = 3), t019 (4.76%, n = 2), t2881 (4.76%, n = 2) and other 14 *spa* types with 2.38% (one strain each: t005, t091, t174, t223, t280, t355, t435, t437, t948, t1211, t1889, t5841, t14512 and t14513). *Spa* type t127 was also the predominant type in the B location (44.83%, n = 13), along with types t005 (6.90%, n = 2), t021 (6.90%, n = 2) and other 12 *spa* types with 3.45% (one strain each: t002, t012, t015, t053, t084, t559, t582, t620, t701, t728, t7585, t15296). The genes responsible for the PVL production were detected in *spa* types t008 and t044 strains, but also in other *spa* types: t019, t284, t355, t435, t437, t1211, t1889, t5841, t14513. In the A strains 12 of the 13 t127 *spa* type strains were MRSA, while in the B strains only 4 of the 13 t127 *spa* type strains were MRSA. In the A location other 5 strains with *spa* types t019, t174, t437, t948, t2881 were MRSA and in the B location a t559 *spa* type strain was MRSA. In the A location t223, t1889 and in the B location t012, t021 (n = 2), t582 strains were positive for *tst*1 gene. In the A location one strain with *spa* type t437 was positive for *se*B gene, while 3 strains with *spa* types t1889 (n = 1) and t2881 (n = 2) presented the *se*C gene. In the B location 3 strains with *spa* types t053, t127, t701 were positive for *se*A gene and 4 strains with *spa* types t015, t582, t620, t728 were positive for *se*C gene. The distribution of *spa* types in the A and B locations are presented in figures 5 and 6.

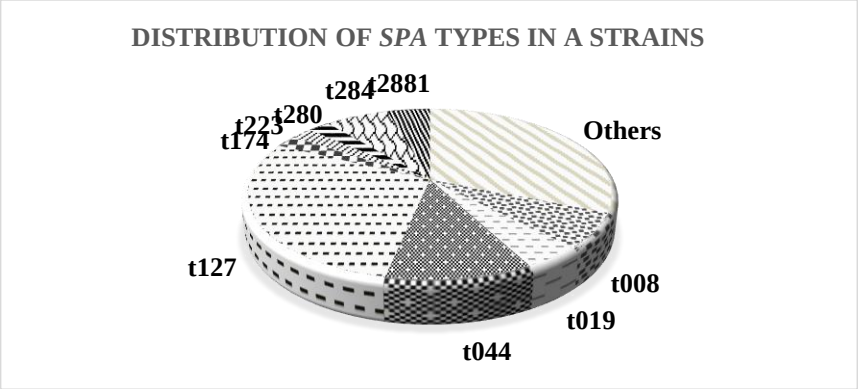

Figure 5. Distribution of *spa* types in A strains.

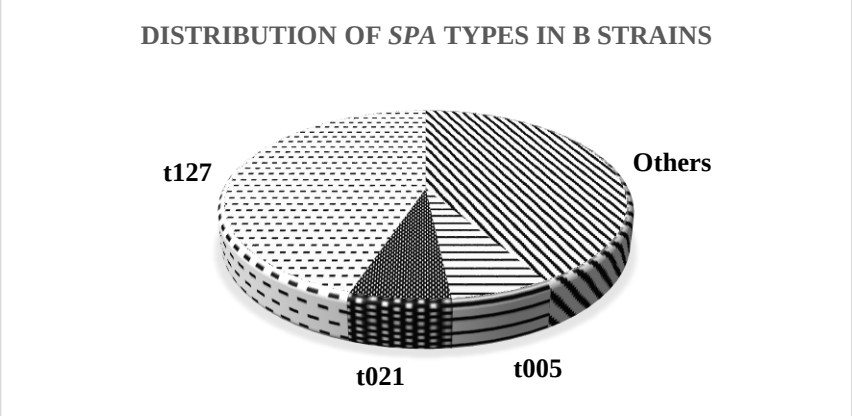

Figure 6. Distribution of *spa* types in B strains.

The *spa* types t14512, t14513 (PVL positive strain) and t15296 were discovered for the first time during this study. The new *spa*-types were assigned through EU Ridom SpaServer database ([51]). Also, all the *spa*-types identified in this study were uploaded in the same database, except one strain's sequence which was not accepted because of a point mutation in the 3' signature changing the original structure 3'TAYAT**G**TCGT into 3'TAYAT**A**TCGT.

The correlations between patterns of antibiotics resistance, *spa* types and PVL-positive MSSA and MRSA strains are presented in tables 3 and 4.

Table 3. The correlations between patterns of antibiotics resistance, *spa* types and PVL-positive MSSA and MRSA strains in the A location

Patterns of antibiotics resistance	No. of strains	MSSA/ *spa* types/ PVL +/-/ (no. of strains)	MRSA/ *spa* types/ PVL +/-/ (no. of strains)
Wild-type	2	t5841 +, t044 +	-
P	14	t005 -, t091 -, t223 -, t280 -, t284 + (3), t355 +, t435 +, t1211 +, t1889 +, t2881 -, t14512 -, t14513 +	-
P, K	1	t127 -	-
P, FOX	2	-	t019 + (2)
P, FOX, E	1	-	t008 +
P, FOX, E, K	1	-	t008 +
P, FOX, E, K, CIP	1	-	t008 +
P, FOX, K, TE	1	-	t044 +
P, FOX, K, TE, FD	1	-	t044 +
P, FOX, E, DA, K, CIP, TE	1	-	t437 +
P, FOX, E, DA (MLSBi), K, QD	3	-	t127 - (2), t2881 -
P, FOX, E, DA (MLSBi), K, TE, QD	11	-	t127 - (9), t174 -, t948 -
P, FOX, E, DA (MLSBi), TE, QD	1	-	t127 -
P, FOX, E, DA (MLSBi), K, TE, QD, FD	2	-	t044 + (2)
	42 strains		

Table 4. The correlations between patterns of antibiotics resistance, *spa* types and PVL-positive MSSA and MRSA strains in the B location

Patterns of antibiotics resistance	No. of strains	MSSA/ *spa* types/ PVL +/-/ (no. of strains)	MRSA/ *spa* types/ PVL +/-/ (no. of strains)
Wild-type	1	t002 -	-
P	3	t701 -, t728 -, t7585 -	-
P, DA(I)*	2	t005 -, t127 -	-
P, TE	1	t084 -	-
P, RD(I)	2	t005 -, t021 +	-
P, TE, RD(I)	1	t021 -	-
P, E(I), TE, QD(I)	1	t582 -	-
P, CIP	1	t012 -	-
P, DA(I), K, TE, RD(I), QD(I)	1	t127 -	-
P, FD	2	t015 -, t620 -	-
CIP, RD, QD(I), FD	1	t053 -	-
P, FOX, E, DA (MLSBi), K, TOB, CN, TE, RD(I), QD	1	-	t127 -

Patterns of antibiotics resistance	No. of strains	MSSA/ *spa* types/ PVL +/-/ (no. of strains)	MRSA/ *spa* types/ PVL +/-/ (no. of strains)
P, FOX, E, DA (MLSBi), K, TOB, CN, RD(I), QD	1	-	t127 -
P, FOX, E, DA (MLSBi), K, TE(I), RD(I), QD	1	-	t559 -
P, FOX, E, DA (MLSBi), K, TE, RD(I), QD	1	-	t127 -
P, FOX, E, DA (MLSBi), K, TE, QD	1	-	t127 -
P, E, DA (MLSBi), K, TE, QD	4	t127 - (3), t15296 -	-
P, E, DA (MLSBi), K, TOB, CN, QD, FD	1	t127 -	-
P, E, DA (MLSBi), K, TOB, CN, TE, QD	1	t127 -	-
P, E, DA (MLSBi), K, TOB, TE(I), QD	1	t127 -	-
P, E, DA (MLSBi), K, TE, RD(I), QD, FD	1	t127 -	-
	29 strains		

(I)* - Intermediate

Discussion

Epidemiological studies have shown an increase not only in healthcare-associated *S. aureus* infections, but also in *S. aureus* infections acquired in the community (DRYDEN [14]).

SSTIs caused by *S. aureus* are a diverse group of infections that can be classified into uncomplicated and complicated infections. Host factors such as co-morbidities and microbial factors, in particular drug resistance, complicate the management of these infections. MRSA is an important pathogen involved in complicated SSTIs (cSSTIs) in Europe and its involvement can be associated with poor patient outcomes. Therapy should be initiated without delay and on an empiric base, before micro-biological analysis have been accomplished, waiting for culture and antimicrobial susceptibility testing for documentation of the presence of MRSA (LEE & al [52]).

In 2008, Europe showed a strong presence of MRSA strains, accounting for approximately 44% of hospital infections (KOCK & al [53]). Other European studies showed an overall oxacillin resistance rate for *S. aureus* of 26.7%. There was a great variation of MRSA rates between countries, the resistance level ranging from over 40% in Great Britain, Belgium, Greece, Ireland and Island to only 0.6% in Sweden (SADER & al [54]). Twenty five % or more of *S. aureus* isolates have been reported in Bulgaria, Croatia, Cyprus, Greece, Israel, Italy, Malta, Portugal, Ireland, Romania, Spain, Turkey and the UK (KOCK & al [53]). According to the ECDC Antimicrobial Resistance Surveillance Network (EARS-Net formerly EARSS) report, in Romania the MRSA rate among invasive infections was 49.5% in 2011, 53.3% in 2012, 64.5% in 2013 and 56% in 2014 ([55]). Fortunately, this is improving because of surveillance programs and stringent outbreak control criteria (HUMPHREYS [56]).

HA-MRSA reach also a high prevalence in Australia (HUMPHREYS [56]), North Africa, the Middle East and East Asia (IPPOLITO & al [57]).

Spa type t030 has been described as the prevalent MRSA *spa* type in Turkey (ALP & al [58]) and Romania (GRUNDMANN & al [3]), being reported by other several studies as the most widespread MRSA in Romanian hospitals (OPREA & al [59]; IONESCU & al [60]), and considered as being endemic (SZEKELY & al [61]). ST239/ t030 highly associated with the SCC*mec* type III is now widely disseminated in many Asian countries and China in particular, although it might have originated from European countries (CHEN & al [62]). In Romanian hospitals, the ST239 clone was reported by the Seqnet.org structured survey (GRUNDMANN & al [63]) and by other authors (CÎRLAN & al [64]). *Spa* type t127 reported frequently in Romanian hospitals (OPREA & al. [59]; SZEKELY & al [61]; CODIȚĂ & al [65]), was described also by Franco & al ([66]) to be associated with animals (e.g., pigs).

In Europe, CA-MRSA was first described at the beginning of this decade. The highest rates of CA-MRSA carriage (>50%) are reported in North and South America, Asia and Malta. Intermediate rates (25-50%) are reported in China, Australia, Africa and some European countries, such as Portugal (49%), Greece (40%), Italy (37%) and Romania (34%). Other European countries, including the Netherlands and Scandinavian Countries reported generally low prevalence rates (ALAKLOBI & al [67]).

CA-MRSA had a higher prevalence in the United States, Canada (CARROLL [68]) and Australia (KOCK & al [53]). Both in Europe and in United States there is a dramatic raise in the number of reports showing the increase of MRSA prevalence in community-associated infections which differs with area and population. Before 2000, in United States, the percent of MRSA strains isolated from SSTIs was very low (3%) and raised in some regions to 30% and then to 64% between 2001-2004, with 97% of isolates belonging to clone USA300 (MORAN & al [7]).

Other United States clones include USA400 (ST1), USA1000 (ST59), and USA1100 (ST30) (PAN & al [69]).

In China, the most common clone among CA-MRSA isolates reported in the case of children with SSTIs was ST59-MRSAIV/V-t437 (WU & al [70]; LI & al [71]). In Beijing, one recent study reported the livestock ST398 clone as having a high prevalence in *S. aureus* SSTIs, where 64.3% were harbouring the *luk*S/F-PV genes (ZHAO & al [72]). In Japan, the most common clone among SSTIs-associated MRSA was MLST-CC8/*spa*-CC008-SCC*mec*-IV (MAEDA & al [73]). In New York, the United States CC8 is the most common type in *S. aureus* SSTIs, especially among PVL-positive *S. aureus* (KALTSAS & al. [74]).

Vandenesch & al ([22]) showed a limited number of clones with a preferential geographic distribution before 2003. Afterwards, a much higher genetic diversity was reported in Europe among the CA-MSSA and the CA-MRSA strains (ROLO & al [75]). It is clear that some strains are found geographically restricted while others are found worldwide, but it is not clear why certain MRSA strains predominate in hospitals and other MRSA strains predominate in community settings (DIEP & al [76]). Some strains showed significant variation in the ability to cause outbreaks.

The most prevalent European CA-MRSA clone detected was ST80/ CC80 and is resistant to kanamycin/ amikacin and fusidic acid (STEGGER & al [77]), while in the United States the most prevalent clones were ST8 (USA300) and ST1 (USA400), and in Oceania ST30. The study by Tristan & al. ([21]) suggests intercontinental exchanges of several continent-specific clones: the ST8 clone from the United States towards Europe, the ST1 clone from the United States towards Europe and Asia, the ST59 clone (USA1000) from the United States towards Asia, the ST80 clone from Europe towards Asia (HSU & al [78]) and the ST30 clone from Oceania towards Europe. The epidemic character of CA-MRSA, especially the geographically widespread clone USA300 is poorly understood. USA300 isolates carry a type IV SCC*mec* element conferring β-lactam antibiotic class resistance and a putative pathogenicity island, arginine catabolic mobile element (ACME), which may be involved in increasing persistance in the skin environment and in inhibiting polymorphonuclear cells function. Physical linkage between SCC*mec* and ACME suggests that selection for antibiotic resistance and for pathogenicity may be interconnected (DIEP & al [79]).

The main European clone, t044/ ST80, was detected in Austria, Belgium, Bulgaria, Chile, Croatia, Cyprus, Czech Republic, Finland, France, Germany, Greece, Hungary, Iceland, Iran, Ireland, Italy, Jordan, Lebanon, Netherlands, Norway, Romania, Spain, Sweden, Switzerland, United Arab Emirates, United Kingdom (EU Ridom SpaServer database), but also in northern Europe (e.g., Denmark), where MRSA strains are rare in hospitals (FARIA & al [80]). ST30 is also a major clone in Asia and Oceania (HO & al [81]; VLACK & al [82]) and is referred to as the South West Pacific clone (VLACK & al [82]).

ST1 and ST30, the PVL-positive clones, can now be considered pandemic. They are detected in America, Europe and Asia. Some continent-specific clones described in 2003, such as clone ST8, have now spread all over the world and the PVL-positive CA-MRSA have spread to several countries on various continents (TRISTAN & al [21]; VANDENESCH & al [22]). In 2002, PVL has been associated with 1.6% of *S. aureus* strains in England and Wales (HOLMES & al [83]). Currently, the prevalence of PVL-positive CA-MRSA varies considerably from continent to continent. In contrast to the United States, where CA-MRSA now accounts for the majority of *S. aureus* infections in the community, in Northern Europe, the prevalence of PVL-positive CA-MRSA is lower, at ≈1–3% (DEL GIUDICE & al [84]), while Southern European countries, such as Greece and countries located at the southern boundaries of Europe, such as Algeria, have a greater prevalence.PVL-positive MSSA/MRSA have also occasionally been described in Romania (IONESCU & al [60]; NĂSTASE & al [85]; CODIȚĂ & al [86]; MONECKE & al [87]). Studies have reported a high prevalence of the PVL-positive strains in our country (31% among MRSA, 14% among MSSA). One study revealed a relatively high rate of PVL-producing strains (23.93%), mainly CA-MRSA (51.11%) in North-Eastern region of Romania (VREMERA & al [88]).

Initially PVL-positive CA-MRSA isolates were susceptible to most antistaphylococcal antimicrobial agents, then they have acquired new antimicrobial resistance determinants. In Asia (Singapore, People's Republic of China) or Africa (Algeria) most PVL-positive CA-MRSA strains showed multiple antimicrobial resistance determinants (TRISTAN & al [21]). One study from 1999 showed the prevalence of resistance to aminoglycosides in *S. aureus* SSTIs: MSSA – gentamicin 2% (4/217), tobramycin 4% (8/217), kanamycin 5% (11/217) and MRSA – gentamicin 67% (39/57), tobramycin 91% (52/57), kanamycin 93% (53/57) (SCHMITZ & al [89]), while another study from 2001 showed the distribution of the tetracycline resistance genes of MRSA and MSSA strains

in Europe (SCHMITZ & al [90]). In Romania, most of the PVL-positive CA-MRSA isolates were resistant to erythromycin (91.30%), and susceptible to clindamycin, fluoroquinolones, rifampicin, chloramphenicol or fusidic acid (VREMERA & al [88]). CA-MSSA/CA-MRSA, PVL-positive strains isolated from skin and soft tissue infections in A and B location proved different patterns of antimicrobial resistance covering a large spectrum, from sensitive to all classes of antimicrobials tested to resistant to one up to all of the following antibiotics: erythromycin, clindamycin, kanamycin, ciprofloxacin, tetracycline, quinupristin-dalfopristin, fusidic acid. In B location, one CA-MSSA PVL-positive strain was intermediate to rifampicin, but in this location we found also this intermediate resistance in other 8 PVL negative strains, amongst 4 were MSSA and 4 MRSA. Three CA-MRSA t044 PVL positive strains isolated in the A location and 5 MSSA PVL negative strains from B location were found resistant to fusidic acid, widely used for skin and soft tissue infections topical therapy.

In countries of high prevalence, PVL-positive CA-MRSA are responsible for an increasing number of HA infections. Among the different clones, sequence variations of PVL have been observed. The clone most frequently observed in Europe, ST80 harbours an H2 haplotype which is described as the ancestral allele that gave rise to the other variants. These trends, although largely regionalised, must be taken into account when targeting skin infections as well as other infections, including CAP. These emerging tendencies are directed towards increasing incidence of CA-MRSA and decreasing susceptibility to antimicrobial agents. It must be considered when choosing empiric treatment options (VANDENESCH [91]).

CA-MRSA strains often cause more severe diseases such as deep skin infections. A clear epidemiological association between PVL-positive *S. aureus* strains and necrotizing pneumonia has been shown (LINA & al [24]). In the United States, the high prevalence of PVL-positive CA-MRSA is mostly responsible for necrotizing infections, whereas in Europe the majority of cases of necrotizing pneumonia are caused by PVL-positive MSSA strains that are as virulent as the PVL-positive CA-MRSA clones (SICOT & al [92]).

In Romania, one study and one communication presented case reports on fatal sepsis due to PVL-positive CA-MRSA *spa* type t044 clone (SZEKELY & al [93]; ALEXANDRESCU & al [94]). Our study allowed us to identify PVL-positive *spa* types t008 and t044 strains, as being implicated in community acquired SSTIs. Over the time, PVL-positive t044 strains have been involved in necrotizing pneumonia, fatal sepsis with community-onset, associated with influenza but also with skin infections evolving in teenagers or preschoolers in Romania (SZEKELY & al [93]; ALEXANDRESCU & al [94]). In a previous study we found PVL-positive *spa* type t008 strains involved in newborns or infants infections with hospital-onset (personal communication). We presume that t008 strain has the capacity to multiply and to disseminate in hospital outbreaks.

However, little is known on the precise Romanian situation. In recent Romanian studies the MRSA rates in hospitals are very high, ranging from approximately 30% up to 85%, whereas CA-MRSA has been identified inside hospitals (COMAN & al [95]; GRIGORE & al [96]; DORNEANU & al [97]; SZEKELY & al [98]; DOROBĂȚ & al [99]; IONESCU & al [60]; KOCK & al [53]; NĂSTASE & al [85]; NICA & al [100]; VREMERA & al [88]; IONESCU & al [101], MEREZEANU & al [102], COTAR & al [103], CRISTEA & al [104]). From our knowledge, there are no national reports on MRSA prevalence among community onset SSTIs. Our study revealed a prevalence of 59.52% in location A and 17.24% in location B.

In our study the prevalent community onset SSTI *spa* type was t127 in strains isolated from outpatients both at the Cantacuzino Institute and at the Elias University Emergency Hospital.

Conclusions

The most prevalent *S. aureus* clone in community onset SSTIs was *spa* type t127, followed by *spa* types t044 and t008. Previous reports on possible link between human and animal t127 strains support future development by using an integrated One health approach. On the other hand, we proved that t127 MRSA was involved in several hospital outbreaks in the last years.

The genes responsible for PVL production were detected in strains of t008 and t044 *spa* types, but also in other *spa* types: t019, t284, t355, t435, t437, t1211, t1889, t5841, t14513.

The detection of PVL, TSST and SEs positive *S. aureus* strains by using molecular methods and anti-microbial resistance testing revealed differencies between mostly recurrent infections (A strains) and mainly not recurrent infections (B strains).

Detection of virulence and AMR genes may support enhanced surveillance and public health interventions aiming to prevent the disemination of clones implicated in necrotizing pneumonia, complicated suppurative skin infections, toxic-septic shock and food poisonings both in community and hospitals settings.Systematic surveillance of both hospital and community isolates is required and should be done regularly for proper treatment, together with measures designed to limit their spread, including an integrated One health approach.

Our study results confirmed that containment of *S. aureus*/ MRSA strains remains a first emergency public health constraint.

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References

1. T.J. FOSTER. Immune evasion by staphylococci. *Nat. Rev. Microbiol*., 3(12): 948, 958 (2005).
2. R.J. GORDON AND F.D. LOWY. Presence of methicillin resistant *Staphylococcus aureus* in hospitals. *J. Clin. Infect. Dis.*, 46: 350, 359 (2008).
3. H. GRUNDMANN, D.M. AANENSEN, C.C. VAN DEN WIJNGAARD, B.G. SPRATT, D. HARMSEN, A.W. FRIEDRICH, THE EUROPEAN STAPHYLOCOCCAL REFERENCE LABORATORY WORKING GROUP. Geographic Distribution of *Staphylococcus aureus* Causing Invasive Infections in Europe: A Molecular-Epidemiological Analysis. *PLoS Med*., 7(1), e1000215 (2010). doi:10.1371/journal.pmed.1000215.
4. T.C. SMITH. Livestock-Associated *Staphylococcus aureus*: The United States Experience. *PLoS Pathog*., 11(2), e1004564 (2015). doi:10.1371/journal.ppat.1004564.
5. M. GOETGHEBEUR, P.-A. LANDRY, D. HAN, C. VICENTE. Methicillin-resistant *Staphylococcus aureus*: A public health issue with economic consequences. *Can. J. Infect. Dis. Med. Microbiol*., 18(1): 27, 34 (2007).
6. C.D. SALGADO, B.M. FARR, D.P. CALFEE. Community-acquired methicillin resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clin. Infect. Dis*., 36: 131, 139 (2003).
7. G.J. MORAN, A. KRISHNADASAN, R.J. GORWITZ, G.E. FOSHEIM, L.K. MCDOUGAL, R.B. CAREY, D.A. TALAN, EMERGENCY ID NET STUDY GROUP. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N. Engl. J. Med*., 355: 666, 674 (2006).
8. M.W. ELLIS, D.R. HOSPENTHAL, D.P. DOOLEY, P.J. GRAY, C.K. MURRAY. Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. *Clin. Infect. Dis.*, 39: 971, 979 (2004).
9. E.M. BEGIER, K. FRENETTE, N.L. BARRETT, P. MSHAR, S. PETIT, D.J. BOXRUD, K. WATKINS-COLWELL, S. WHEELER, E.A. CEBELINSKI, A. GLENNEN, D. NGUYEN, J.L. HADLER, CONNECTICUT BIOTERRORISM FIELD EPIDEMIOLOGY RESPONSE TEAM. A high-morbidity outbreak of methicillin-resistant *Staphylococcus aureus* among players on a college football team, facilitated by cosmetic body shaving and turf burns. *Clin. Infect. Dis*., 39: 1446, 1453 (2004).
10. A. SCANVIC, L. DENIC, S. GAILLON, P. GIRY, A. ANDREMONT, J.C. LUCET. Duration of colonization by methicillin-resistant *Staphylococcus aureus* after hospital discharge and risk factors for prolonged carriage. *Clin. Infect. Dis*., 32: 1393, 1398 (2001).
11. B.E. GONZALEZ, A.M. RUEDA, S.A. SHELBURNE III, D.M. MUSHER, R.J. HAMILL, K.G. HULTEN, Community-associated strains of methicillin-resistant *Staphylococccus aureus* as the cause of healthcare-associated infection. *Infect. Control. Hosp. Epidemiol*., 27: 1051, 1056 (2006).
12. J.T. WEBER. Community-associated methicillin-resistant *Staphylococcus aureus*. *Clin. Infect. Dis*., 41(Suppl 4): S269, S272 (2005).
13. A.L. CAMPBELL, K.A. BRYANT, B. STOVER, G.S. MARSHALL. Epidemiology of methicillin-resistant *Staphylococcus aureus* at a children's hospital. *Infect. Control. Hosp. Epidemiol*., 24: 427, 430 (2003).
14. M.S. DRYDEN. Skin and soft tissue infection: microbiology and epidemiology. *Int. J. Antimicrob. Agents*, 34(1): S2, S7 (2009).
15. H.A. CARLETON, B.A. DIEP, E.D. CHARLEBOIS, G.F. SENSABAUGH, F. PERDREAU-REMINGTON. Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an

expanding community reservoir of MRSA. *J. Infect. Dis*., 190: 1730, 1738 (2004).
16. F.C. TENOVER, L.K. MCDOUGAL, R.V. GOERING, G. KILLGORE, S.J. PROJAN, J.B. PATEL, P.M. DUNMAN. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. *J. Clin. Microbiol*., 44: 108, 118 (2006).
17. A.R. LARSEN, R. GOERING, M. STEGGER, J.A. LINDSAY, K.A. GOULD, J. HINDS, M. SØRUM, H. WESTH, K. BOYE AND R. SKOV. Two Distinct Clones of Methicillin-Resistant *Staphylococcus aureus* (MRSA) with the Same USA300 Pulsed-Field Gel Electrophoresis Profile: a Potential Pitfall for Identification of USA300 Community-Associated MRSA. *J. Clin. Microbiol*., 47(11): 3765, 3768 (2009).
18. R.M. KLEVENS, M.A. MORRISON, J. NADLE, S. PETIT, K. GERSHMAN, S. RAY, L.H. HARRISON, R. LYNFIELD, GH. DUMYATI, J.M. TOWNES, A.S. CRAIG, E.R. ZELL, G.E. FOSHEIM, L.K. MCDOUGAL, R.B. CAREY, S.K. FRIDKIN, FOR THE ACTIVE BACTERIAL CORE SURVEI-LLANCE (ABCS) MRSA INVESTIGATORS. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA*, 298: 1763, 1771 (2007).
19. D.R. CAMERON, B.P. HOWDEN AND A.Y. PELEG. The Interface Between Antibiotic Resistance and Virulence in *Staphylococcus aureus* and Its Impact Upon Clinical Outcomes. *CID*, 53(6): 576, 582 (2011).
20. A.N. SPAAN, T. HENRY, W.J. VAN ROOIJEN, M. PERRET, C. BADIOU, P.C. AERTS, J. KEMMINK, C.J. de HAAS, K.P. van KESSEL, F. VANDENESCH, G. LINA, J.A. van STRIJP. The staphylococcal toxin PVL targets human C5a receptors. *Cell Host Microbe*, 13: 584, 594 (2013).
21. A. TRISTAN, M. BES, H. MEUGNIER, G. LINA, B. BOZDOGAN, P. COURVALIN, M.-E. REVERDY, M.C. ENRIGHT, F. VANDENESCH, J. ETIENNE. Global distribution of Pantone-Valentine leukocidine positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerg. Infect. Dis*., 13: 594, 600 (2007).
22. F. VANDENESCH, T. NAIMI, M.C. ENRIGHT, G. LINA, G.R. NIMMO, H. HEFFERNAN, N. LIASSINE, M. BES, T. GREENLAND, M.E. REVERDY, J. ETIENNE. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Pantone-Valentine leukocidin genes: worldwide emergence. *Emerg. Infect. Dis*., 9: 978, 984 (2003).
23. L.J. SHALLCROSS, K. WILLIAMS, S. HOPKINS, R.W. ALDRIDGE, A.M. JOHNSON, A.C. HAYWARD. Pantone-Valentine leukocidin associated staphylococcal disease: a cross-sectional study at a London hospital. *Engl. Clin. Microbiol. Infect*., 16: 1644, 1648 (2010).
24. G. LINA, Y. PIEMONT, F. GODAIL-GAMOT, M. BES, M.O. PETER, V. GAUDUCHON, F. VANDENESCH, J. ETIENNE. Involvement of Pantone-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis*., 29: 1128, 1132 (1999).
25. L. KREIENBUEHL, E. CHARBONNEY AND PH. EGGIMANN. Community-acquired necrotizing pneumonia due to methicillin-sensitive *Staphylococcus aureus* secreting Panton-Valentine leukocidin: a review of case reports. *Annals of Intensive Care*, 1: 52 (2011).
26. O. DUMITRESCU, S. BOISSET, C. BADIOU, M. BES, Y. BENITO, M.-E. REVERDY, F. VANDENESCH, J. ETIENNE AND G. LINA. Effect of Antibiotics on *Staphylococcus aureus* Producing Panton-Valentine Leukocidin. *Antimicrob. Agents Chemother*., 51(4): 1515, 1519 (2007).
27. P.N. PANTON AND F.C.O. VALENTINE. Staphylococcal toxin. *Lancet*, i, 506, 508 (1932).
28. J.A. LINDSAY AND M.T. HOLDEN. *Staphylococcus aureus*: superbug, super genome? *Trends Microbiol*., 12: 378, 385 (2004).
29. K.N. SHANDS, G.P. SCHMID, B.B. DAN, D. BLUM, R.J. GUIDOTTI, N.T. HARGRETT, R.L. ANDERSON, D.L. HILL, C.V. BROOME, J.D. BAND AND D.W. FRASER. Toxic-Shock Syndrome in Menstruating Women — Association with Tampon Use and *Staphylococcus aureus* and Clinical Features in 52 Cases. *N. Engl. J. Med*., 303: 1436, 1442 (1980).
30. M.S. BERGDOLL, B.A. CRASS, R.F. REISER, R.N. ROBBINS, J.P. DAVIS. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome *Staphylococcus aureus* isolates. *Lancet*, 1(8228): 1017, 1021 (1981).
31. M.S. BERGDOLL, F.S. CHU, C.R. BORJA, I-Y. HUANG AND K.F. WEISS. The Staphylococcal Enterotoxins. *Japan. J. Microbiol*., 11 (4): 358, 368 (1967).
32. J. KADARIYA, T.C. SMITH AND D. THAPALIYA. *Staphylococcus aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. *BioMed Research International*, 2014: 1, 9 (2014).
33. M.J. BETLEY AND J.J. MEKALANOS. Staphylococcal enterotoxin A is encoded by a phage. *Science*. 229: 185, 187 (1985).
34. W.M. SHAFER AND J.J. IANDOLO. Chromosomal locus for staphylococcal enterotoxin B. *Infect. Immun*., 20: 273, 278 (1978).
35. Z. SHALITA, I. HERTMAN, S. SAND. Isolation and characterisation of a plasmid involved with enterotoxin B production in *Staphylococcus aureus*. *J. Bacteriol*., 129: 317, 325 (1977).
36. J.R. FITZGERALD, S.R. MONDAY, T.J. FOSTER, G.A. BOHACH, P.J. HARTIGAN, W.J. MEANEY,

C.J. SMITH. Characterization of putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. *J. Bacteriol*., 183: 63, 70 (2001).
37. K.W. BAYLES AND J.J. IANDOLO. Genetic and molecular analysis of the gene encoding staphylococcal enterotoxin D. *J. Bacteriol*., 171: 4799, 4806 (1989).
38. J.L. COUCH, M.T. SOLTIS, M.J. BETLEY. Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. *J. Bacteriol*., 170: 2954, 2960 (1988).
39. M. BUKOWSKI, B. WLADYKA AND G. DUBIN. Exfoliative Toxins of *Staphylococcus aureus*, *Toxins*, 2: 1148, 1165 (2010).
40. R.R. McDONALD, N.A. ANTONISHYN, T. HANSEN, L.A. SNOOK, E. NAGLE, M.R. MULVEY, P.N. LEVETT AND G.B. HORSMAN. Development of a Triplex Real-Time PCR Assay for Detection of Panton-Valentine Leukocidin Toxin Genes in Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*. *J. Clin. Microbiol*., 43(12): 6147, 6149 (2005).
41. "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0, 2014. http://www.eucast.org."
42. "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015. http://www.eucast.org."
43. E.C. DRĂGULESCU, M. OPREA, M. STRĂUȚ, I. CODIȚĂ. Detectarea rapidă a tulpinilor de *Staphylococcus aureus* comunitar meticilino-rezistent folosind tehnica PCR multiplex, A XI-a Reuniune Anuală de Microbiologie, 24-26 mai 2007, Mamaia, Romania – poster.
44. F. MARTINEAU, F.J. PICARD, N. LANSAC, C. MENARD, P.H. ROY, M. OUELLETTE AND M.G. BERGERON. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob. Agents Chemother*., 44: 231, 238 (2000).
45. B. STROMMENGER, CH. KETTLITZ, G. WERNER, W. WITTE. Multiplex PCR Assay for Simultaneous Detection of Nine Clinically Relevant Antibiotic Resistance Genes in *Staphylococcus aureus*. *J. Clin. Microbiol*., 41: 4089, 4094 (2003).
46. O.G. BRAKSTAD, K. AASBAKK AND J.A. MAELAND. Detection of *Staphylococcus aureus* by Polymerase Chain Reaction Amplification of the *nuc* Gene. *J. Clin. Microbiol*., 30: 1654, 1660 (1992).
47. M. MEHROTRA, G. WANG AND W.M. JOHNSON. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. *J. Clin. Microbiol*., 38(3): 1032, 1035 (2000).
48. W. JOHNSON, M.S. TYLER, S.D. EWAN, E.P. ASHTON, F.E. POLLAND AND K.R. ROZEE. Detection of genes for enterotoxins, exofoliative toxins and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by polymerase chain reaction. *J. Clin. Microbiol*., 29: 426, 430 (1991).
49. C. MILHEIRICO, D.C. OLIVEIRA AND H. DE LENCASTRE. Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrob. Agents Chemother*., 51: 3374, 3377 (2007).
50. D. HARMSEN, H. CLAUS, W. WITTE, J. ROTHGANGER, H. CLAUS, D. TURNWALD and U. VOGEL. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting using a novel software for spa-repeat determination and database management. *J. Clin. Microbiol*., 41: 5442, 5448 (2003).
51. http://www.spaserver.ridom.de/
52. M.C. LEE, A.M. RIOS, M.F. ATEN, A. MEJIAS, D. CAVUOTI, G.H. MCCRACKEN JR. AND R.D. HARDY. Management and outcome of children with skin and soft tissue abscesses caused by community-acquired methicillin-resistant *Staphylococcus aureus*. *Pediatr. Infect. Dis. J*., 23: 123, 127 (2004).
53. R. KOCK, K. BECKER, B. COOKSON, J.E. VAN GEMERT-PIJNEN, S. HARBARTH, J. KLUYTMANS, M. MIELKE, G. PETERS, R.L. SKOV, M.J. STRUELENS, E. TACCONELLI, T.A. NAVARRO, W. WITTE, A.W. FRIEDRICH. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Euro Surveill*., 15(41): 19688 (2010).
54. H.S. SADER, T.R. FRITSCHE, R.N. JONES. Daptomycin bactericidal activity and correlation between disk and broth microdilution method results in testing of *Staphylococcus aureus* strains with decreased susceptibility to vancomycin, *Antimicrob. Agents Chemother.*, 50(7): 2330, 2336 (2006).
55. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). ECDC website. Available at http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf.
56. H. HUMPHREYS. National guidelines for the control and prevention of methicillin-resistant *Staphylococcus aureus* — what do they tell us? *Clin. Microbiol. Infect*., 13(9): 846, 853 (2007).
57. G. IPPOLITO, S. LEONE, F.N. LAURIA, E. NICASTRI, R.P. WENZEL. Methicillin-resistant *Staphylococcus aureus*: the superbug. *Int. J. Infect. Dis*., 14(Suppl 4): S7, S11 (2010).

58. E. ALP, C.H. KLAASSEN, M. DOGANAY, U. ALTOPARLAK, K. AYDIN, A. ENGIN, C. KUZUCU, C. OZAKIN, M.A. OZINEL, O. TURHAN, A. VOSS. MRSA genotypes in Turkey: persistence over 10 years of a single clone of ST239. *J. Infect*., 58(6): 433 (2009).
59. M. OPREA, E.C. DRĂGULESCU, I.L. COLDEA, I. CODIȚĂ, C. SZMAL, M. STRĂUȚ. Molecular characterization of *Staphylococcus aureus* isolates belonging of to most prevalent *spa*-types recovered from Romanian hospitals during 2006-2007. 18th European Conference of Clinical Microbiology and Infectious Diseases, 19-22 April, 2008, Barcelona, Spain, P1436.
60. R. IONESCU, J.R. MEDIAVILLA, L. CHEN, D.O. GRIGORESCU, M. IDOMIR, B.N. KREISWIRTH AND R.B. ROBERTS. Molecular Characterization and Antibiotic Susceptibility of *Staphylococcus aureus* from a Multidisciplinary Hospital in Romania. *MICROBIAL DRUG RESISTANCE*. 16(4): 263, 272 (2010).
61. E. SZEKELY, A. MAN, A. MARE, K.E. VAS, S. MOLNAR, D. BILCA, J. SZEDERJESI, F. TOMA, L. LORINCZI. Molecular epidemiology and virulence factors of methicillin-resistant *Staphylococcus aureus* strains in a Romanian university hospital. *Revista Română de Medicină de Laborator*, 20(4/4): 371, 382 (2012).
62. Y. CHEN, Z. LIU, L. DUO, J. XIONG, Y. GONG, J. YANG, Z. WANG, X. WU, Z. LU, X. MENG, J. ZHAO, C. ZHANG, F. WANG, Y. ZHANG, M. ZHANG, L. HAN. Characterization of *Staphylococcus aureus* from Distinct Geographic Locations in China: An Increasing Prevalence of *spa*-t030 and SCC*mec* Type III. *PLoS ONE*, 9(4): e96255 (2014). doi:10.1371/journal.pone.0096255.
63. H. GRUNDMANN, L.M. SCHOULS, D.M. AANENSEN, G.N. PLUISTER, A. TAMI, M. CHLEBOWICZ, C. GLASNER, A.J. SABAT, K. WEIST, O. HEUER, A.W. FRIEDRICH, on behalf of the ESCMID Study Group on Molecular Epidemiological Markers and the European Staphylococcal Reference Laboratory Working Group. The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: Results of a second structured survey. *Euro Surveill*., 19(49): 20987 (2014).
64. M. CÎRLAN, M. SAAD, G. COMAN, N.E. BILAL, A.M. ELBASHIER, D. KREFT, S. SNIJDERS, W. VAN LEEUWEN, A. VAN BELKUM. International spread of major clones of methicillin resistant *staphylococcus aureus*: nosocomial endemicity of multi locus sequence type 239 in Saudi Arabia and Romania. *Infection, Genetics and Evolution*, 5: 335, 339 (2005).
65. I. CODIȚĂ, E.C. DRĂGULESCU, S. DINU, B.E. LIXANDRU, R.I. ȘERBAN. t127 *spa* type MDR MRSA strains isolated in 2010/2011 from pediatric and new born units in Bucharest, 7th Conference of the Romanian Association of Medical Laboratories with international participation, 10-12th May 2012, Brașov, România, Romanian Review of Laboratory Medicine, 21(2/4), May 2011 (abstract) – oral presentation.
66. A. FRANCO, H. HASMAN, M. IURESCIA, R. LORENZETTI, M. STEGGER, A. PANTOSTI, F. FELTRIN, A. IANZANO, M.C. PORRERO, M. LIAPI and A. BATTISTI. Molecular characterization of *spa* type t127, sequence type 1 methicillin-resistant *Staphylococcus aureus* from pigs. *J. Antimicrob. Chemother*., 66: 1231, 1235 (2011).
67. F. ALAKLOBI, F. ALJOBAIR, A. ALRASHOD, R. ALHABABI, M. ALSHAMRANI, W. ALAMIN, L. LYTVYN, F. ALROUKI, D. MERTZ. The prevalence of community-associated methicillin-resistant *Staphylococcus aureus* among outpatient children in a tertiary hospital: A prospective observational study in Riyadh, Saudi Arabia, *International Journal of Pediatrics and Adolescent Medicine*, 2: 136e140 (2015).
68. K.C. CARROLL. Rapid diagnostics for methicillin-resistant *Staphylococcus aureus*: current status. *Mol. Diagn. Ther*., 12(1): 15, 24 (2008).
69. E.S. PAN, B.A. DIEP, E.D. CHARLEBOIS, C. AUERSWALD, H.A. CARLETON, G.F. SENSABAUGH, F. PERDREAU-REMINGTON. Population dynamics of nasal strains of methicillin-resistant *Staphylococcus aureus* – and their relation to community-associated disease activity, *J. Infect. Dis*., 192: 811, 818 (2005).
70. D. WU, Q. WANG, Y. YANG, W. GENG, S. YU, K. YAO, L. YUAN, X. SHEN. Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* from skin/soft tissue infections in a children's hospital in Beijing, China. *Diagn. Microbiol. Infect. Dis*., 67(1): 1, 8 (2010) doi: 10.1016/j.diagmicrobio.2009.12.006 PMID: 20227225.
71. T. LI, X. YU, J. XIE, Y. XU, Y. SHANG, Y. LIU et al. Carriage of virulence factors and molecular characteristics of *Staphylococcus aureus* isolates associated with bloodstream, and skin and soft tissue infections in children. *Epidemiol. Infect*., 1, 5 (2012).
72. C. ZHAO, Y. LIU, M. ZHAO, Y. LIU, Y. YU, H. CHEN, Q. SUN, H. CHEN, W. JIANG, Y. LIU, S. HAN, Y. XU, M. CHEN, B. CAO, H. WANG. Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. *PLoS One*. 2012; 7(6): e38577 (2012). doi:10.1371/journal.pone.0038577 PMID: 22701673.

73. T. MAEDA, T. SAGA, T. MIYAZAKI, Y. KOUYAMA, S. HARADA, M. IWATA, S. YOSHIZAWA, S. KIMURA, Y. ISHII, Y. URITA, M. SUGIMOTO, K. YAMAGUCHI, K. TATEDA. Genotyping of skin and soft tissue infection (SSTI)-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains among outpatients in a teaching hospital in Japan: application of a phage-open reading frame typing (POT) kit. *J. Infect. Chemother*., 18(6): 906, 914 (2012). doi: 10.1007/s10156-012-0506-4 PMID: 23150115.
74. A. KALTSAS, A. GUH, J.R. MEDIAVILLA, A.K. VARSHNEY, N. ROBIOU, P. GIALANELLIA, M. HENRY, M.H. LEVI, B.C. FRIES. Frequency of panton-valentine leukocidin-producing methicillin-sensitive *Staphylococcus* strains in patients with complicated skin and soft tissue infection in bronx, New York. *J. Clin. Microbiol*., 49(8): 2992, 2995 (2011). doi: 10.1128/JCM.00704-11 PMID: 21653777.
75. J. ROLO, M. MIRAGAIA, A. TURLEJ-ROGACKA, J. EMPEL, O. BOUCHAMI, N.A. FARIA, A. TAVARES, W. HRYNIEWICZ, A.C. FLUIT, HERMINIA DE LENCASTRE and THE CONCORD WORKING GROUP. High Genetic Diversity among Community-Associated *Staphylococcus aureus* in Europe: Results from a Multicenter Study. *PLoS ONE* 7(4): e34768 (2012) doi:10.1371/journal.pone.0034768.
76. B.A. DIEP, H.A. CARLETON, R.F. CHANG, G.F. SENSABAUGH, F. PERDREAU-REMINGTON. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J. Infect. Dis*., 193(11): 1495, 1503 (2006).
77. M. STEGGER, T. WIRTH, P.S. ANDERSEN, R.L. SKOV, A. DE GRASSI, P.M. SIMOES, A. TRISTAN, A. PETERSEN, M. AZIZ, K. KIIL, I. CIRKOVIC, E.E. UDO, R. DEL CAMPO, J. VUOPIO-VARKILA, N. AHMAD, S. TOKAJIAN, G. PETERS, F. SCHAUMBURG, B. OLSSON-LILJEQUIST, M. GIVSKOV, E.E. DRIEBE, H.E. VIGH, A. SHITTU, N. RAMDANI-BOUGESSA, J. RASIGADE, L.B. PRICE, F. VANDENESCH, A.R. LARSEN, F. LAURENT. Origin and evolution of European community-acquired methicillin-resistant *Staphylococcus aureus*. *mBio*, 5(5): e01044-14 (2014). doi:10.1128/mBio.01044-14.
78. L.Y. HSU, A. TRISTAN, T.H. KOH, M. BES, J. ETIENNE, A. KURUP, T. THUAN-TONG, T. BAN-HOCK. Community associated methicillin-resistant *Staphylococcus aureus*, Singapore. *Emerg. Infect. Dis*., 11: 341, 342 (2005).
79. B.A. DIEP, G.G. STONE, LI BASUINO, CH.J. GRABER, A. MILLER, SHELLEY-ANN DES ETAGES, A. JONES, A.M. PALAZZOLO-BALLANCE, F. PERDREAU-REMINGTON, G.F. SENSABAUGH, F.R. DELEO AND H.F. CHAMBERS. The Arginine Catabolic Mobile Element and Staphylococcal Chromosomal Cassette *mec* Linkage: Convergence of Virulence and Resistance in the USA300 Clone of Methicillin-Resistant *Staphylococcus aureus*. *The Journal of Infectious Diseases*, 197: 1523, 1530 (2008).
80. N.A. FARIA, D.C. OLIVEIRA, H. WESTH, D.L. MONNET, A.R. LARSEN, R. SKOV, H. De LENCASTRE. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. *J. Clin. Microbiol*., 43: 1836, 1842 (2005).
81. P.L. HO, C. CHEUNG, G.C. MAK, C.W. TSE, T.K. NG, C.H. CHEUNG, T.L. QUE, R. LAM, R.W. LAI, R.W. YUNG, K.Y. YUEN. Molecular epidemiology and household transmission of community-associated methicillin-resistant *Staphylococcus aureus* in Hong Kong. *Diagn. Microbiol. Infect. Dis*., (2006).
82. S. VLACK, L. COX, A.Y. PELEG, C. CANUTO, C. STEWART, A. CONLON, A. STEPHENS, PH. GIFFARD, F. HUYGENS, A. MOLLINGER, R. VOHRA and J.S MCCARTHY. Carriage of methicillin-resistant *Staphylococcus aureus* in a Queensland indigenous community. *Med. J. Aust*., 184: 556, 559 (2006).
83. A. HOLMES, M. GANNER, S. MCGUANE, T.L. PITT, B.D. COOKSON AND A.M. KEARNS. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *J. Clin. Microbiol*., 43: 2384, 2390 (2005).
84. P. DEL GIUDICE, V. BLANC, F. DURUPT, M. BES, J.P. MARTINEZ, E. COUNILLON, G. LINA, F. VANDENESCH, J. ETIENNE. Emergence of two populations of methicillin-resistant *Staphylococcus aureus* with distinct epidemiological, clinical and biological features, isolated from patients with community-acquired skin infections. *Br. J. Dermatol*., 154: 118, 124 (2006).
85. E. NĂSTASE, O. DORNEANU, T. VREMERA, C. LOGIGAN, E. MIFTODE, C.M. DOROBĂȚ. *Mec*A and *pvl* genes detection in *Staphylococcus aureus* strains isolated from lower respiratory tract infections. *Rev. Med. Chir. Soc. Med. Nat. Iasi*, 114: 1162, 1168 (2010).
86. I. CODIȚĂ, M. OPREA, C. SZMAL, E.C. DRĂGULESCU, M. TRAŞCĂ, D. BREHAR CIOFLEC, G. CHICIN, R. ŞERBAN, A. PISTOL. Panton-Valentine leukocidin positive, t008 *spa*-type meticillin-resistant *Staphylococcus aureus* in a Romanian western county hospital, 18th European Congress of Clinical Microbiology and Infections

Diseases, 19-22 april 2008, Barcelona, Spain – publication only.
87. S. MONECKE, E. MULLER, O.S. DORNEANU, T. VREMERA, R. EHRICHT. Molecular Typing of MRSA and of Clinical *Staphylococcus aureus* Isolates from Iasi, Romania. *PLoS ONE*, 9(5): e97833 (2014).
88. T. VREMERA, L.S. IANCU, E.V. NĂSTASE, C. LOGIGAN, E.G. MIFTODE, A.I. MEREUȚĂ, C. LUNCĂ, O.S. DORNEANU. TRIPLEX REAL-TIME PCR FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND PANTON-VALENTINE LEUKOCIDIN IN NORTH-EAST ROMANIA. *Rev. Med. Chir. Soc. Med. Nat*., 116(4): 1171, 1176 (2012).
89. F.J. SCHMITZ, A.C. FLUIT, M. GONDOLF, R. BEYRAU, E. LINDENLAUF, J. VERHOEF, H.P. HEINZ AND M.E. JONES. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. *J. Antimicrob. Chemother*., 43: 253, 259 (1999).
90. F.J. SCHMITZ, A. KREY, R. SADURSKI, J. VERHOEF, D. MILATOVIC AND A.C. FLUIT. Resistance to tetracycline and distribution of tetra-cycline resistance genes in European *Staphylococcus aureus* isolates. *J. Antimicrob. Chemother*., 47: 239, 240 (2001).
91. F. VANDENESCH. European CA-MRSA: a singular situation?, 18th European Congress of Clinical Microbiology and Infectious Diseases Barcelona, Spain, 19-22 April 2008 – Abstract number: S468.
92. N. SICOT, N. KHANAFER, V. MEYSSONNIER, O. DUMITRESCU, A. TRISTAN, M. BES, G. LINA, F. VANDENESCH, P. VANHEMS, J. ETIENNE, Y. GILLET. Methicillin resistance is not a predictor of severity in community-acquired *Staphylococcus aureus* necrotizing pneumonia-results of a prospective observational study. *Clin. Microbiol. Infect*., 19(3): E142, E148 (2013).
93. E. SZEKELY, L.S. ENACHE, S. MARINESCU, E. UNGVARI, A. TOTH, J. PASZTI. Fatal sepsis due to community-associated methicillin resistant *Staphylococcus aureus* – a case report. *Rev. Română Med. Lab.*, 18(2/4): 29, 33 (2010).
94. V. ALEXANDRESCU, I. CODIȚĂ, A. BĂEȚEL, E.C. DRĂGULESCU, I.L. COLDEA. Caz letal de gripă sezonieră suprainfectată cu *S. aureus* rezistent la meticilină producător de leucocidină Panton-Valentine – România 2009, Simpozionul tematic „Ziua Europeană a Informării despre Antibiotice", 18 noiembrie 2009, I.N.C.D.M.I. Cantacuzino, Bucureşti, România, 14 – oral presentation.
95. G. COMAN, M. CÎRLAN, E. PETRARU, C. DAHOREA, F. BUTNARU. Antibiotic resistance phenotypes of *S. aureus* from pediatric infections. *Rev. Medico-Chirurgicala*, 106: 46, 52 (2002).
96. L. GRIGORE, V. DUMITRESCU, S. SFARTZ, I. CODIȚĂ. The antibiotic resistance of *Staphylococcus aureus* strains isolated in units with an elevated nosocomial risk and in outpatient facilities in 1995. *Bacteriol. Virusol. Parazitol. Epidemiol*., 42: 51, 54 (1997).
97. O. DORNEANU, E. MIFTODE, T. VREMERA, E. NĂSTASE, O. FILIP, V. LUCA. Prevalence and characteristics of *Staphylococcus aureus* isolated from infections in Northeast Romania. *Journal of Preventive Medicine*, 14: 66, 70 (2006).
98. E. SZEKELY, L. LORINCZI, D. BILCA, E. FODOR, J. SOKI, M. SABĂU. Incidence, antibiotic resistance and clonal relations of MRSA strains isolated from a Romanian university hospital. *Acta Microbiol. Immunol. Hung*., 55: 1, 13 (2008).
99. O.M. DOROBĂȚ, I. BĂDICUȚ, D. TĂLĂPAN, C. TENEA, A. RAFILA. Antibiotic resistance of Gram-positive cocci isolated in 2008. *Bacteriol. Virusol. Parazitol. Epidemiol*., 55: 83, 92 (2010).
100. M. NICA, T. BIOLAN, A. DASCĂLU, E. MOZES, A. TODERAN ET AL. Bacterial strains isolated from systemic infections and reported for evaluation and antibiotic resistance surveillance by the ''Dr. Victor Babes'' Clinical Hospital for Infectious and Tropical Diseases, Bucharest. *Bacteriol. Virusol. Parazitol. Epidemiol*., 55: 161, 168 (2010).
101. B. IONESCU, D. IONESCU, I. GHEORGHE, C. CURUȚIU, O. BANU, C. BLEOTU, G. MIHĂESCU, V. LAZĂR, R. GRIGORE, E. BEZIRTZOGLOU, Ș. BERTEȘTEANU. Virulence patterns of *Staphylococcus aureus* hospital strains isolated in Bucharest, Romania. *Rom Biotechnol Lett*, 20(3): 10536, 10546 (2015).
102. N. MEREZEANU, I. GHEORGHE, M. POPA, V. LAZĂR, O. BANU, A. BOLOCAN, R. GRIGORE, Ș.V. BERTEȘTEANU, O. PÂNTEA. Phenotypic and Genotypic Investigation of Resistance and Virulence Features of Methicillin Resistant *Staphylococcus aureus* strains isolated from Hospitalized Patients. *Rom Biotechnol Lett*, 21(3): 11591-98 (2016).
103. A.I. COTAR, S. DINU, M.C. CHIFIRIUC, O. BANU, C. IORDACHE, M. LIXANDRU, O. DRACEA, M. BUCUR, V. LAZAR. Molecular markers of quorum-sensing and virulence gene regulators in *Staphylococcus aureus* strains isolated from biofilm associated infections *Rom Biotechnol Lett*, 13(3): 1-7 (2008).
104. V.C. CRISTEA, I. GHEORGHE, G. NEACȘU, R. GÂLCĂ, I. CZOBOR, I. ALEXANDRU, M.C. CHIFIRIUC, V. LAZĂR, M.I. POPA. *Staphylococcus sp.* Involvement in Community Acquired Urinary Tract Infections. *Rom Biotechnol Lett*, 21(2): 11413-17 (2016).