
CHARACTERIZATION OF ROMANIAN VACCINAL *STAPHYLOCOCCUS AUREUS* STRAINS

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ABSTRACT

Introduction: Corpuscular staphylococcal vaccine was manufactured at Cantacuzino Institute, since the 1960s, with several changes in bacterial strain composition.

Objectives: The aim of this study is the phenotypic and molecular characterization of eleven *S. aureus* strains that were included in the last formulation of the Romanian corpuscular staphylococcal vaccine.

Methods: *S. aureus* isolates were phenotypically characterized (antimicrobial susceptibility testing and bacteriophage typing) and the results were correlated with those obtained using conventional PCR-based methods for *nuc*, *mecA*, *lukS/E*, *sea*, *seb*, *sed*, *seh*, *sei*, *sej*, *sem*, *tst*, *etd* genes and *agr* locus identification with specific primers. Clonal relationships of isolates were established by pulsed field gel electrophoresis (PFGE), *spa*-typing and multilocus sequence typing (MLST).

Results: Only one methicillin resistant strain, *mecA* gene positive, was identified. MRSA isolate harboured a non-typable SCC_{mec} structure with typing assay used. Different combinations of virulence genes and all the four *agr* types were represented among these strains. Six isolates were non-typable by bacteriophage typing. The studied strains presented eleven distinct *Sma*I macrorestriction patterns and nine MLST sequence types.

Conclusion: The results of this study may contribute to the Romanian staphylococcal vaccine regulations development by improving the degree of characterization, and to a better understanding of the historical data related to its benefits.

Keywords: *Staphylococcus aureus*, phenotypical and molecular characterization, virulence genes, typing.

REZUMAT

Introducere: Vaccinul stafilococic corpuscular a fost produs în Institutul Cantacuzino, începând cu anii 1960, cu câteva schimbări în compoziția bacteriană.

Obiective: Scopul acestui studiu a fost caracterizarea fenotipică și moleculară a unsprezece tulpini de *S. aureus* care au fost incluse în ultima formulă a vaccinului corpuscular stafilococic românesc.

Metode: Izolatele de *S. aureus* au fost caracterizate fenotipic (testarea sensibilității la antibiotice și tipizare bacteriofagică) și rezultatele au fost corelate cu cele obținute prin metode bazate pe PCR pentru identificarea genelor *nuc*, *mecA*, *lukS/E*, *sea*, *seb*, *sed*, *seh*, *sei*, *sej*, *sem*, *tst*, *etd* și locusului *agr*, utilizând primeri specifici. Relațiile clonale dintre izolate au fost stabilite prin electroforeza în câmp pulsator (PFGE), tipizare *spa* și tipizare multilocus (MLST).

Rezultate: A fost identificată o singură tulpină rezistentă la metilicină, pozitivă pentru gena *mecA*. Izolatul rezistent la metilicină a prezentat o structură SCC_{mec} netipabilă prin metoda utilizată. La aceste tulpini au fost identificate diferite combinații de gene de virulență și toate cele patru tipuri *agr*. Șase izolate nu au fost tipabile prin tipizarea bacteriofagică. Tulpinile izolate au prezentat opt profiluri distincte de macrorestricție cu *Sma*I și nouă tipuri MLST.

Concluzie: Rezultatele acestui studiu pot contribui la fundamentarea unor reglementări, prin îmbunătățirea gradului de caracterizare a vaccinului stafilococic românesc, precum și a unei mai bune înțelegeri a datelor istorice legate de beneficiile acestuia.

Cuvinte-cheie: *Staphylococcus aureus*, caracterizare fenotipică și moleculară, gene de virulență, tipizare.

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium, notable for the frequency and severity of infections that it causes in both

hospitals and communities. These infections range from localized skin infections to bacteraemia and septic shock, due to a wide variety of virulence factors. Some virulence

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factors received more attention, as they seem to be involved in more challenging infections [1, 2].

Panton-Valentine leukocidin (PVL) is predominantly associated with severe skin infections and necrotizing pneumonia. PVL and Staphylococcal Chromosomal Cassette *mec* (SCC*mec*) type IV are suggested to be markers for community-acquired methicillin resistant *S. aureus*, although there are also exceptions [3].

S. aureus strains produce a wide variety of staphylococcal enterotoxins, exoproteins structurally related, which together with toxic shock syndrome toxin-1 (TSST-1), belong to the pyrogenic toxin staphylococcal superantigens (PTSAgs) family [4]. These superantigens (SAGs) can cause food poisoning and shock symptoms in humans. A repertoire including more than 20 types of staphylococcal enterotoxins have been described and it is common for *S. aureus* strains to produce one or more types of these exotoxins. Staphylococcal enterotoxin B (SEB), the best characterized among the staphylococcal enterotoxins, was considered to be an incapacitating agent during the 1960's and categorized as a category B agent because it is one of the most potent bacterial superantigens. It is often associated with important toxic effects upon the immune system, leading to stimulation of cytokine release and inflammation [5]. SEB is responsible for food poisoning in humans, its toxic capacity being expressed after ingestion of contaminated water and foods or inhalation of aerosols. SEB causes fever, severe respiratory distress, headache, and sometimes nausea and vomiting [6].

In *S. aureus*, the accessory gene regulator (*agr*) globally controls the coordinated production of virulence factors. *S. aureus* strains were divided into four major *agr* types. Links between an *agr* type and a specific staphylococcal disease have been documented [7].

The emergence and disease-causing capacity of staphylococci are strongly related to the widespread use of antibiotics combined with the enormous potential of this bacterium to develop multidrug resistance [1]. Methicillin resistant *S. aureus* (MRSA) infections are observed primarily in hospital settings but the rate of community resistant isolates is continuously increasing. Romania is in “the

red zone” regarding MRSA in Europe, with a 57.2% resistance rate, compared to an EU/EEA average of just 16.8% in 2015 [8].

Methicillin resistance in staphylococci is caused, mostly, by expression of an additional modified penicillin binding protein named PBP2a (PBP2'), encoded by the *mecA* gene. The PCR targeting the *mecA* gene represents the method of choice for MRSA detection. *mecA* is located on a mobile genetic element called the Staphylococcal Chromosomal Cassette (SCC). SCC*mec* is characterized by the presence of terminal inverted and direct repeats, two essential genetic components (the *mec* genes complex and the *ccr* genes complex) and the junkyard regions (J regions). In staphylococci, different SCC*mec* elements have been classified according to the combination of the two components: the *ccr* complex (8 types) and the *mec* complex (11 classes) (http://www.sccmec.org/Pages/SCC_TypesEN.html), resulting eleven SCC*mec* types, but five of them are more frequent. A relatively newly discovered gene, *mecC*, has proved also to confer resistance to beta-lactamic antibiotics. It has a 70% homology with *mecA* gene and was detected in strains recovered from humans and animals [9, 10].

Resistance to methicillin is often accompanied by resistance to other antibiotics: aminoglycosides, quinolones, macrolides, etc. Also, *S. aureus* strains with intermediate susceptibility or high-level resistance to vancomycin have been reported. Infections caused by multidrug-resistant *S. aureus* limit therapeutic options, and they may be associated with important mortality and higher hospitalization costs [11, 12].

Bacterial typing is an important prerequisite for targeted infection control measures. Numerous techniques are available to differentiate *S. aureus* isolates. Historically, isolates were distinguished by phenotypic methods, including antibiotic susceptibility testing and bacteriophage typing. Both methods have limitations, as genetically unrelated isolates commonly have the same susceptibility pattern, and many *S. aureus* isolates are non-typable by bacteriophage typing.

With the advent of molecular biology, strain typing focused on DNA-based methods [13]. DNA bands-based methods (e.g. PCR-

based approaches and pulsed-field gel electrophoresis – PFGE), require subjective interpretation and comparison of patterns and fingerprint images and, despite the innovative software programs, are difficult to standardize. PFGE method for *S. aureus* is based on the digestion of bacterial DNA by rare-cutting *SmaI* restriction endonuclease that recognise a specific site, generating large fragments of DNA that can not be separated effectively by conventional electrophoresis [2].

DNA sequence-based typing has become more popular due to the progress in large-scale sequencing Sanger methodology. DNA sequence analysis is an objective genotyping method. Two different strategies have been used to provide genotyping data: multilocus sequence typing (MLST) and single-locus sequence typing (SLST). MLST is a good discriminatory method of characterizing bacterial isolates on the basis of the sequences of internal fragments of seven house-keeping genes. For each gene fragment, the different sequences are assigned as distinct alleles, and each isolate is defined by combination of the seven alleles (the allelic profile or sequence type - ST). As there are many alleles at each of seven loci, isolates are highly unlikely to have identical allelic profiles by chance and isolates with the same allelic profile can be assigned as members of the same clone. Sequence data are readily compared between laboratories and a major advantage of MLST is the ability to compare the results obtained in different studies via the Internet [14]. The MLST approach is labour-intensive, time-consuming, and costly for using in clinical setting. However, a single-locus target, if discriminating, provides an inexpensive, rapid, objective, and portable genotyping method.

Among SLST approaches, *spa*-typing is the most commonly used for *S. aureus* strains. The X region of the protein A encoding gene (*spa*) consists of direct repeats exhibiting an extensive polymorphism based on point mutations, deletions, duplications, and insertions. To the different repeats can be assigned an alpha-numeric code, and the order of specific repeats defines the *spa*-type. Ridom StaphType (Ridom GmbH, Würzburg, Germany) provides a software tool enabling straightforward

sequence analysis and designation of *spa*-types via synchronization to a central server [15, 16].

With the development of benchtop high throughput sequencing platforms and advances in microbial bioinformatics, performance of whole-genome sequencing (WGS) - based typing has been attracting increased interest in clinical microbiology laboratory. The data rendered from these experiments allow the comparison to global typing schemes, high-resolution typing, and resistance and virulence profiling [17, 18].

Adequate and precise characterization and typing of *S. aureus* isolates, which allows monitoring of local outbreaks and wider-scale discrimination of clones, is of public health great concern.

The aim of this study is the phenotypic and molecular characterization of eleven *S. aureus* strains that were included in the last formulation of the Romanian corpuscular staphylococcal vaccine. This vaccine has been manufactured at Cantacuzino Institute since 1960s, with several changes in bacterial strain composition. These findings will help the decision-making authorities to establish the representativeness of vaccinal strains among the current *S. aureus* circulating strains in Romania. Also, the molecular characterization of these strains will be beneficial, since more recommendations and requirements of modern vaccine monographs imply molecular biology methods for seed identity tests.

MATERIAL AND METHODS

Bacterial strains

The strains of *S. aureus* (designated as VS1, VS2, VS3, VS4, VS5, VS7, VS8, VS9, VS10, VS11, VS14), were isolated in 1994 from different sites of infection (skin lesions, soft tissues infections and blood) and were epidemiologically unrelated.

The following reference strains were used: *S. aureus* ATCC 25923 for antimicrobial susceptibility testing with disk diffusion method, thermonuclease (TNase) gene and Panton-Valentine Leukocidin (PVL) genes amplification, EMRSA-3 for *mecA* detection, *S. aureus* FRI S6 and *S. aureus* 920, *S. aureus* 75916, *S. aureus* 90/10685 for SAg genes detection, EMRSA-3, EMRSA-1, EMRSA-15

and EMRSA-16 for SCC_{mec} typing and *S. aureus* NCTC 8325 for PFGE typing. All strains were phenotypically identified as *S. aureus* by conventional bacteriology methods using Gram stained smear, catalase, coagulase, and TNase production tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for penicillin, cefoxitin, tetracycline, erythromycin, clindamycin, kanamycin, tobramycin, gentamicin, chloramphenicol, rifampin, ciprofloxacin, cotrimoxazole, vancomycin and linezolid was performed using discs impregnated with the antibiotics specified above (Oxoid Limited, Basingstoke, Hampshire, England) on Mueller-Hinton agar (Merck KgaA, Darmstadt, Germany), according to CLSI (Clinical and Laboratory Standards Institute) recommendations. [19].

Bacteriophage typing

The strains were bacteriophage typed according to the method of Blair and Williams, using the basic international set of typing bacteriophages, plus 6 additional bacteriophages. All bacteriophages were used at routine test dilution and at 100 X routine test dilution [20].

DNA extraction

Strains were grown in BHI broth at 35°C. Genomic DNA was extracted using a commercial extraction kit, NucleoSpin Tissue kit (Macherey-Nagel GmbH&Co.KG, Düren, Germany), according to the recommendations of the manufacturer.

Detection of methicillin resistance and virulence genes

Specific sequences for *nuc* (thermonuclease gene, *S. aureus* specific gene), *mecA* and *lukS/F*

(encoding for PVL) genes were amplified in a multiplex PCR reaction. The expected products were 279 bp for *nuc* gene [21], 433 bp for *lukS/F* gene [22] and 532 bp for *mecA* gene [23]. The multiplex reaction was performed in a final volume of 25 µl with 0.2 µM *nuc* and *mecA* primers, 0.4 µM *lukS/F* primers, 1.5 mM MgCl₂ and 1.5 U of Flexi Go Taq Polymerase (Promega, Madison, USA).

Genes encoding eight SAgS (*sea*, *seb*, *sed*, *seh*, *sei*, *sej*, *sem*, and *tst*) and exfoliatin D (*etd*) were determined in PCR reactions according to Holtfreter [24].

SCC_{mec} typing

The MRSA strain VS14 was subjected to SCC_{mec} typing in a multiplex PCR using six pairs of primers targeting the most frequent SCC_{mec} types (I to V) [25-27]. The primers are designed in order to amplify two specific targets for each SCC_{mec} type (Table 1). The primers concentrations were as follows: primers α2 and β, 0.28 µM each; *ccrA1* F and *ccrA1* R, 0.08 µM each; *ccrC* F and *ccrC* R, 0.17µM each, 1272 F, 1272R, 5*RmecA*, 5*R431*, *mecI* F and *mecI* R, 0.07 µM each. Amplification comprised 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C, with a final extension for 4 min at 72°C, in a final volume of 50 µl.

agr locus typing

agr types were identified by PCR amplification as previously described elsewhere [24].

PFGE typing

The isolates were subjected to PFGE, which was performed according to HARMONY protocol [28]. Shortly, the bacterial DNA, included in agarose plugs, was digested with *Sma*I and the macrorestriction fragments were separated using Chef Mapper system (Bio-Rad

Table 1 - Targets and corresponding amplicons to each SCC_{mec} type

Target	Size of amplicons	SCC _{mec} type				
		I	II	III	IV	V
<i>ccrA2B2</i> ^{a)}	937bp		x		x	
<i>ccrA1</i> ^{b)}	721bp	x		x		
<i>ccrC</i> ^{a)}	518bp					x
<i>IS1272</i> ^{a)}	415bp	x			x	
<i>mecA-IS431</i> ^{a)}	359bp					x
<i>mecI</i> ^{c)}	209bp		x	x		

a) [25] Boye *et al.* (2007); b) [26] Oliveira and de Lencastre (2002); c) [27] Hanssen *et al.* (2004)

Laboratories, Hercules, CA). The recommended reference standard, *S. aureus* NCTC 8325, was positioned in every fifth or sixth lane to allow later normalization of electrophoretic patterns across the gel. The profiles were analyzed using DNA Fingerprinting II software (Bio-Rad Laboratories, Hercules, CA).

spa typing

spa typing was performed using the primers described by Harmsen [15]. PCR amplification was performed in a 50 µl reaction mixture containing 0.2 µM of each primer. The thermal cycling reactions consisted of an initial denaturation (10 min at 80°C) followed by 35 cycles of denaturation (45 s at 94°C), annealing (45 s at 60°C), and extension (90 s at 72°C), with a single final extension (10 min at 72°C). The amplified products were purified and subsequently sequenced. All sequencing reactions were carried out using BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). The unincorporated labelled ddNTPs were removed with DyeEX 2.0 Spin kit (Qiagen, Hilden, Germany), in accordance with manufacturer's instructions. The sequences were analyzed with Ridom StaphType software and a *spa*-type was assigned for every *S. aureus* isolate [15].

MLST was performed and interpreted according to MLST website (<https://pubmlst.org>).

All the PCR and sequencing reactions in this study were conducted on a AmpGene PCR System 2001 (Applied Biosystems, Warrington, UK) and all the PCR products were analyzed by electrophoresis through 1.5% agarose gels (Bio-Rad Laboratories, Hercules, CA). The molecular weight marker 100bp DNA StepLadder (Promega, Madison, USA) was used. All the sequences were obtained by using Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Warrington, UK).

RESULTS AND DISCUSSION

Antimicrobial susceptibility pattern revealed ten susceptible strains and only one MRSA strain, namely *S. aureus* VS14. This isolate is multiresistant, being resistant also to tetracycline, aminoglycosides, chloramphenicol, rifampin, trimethoprim-sulfamethoxazole, macrolides and with

inducible resistance to lincosamides and streptogramins B (MLS_{Bi} phenotype).

All the strains, except *S. aureus* VS3 isolate, were penicillin resistant. Seven strains were resistant to tetracycline, whereas 3 strains were erythromycin resistant, 2 strains were kanamycin resistant and only one was resistant to rifampin (Table 2).

Six out of the 11 strains were found to be non-typable by bacteriophage typing (Table 3). Bacteriophage typing was not relevant for the analysis of *S. aureus* vaccinal isolates, since most of the strains were non-typable. This technique has been used for many years as a standard procedure for typing of *S. aureus* strains involved in outbreaks, but its limitations (poor reproducibility, it does not type all isolates), also noticed in this study, has led to the replacement of this technique with more discriminatory techniques.

All the isolates tested in this study presented a specific amplification for *nuc* gene, confirming species assignment.

The methicillin resistant *S. aureus* strain (VS14) was confirmed by PCR, a specific amplicon for *mecA* gene being obtained.

lukS/F genes were present in six isolates and four isolates were positive for *seb* gene. Two isolates were positive for both *lukS/F* and *seb* genes. The isolates *lukS/F* positive were recovered from skin lesions and soft tissues infections. No isolates from blood culture presented amplification for *lukS/F* gene. It is well documented that in very few cases the strains isolated from blood culture are *lukS/F* positive [22]. In all the analyzed strains, at least one gene encoding SAgS was identified. The gene for staphylococcal enterotoxin I (SEI) was detected in all the strains. The virulence conferred by these factors, along with antibiotic resistance, can be important factors that contribute to the course of infections.

All the *agr* types were identified in these eleven strains, revealing the diversity of the isolates. The results are summarized in Fig. 1.

SCC_{mec} typing for MRSA strain *S. aureus* VS14 failed to detect one specific type among the five types targeted by the PCR multiplex used in this study, suggesting that this MRSA strain is harbouring a less frequent SCC_{mec} type.

Table 2 - Antibiotic susceptibility pattern of vaccinal *S. aureus* strains

Bacterial strains	Antibiotics													
	Penicillin	Cefoxitin	Tetracycline	Erythromycin	Clindamycin	Kanamycin	Tobramycin	Gentamicin	Chloramphenicol	Rifampin	Ciprofloxacin	Cotrimoxazole	Vancomycin	Linezolid
VS1	R	S	S	S	S	S	S	S	S	s	S	S	S	S
VS2	R	S	R	S	S	S	S	S	S	S	S	S	S	S
VS3	S	S	S	S	S	S	S	S	S	S	S	S	S	S
VS4	R	S	S	S	S	S	S	S	S	S	S	S	S	S
VS5	R	S	R	R	S (MLSBI phenotype)	S	S	S	S	R	S	S	S	S
VS7	R	S	R	S	S	R	S	S	S	S	S	S	S	S
VS8	R	S	R	S	S	S	S	S	S	S	S	S	S	S
VS9	R	S	S	S	S	S	S	S	S	S	S	S	S	S
VS10	R	S	R	S	S	S	S	S	S	S	S	S	S	S
VS11	R	S	R	R	S (MLSBI phenotype)	S	S	s	S	S	S	S	S	S
VS14	R	R	R	R	S (MLSBI phenotype)	R	I	I	R	R	R	R	S	S

 Table 3 - Phage patterns of vaccinal *S. aureus* strains

<i>S. aureus</i> isolate	VS1	VS2	VS3	VS4	VS5	VS7	VS8	VS9	VS10	VS11	VS14
Bacteriophage pattern	NT ^{a)}	NT	NT	96 ^{b)} _{CL}	47 ^{c)} _{SCL}	NT	3C ^{d)} ₊₊ ; 55 ^{e)} ₊ ; 71 ^{f)} _± ; 6 ⁺ ₊ ; 42E ⁺⁺ ₊₊ ; 47 ⁺⁺ ₊₊ ; 54 [±] _± ; 75 [±] _± ; 77 [±] _± ; 78 [±] _± ; 3A [±] _±	3C ^{SCL} _{SCL} ; 55 ^{SCL} _{SCL} ; 71 ^{SCL} _{SCL} ; 83A ^{SCL} _{SCL} ; 84 ^{SCL} _{SCL} ; 85 ⁺⁺ ₊₊ ; 94 ^{SCL} _{SCL} ; 81 ⁺⁺ ₊₊ ; 95 ⁺⁺ ₊₊ ; 90 ⁺⁺ ₊₊ ; HK ⁺⁺ ₊₊ ; 78 ⁺⁺ ₊₊ ; 52A ⁺⁺ ₊₊ ; 79 [±] _± ; 80 [±] _± ; 53 ⁺⁺ ₊₊ ; 6 ⁺⁺ ₊₊ ; 42E [±] _±	NT	3C ⁺⁺ ₊₊	NT

a) NT = non-typable; b) CL = confluent lysis; c) SCL = semi-confluent lysis; d) ++ = > 50 lysis plaques; e) + = 20-50 lysis plaques; f) ± = 1-19 lysis plaques

Eleven distinct PFGE patterns were obtained (Fig. 1). Using DNA Fingerprinting II software, 95% identity between isolates was observed in three groups of isolates: *S. aureus* VS2 – *S. aureus* VS10, *S. aureus* VS9 – *S. aureus* VS11 and *S. aureus* VS5 – *S. aureus* VS14, the isolates included in this groups being genetically closely related. The isolates *S. aureus* VS9 and *S. aureus* VS11 are also related to isolate *S. aureus* VS8. The remaining isolates had a similarity under 85% (Fig. 1).

PFGE showed a high discriminatory power and despite the specific limitation of this method (labour- and time-consuming) is still considered the method of choice for *S. aureus* typing [28].

spa typing analysis yielded 10 *spa* types among the 11 isolates. MRSA strain *S. aureus* VS14 presented *spa* type t008 which is very well represented in the international database (approx. 6.03% on Ridom SpaServer - <http://spa.ridom.de/frequencies.shtml>), being often associated with community-acquired MRSA strains. VS3 strain belongs to *spa* type t044 clone which is also frequent among community-associated strains and widely disseminated in Europe. Although the great majority of t044 isolates submitted through SpaServer are MRSA, the VS3 strain has proved to be susceptible to methicillin. Two isolates, namely *S. aureus* VS2 and *S. aureus* VS10 shared the same *spa* type – t300. Other *spa*-types identified

in this study were: t085, t435, t284, t159, t258 and t197 (Fig. 1). A new type submitted online to Ridom SpaServer through Ridom StaphType software was assigned - t2172. *Spa*-typing did not discriminate between VS2 and VS10 strains. BURP (Based Upon Repeat Patterns) algorithm, with a default cost of 5, identified only one cluster – clonal complex 435 (strains VS11, VS9, VS8) and 11 singletons, suggestive for genetically distant strains.

Following the MLST site submission, nine ST were identified (Fig. 1). The two strains that shared de t300 *spa*-type had also the same ST37. Strains VS9 and VS11 had different *spa*-types, but identical ST121. Comparing with PFGE and *spa*-typing, the discriminatory power of MLST is slightly reduced, but still, is the preferred method for long-term and global epidemiology.

Characterization and typing of *S. aureus* are used to support infection control measures. The phenotypic typing assays are not valuable methods to establish epidemiological correlation between different isolates. PFGE is a highly discriminative molecular method, implemented worldwide for *S. aureus* typing in epidemiological studies. *Sma*I macrorestriction analysis is accepted for outbreak investigations, but some authors question its use for phylogenetic analyses [29]. Instead, DNA sequence-based approaches are more frequently used because sequence data can

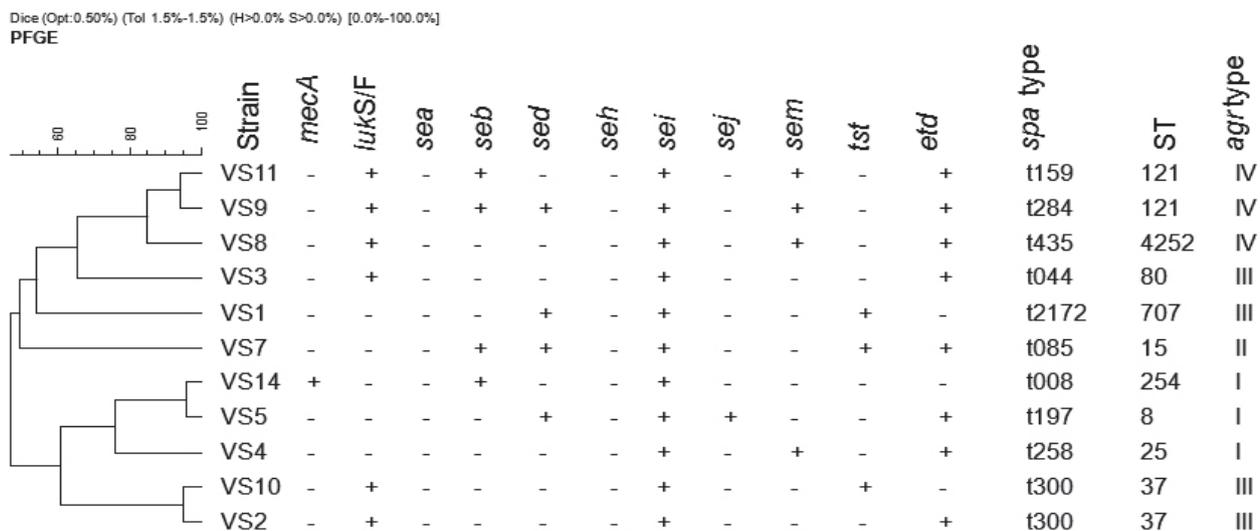


Fig. 1 - PFGE dendrogram representing the genetic relatedness of *S. aureus* isolates and distribution of different genetic markers over the isolates

easily be transferred and compared between laboratories via the Internet and the results are reproducible, rendering these types of methods useful for outbreak and phylogenetic investigations. In contrast to MLST which is widely used for evolutionary investigation in *S. aureus*, *spa*-typing proved to be a more accessible tool for routine investigation. The high throughput sequencing platforms are valuable tools for a complete characterization of *S. aureus* and for typing purposes, also. The whole genome sequencing-based approach is considered now the new gold standard in the analysis of hospital outbreaks and can be used in infection control to prevent MRSA transmission, as well as to predict resistance and virulence [30]. However, the technology is still expensive and the analysis of data is very difficult, restricting this technology only to laboratories with good infrastructure.

In this study, the vaccinal *S. aureus* strains, historically included in the corpuscular *S. aureus* vaccine manufactured at Cantacuzino Institute were characterized, using molecular methods, in terms of virulence factors, methicillin resistance mechanism and genotype. Our results, recorded in international databases, have allowed a modern description of these strains and helped to depict their relatedness to clones circulating worldwide. Until this study, those strains were characterized only based on phenotypical data with the local available tools at the moment of the vaccine launching. The molecular methods used in this study showed a superior discriminating power compared with the phenotypical methods and revealed the diversity of the studied strains.

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