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# Spa Diversity and Genetic Characterization of t127 Methicillin-resistant Staphylococcus Aureus in a Tertiary Greek Hospital

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# Abstract

**Introduction:** Methicillin-resistant *Staphylococcus aureus* (MRSA) causes severe community and hospital acquired infections. Identification of staphylococcal cassette chromosome *mec* (SCC*mec*), multilocus-sequence typing, and sequencing of *S. aureus* protein A (spa) gene are used for MRSA typing. The aim was to investigate the *spa* types of MRSA isolates in a tertiary hospital in Greece and analyse the whole genome sequences of two t127 MRSA isolates.

**Methods:** Totally, 39 MRSA isolates collected during July 2019 to June 2020 in "Georgios Gennimatas" General Hospital of Thessaloniki, Greece, were included in the study. Identification and antimicrobial susceptibility testing were performed using VITEK II automated system, and *spa* typing was performed. A minimum spanning tree was used to display the *spa* type frequencies and the genetic distances among them. Two t127-MRSA isolates (IM-MRSA and PD-MRSA) were selected for WGS.

**Results:** Twenty-two different *spa* types were detected, with t002, t003, and t422 being the most frequent (5/39, 12.8% each), followed by t1994 (4/39, 10.3%). The isolates presented high genetic diversity and, taking into account the time between hospital admission and sampling, intrahospital spread did not occur. Even the two t127 isolates were assigned to different sequence types, ST9-XII-t127 and ST1-IVa-t127. Plasmids and genes conferring antimicrobial resistance and virulence were also identified.

**Conclusions:** Various *spa* types were identified and together with the information about the time between hospital admission and sampling supports polyclonal MRSA spread in the hospital excluding a nosocomial infection. WGS provides a more detailed analysis distinguishing even the isolates belonging to the same *spa* type.


# Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium causing community-acquired and nosocomial infections worldwide (Tong et al. 2015). According to the latest epidemiological report of the European Centre for Disease Prevention and Control (ECDC), the prevalence of MRSA in Greece is among the highest in Europe (European Centre for Disease Prevention and Control. 2020).

Various methods are used for MRSA typing, such as the identification of staphylococcal cassette chromosome *mec* (SCC*mec*), the multilocus-sequence typing (MLST), and the sequencing of the highly polymorphic repeat region of *S. aureus* protein A (*spa*) gene (Enright et al. 2000; Bosch et al. 2016; Kaya et al. 2018). The detection of *spa*-clusters could be used as a rapid tool for the study of MRSA epidemiology in a hospital and for separation between relapse and re-infection (Satta et al. 2013).

Previous studies in Greece showed that *spa* types t044, t003 and t037 are the predominant types among MRSA isolated from clinical sources (Kachrimanidou et al. 2014; Nikolaras et al. 2019). In Europe, the most prevalent are the spa-types t032, t008 and t067, while t011, t108 and t034 predominate among livestock-associated (LA)-MRSA (Asadollahi et al. 2018). LA-MRSA were first detected in 2003 in pigs (Voss et al. 2005), while later, they have been isolated also in patients with no previous contact with livestock (Larsen et al. 2017). Knowledge of MRSA epidemiology is of great importance in organising and implementing infection control measures, especially in hospital settings (Leekha et al. 2020). Over the last decade, whole-genome sequencing (WGS) is an

added value for the investigation of outbreaks caused by MRSA (McManus et al. 2021). Although limited, there are reports in Greece based on whole genome MRSA sequences (Sabat et al. 2015; Sarrou et al. 2019; Karampatakis et al. 2021a; Karampatakis et al. 2021b).

The aim of the present study was to evaluate the antimicrobial resistance patterns and the distribution and prevalence of *spa* types of MRSA isolated from patients hospitalized in various wards of a tertiary hospital in Greece, and to further characterize the whole genome sequences of two isolates belonging to *spa* type t127, which is often associated with LA-MRSA.

## Material And Methods

# Bacterial isolates

Thirty-nine MRSA isolates collected during one-year period (July 2019 to June 2020) from 39 patients (18 males, 46%) hospitalized in various wards of “Georgios Gennimatas” General Hospital of Thessaloniki in Greece, were included in the study. The median age of the patients was 59 years (range 0.25 - 91 years). The isolates were recovered from wound (18, 46.2%), ear swab (five, 12.8%), blood, throat and nasal swabs (four, 10.3% each), central intravenous catheter (two, 5.1%), urine and eye swab samples (one, 2.5%, each) (Table 1). Eight isolates (20.5%) were taken through testing for colonization, while 29 (79.5%) were collected from infection sites. The distinction between infection and colonization was performed using previously described criteria (Geladari et al. 2017). The time between patients’ admission to the hospital and sampling was estimated in order to associate or not with nosocomial infection (>48 or <48 hours, respectively) (Table 1).

# Microbiological methods

All samples were cultured in blood agar, and strain identification and antimicrobial susceptibility testing were performed using the GP ID and AST-P659 cards in VITEK II automated system, respectively (BioMérieux, Marcy-l’Étoile, France). The minimum inhibitory concentration (MIC) was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints reported in January 2019 (CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement M100-S29. CLSI. Wayne). MRSA isolates were defined as resistant to oxacillin (Lee et al. 2018). Cefoxitin-screen test in VITEK 2 was used as a surrogate marker for the detection of *mec*A gene (John et al. 2009).

# DNA extraction – Spa typing

DNA was extracted using the DNA extraction kit (Qiagen, Hilden, Germany). The *spa* gene was amplified and sequenced. Typing was performed through the Rindom Spa server (spaserver.ridom.de). A minimum spanning tree (MST) was generated using the *spa*-clustering method of the *spa*-typing plugin of BioNumerics v.7.1 software (Applied Maths, Sint-Martens-Latem, Belgium), which is connected to the SeqNet/Ridom *Spa* Server (https://www.spaserver.ridom.de/).

# Whole genome sequencing - Bioinformatic analysis

Since t127 is often related with LA-MRSA, two t127 isolates [one collected from a patient hospitalized in internal medicine (IM-MRSA), and a second one from a patient hospitalized in the pediatric ward (PD-MRSA)] were selected for WGS analysis. WGS was performed on Ion Torrent PGM Platform (Life Technologies Corporation,

Grand Island, NY, USA). All procedures were conducted according to manufacturer's guidelines. PCR products were loaded on Ion-316$^{TM}$ chip kit V2BC. The Ion PGM Hi-Q (200) chemistry (Ion PGM Hi-Q Sequencing kit, A25592) was applied.

The consensus sequence was taken using *S. aureus* strain WHC09 (GenBank accession number CP077755) as reference sequence. MLST analysis was performed using the web-based MLST-DTU tool (Larsen et al. 2012). Resfinder version 4.1 and the Comprehensive Antibiotic Resistance Database (CARD) were used for the detection of antimicrobial resistance genes (Alcock et al. 2020; Bortolaia et al. 2020), while the virulence genes were detected using the Virulence Finder v. 2.0 software (Joensen et al. 2014). The SCC*mec* elements of the isolates were identified through the SCC*mec*Finder database version 1.2 (Kaya et al. 2018). PlasmidFinder version 2.1 was used for the identification of plasmids (Carattoli et al. 2014).

## Results

## *Antimicrobial resistance*

The antimicrobial resistance patterns are seen in Table 1. Specifically, 18 isolates (46.2%) displayed resistance to fusidic acid, 16 (41%) to levofloxacin, seven (17.9%) to tetracycline, six (15.4%) to mupirocin, five (12.8%) to trimethoprim/ sulfamethoxazole, three (7.7%) to vancomycin and two (5.1%) to teicoplanin. Thirty-seven isolates (94.9%) were resistant to cefoxitin (cefoxitin-screen positive); two isolates (5.1%) were resistant to cefoxitin and sensitive to oxacillin, while one isolate (2.6%) was sensitive to cefoxitin and resistant to oxacillin.

## *Spa typing*

Twenty-two different *spa* types were detected, with t002, t003, and t422 being the most frequent (5/39, 12.8% each), followed by t1994 (4/39, 10.3%), t127 and t328 (2/39, 5.1% each) (Table 1). Different *spa* types were detected in the various wards, except the paediatric ward where t1994 (3/4, 75%) predominated. Most of the samples were taken <48h after admission; this was the case also for the t1994 isolates, suggesting that they were not associated with nosocomial infection (Table 1, Figure 1).

**Table 1**

Characteristics of the 39 MRSA strains included in the study

| ID | Sex | Age (years) | Sample | Colonization /Infection | Ward | Isolation Date | >48h | Spa type | Antimicrobial Resistance Profile |
|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 59 | CVC | Infection | Cardiology | May 2020 | YES | t653 | Ox-Fox-Fu-E |
| 2 | F | 45 | urine | Infection | ER | Apr 2020 | NO | t442 | Ox-Fox-Fu-Le-Mu-Sxt-G |
| 3 | F | 72 | throat swab | Colonization | ICU | May 2020 | NO | t2986 | Ox-Fox |
| 4 | F | 81 | throat swab | Colonization | ICU | Jun 2020 | NO | t008 | Ox-Fox-Le-E |
| 5 | F | 70 | throat swab | Colonization | Internal medicine | Sept 2019 | NO | t422 | Ox-Fox-Fu-Le-E |
| 6 | M | 78 | blood | Infection | Internal medicine | Dec 2019 | NO | t267 | Ox-Fox-Fu |
| 7 | M | 91 | blood | Infection | Internal medicine | Jan 2020 | NO | t328 | Ox-Fox-Le |
| 8 | F | 82 | throat swab | Colonization | Internal medicine | Apr 2020 | NO | t003 | Ox-Fox-Fu-Le-Tet-E |
| 9 | M | 84 | wound | Infection | Internal medicine | Apr 2020 | NO | t127 | Ox-Fox-Tet-E |
| 10 | M | 75 | wound | Infection | Internal medicine | Jun 2020 | NO | t002 | Ox-Fox-Le-E |
| 11 | F | 55 | nasal swab | Colonization | Orthopaedic | Jul 2019 | YES | t5452 | Ox-Fox-E |
| 12 | F | 86 | wound | Infection | Orthopaedic | Nov 2019 | YES | t003 | Ox-Fox |
| 13 | M | 62 | wound | Infection | Orthopaedic | Nov 2019 | YES | t002 | Ox-Fox |
| 14 | M | 84 | wound | Infection | Orthopaedic | Dec 2019 | NO | t003 | Ox-Fox-Fu-Le |
| 15 | M | 79 | nasal swab | Colonization | Orthopaedic | Jan 2020 | YES | t002 | Ox-Fox |
| 16 | F | 65 | wound | Infection | Outpatient | Sept 2019 | NO | t422 | Ox-Fox-Fu-Le-Mu-Sxt-E-G |
| 17 | M | 70 | wound | Infection | Outpatient | Oct 2019 | NO | t440 | Ox-Fox-Va-Teic |
| 18 | F | 47 | ear swab | Infection | Outpatient | Oct 2019 | NO | t084 | Fox-Sxt |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 19 | F | 28 | ear swab | Infection | Outpatient | Oct 2019 | NO | t422 | Ox-Fox-Fu-Le |
| 20 | F | 0,5 | wound | Infection | Outpatient | Jan 2020 | NO | t328 | Ox-Fox |
| 21 | M | 0,42 | wound | Infection | Outpatient | Jan 2020 | NO | t1814 | Ox-Fox-Sxt |
| 22 | M | 0,25 | ear swab | Infection | Outpatient | Apr 2020 | NO | t131 | Ox-Fox-Fu-Tet |
| 23 | F | 73 | ear swab | Infection | Outpatient | May 2020 | NO | t422 | Ox-Fox-Le-E |
| 24 | F | 7 | nasal swab | Colonization | Outpatient | Jun 2020 | NO | t1994 | Ox-Fox-Fu-Mu-E |
| 25 | M | 11 | ear swab | Infection | Outpatient | Jun 2020 | NO | t012 | Ox-Fox-E |
| 26 | F | 0,83 | wound | Infection | PD | Jul 2019 | NO | t1994 | Fox-Fu-Mu-E |
| 27 | F | 0,42 | wound | Infection | PD | Jul 2019 | NO | t127 | Ox-Fox-Tet-E |
| 28 | M | 4 | eye swab | Infection | PD | Oct 2019 | NO | t1994 | Ox-Fox-Fu-Mu-Va |
| 29 | F | 0,58 | nasal swab | Colonization | PD | Nov 2019 | NO | t1994 | Ox-Fox-Fu-Mu |
| 30 | F | 59 | wound | Infection | Surgery | Aug 2019 | YES | t002 | Ox-Fox-Fu-Le-E |
| 31 | M | 40 | blood | Infection | Surgery | Sept 2019 | YES | t002 | Ox-Fu |
| 32 | F | 21 | wound | Infection | Surgery | Oct 2019 | NO | t091 | Ox-Fox-Fu-Le-Tet-Sxt-Va-Teic |
| 33 | M | 65 | wound | Infection | Surgery | Nov 2019 | YES | t726 | Ox-Fox |
| 34 | M | 22 | wound | Infection | Surgery | Mar 2020 | NO | t1309 | Ox-Fox-Le |
| 35 | M | 16 | wound | Infection | Surgery | Mar 2020 | NO | t044 | Ox-Fox-Fu-Tet |
| 36 | F | 28 | CVC | Infection | Surgery | Mar 2020 | NO | t034 | Ox-Fox-Tet |
| 37 | F | 59 | wound | Infection | Surgery | Jun 2020 | NO | t003 | Ox-Fox-Le-E |
| 38 | M | 81 | blood | Infection | Urology | Aug 2019 | NO | t422 | Ox-Fox-Fu-Le-E |
| 39 | M | 45 | wound | Infection | Urology | Feb 2020 | NO | t003 | Ox-Fox-E |

M: Male, F: Female, CVC: Central Venous Catheter, ER: Emergency Room, ICU: Intensive Care Unit, PD: Paediatric ward

Ox: Oxacillin, Fox: Cefoxitin, Fu: Fusidic acid, Le: Levofloxacin, Mu: Mupirocin, Teic: Teicoplanin,

Te: Tetracycline, SXT: Trimethoprim/Sulfamethoxazole, G: Gentamicin, Va: Vancomycin

*Erythromycin susceptibility results for strains with ID 6,7,12-15,17-21,28,29, and 31-33 are not available.

## *Analysis of whole genome sequences*

IM-MRSA and PD-MRSA isolates were assigned to ST9 and ST1, respectively. They were carrying SCC*mec* elements belonging to XII and IVa types, respectively.

The antimicrobial resistance and virulence genes, and the plasmids carried by the two isolates are seen in Table 2. Specifically, both had antimicrobial resistance genes for β-lactams (*mec*A and *bla*Z), aminoglycosides [*aph*(3′)-IIIa], and macrolide, lincosamide and streptogramin B (*erm*C); IM-MRSA carried three additional aminoglycoside resistance genes [*ant*(6)-Ia, *aad*D and *aac*(6′)-*aph*(2′′)], while PD-MRSA harbored one additional aminoglycoside resistance gene [*aad*(6)]; *dfr*G gene conferring resistance to trimethoprim was detected only in IM-MRSA. Both isolates carried several efflux pump protein genes conferring resistance to streptogramin A, tetracycline, fluoroquinolones, cephalosporins and lincosamides.

Regarding plasmid content, IM-MRSA carried a plasmid replicon type *rep*22 (of the pGSA11 group), while PD-MRSA transferred plasmid replicon types *rep*7a (of the pGSA2 group, which includes the *rep*C cassette), *rep*7c, *rep*10 (of the pGSA3 group which includes the *erm*C gene) and *rep*5 along with *rep*16 (of the pGSA22 group which includes the *bla*Z gene) (Table 2).

**Table 2**

Genetic characteristics of two t127 MRSA strains of the study.

| | PD-MRSA | IM-MRSA |
|---|---|---|
| **Size** (bp) | 2,971,258 | 2,849,316 |
| **GC content** (%) | 34.2 | 31.0 |
| **Number of contigs (with PEGs)**<br>**MLST**<br>**SCC*mec* element** | 530<br>ST1<br>IVa | 5,636<br>ST9<br>XII |
| **Antimicrobial resistance genes**<br><br><br><br><br>**Efflux pump genes** | *mec*A, *bla*Z,<br>*aad(6)*, *aph(3')-IIIa*,<br>*erm*C,<br><br><br>*mep*R, *nor*A, *mgr*A, *arl*R, *arl*S, *Lmr*S, *tet(45)* | *mec*A, *bla*Z, ,<br>*ant(6)-Ia, aph(3')-IIIa,*<br>*aad*D, *aac(6')-aph(2")*,<br>*dfr*G, *ermC*<br><br>*tet*L, *fex*A, *Isa*E, *Inu*B |
| | | |
| **Plasmid group (rep family)** | pGSA$_2$ (rep7)<br>pGSA$_3$ (rep10)<br>pGSA$_{22}$ (rep5, rep16) | pGSA$_{11}$ (rep22) |
| **Virulence factor (*gene*)** | | |
| Chemotaxis inhibitory protein (*chp*) | - | - |
| Aureolysin (*aur*) | + | + |
| Serine protease (*spl*A, *spl*B) | + | - |
| Staphylococcal complement inhibitor (*scn*) | + | - |
| Staphylokinase (*sak*) | + | - |
| γ-hemolysin (*hlgA, hlgB, hlgC*) | + | + |
| Other leukocidin components<br>(*luk*AB, *luk*ED) | + (*luk*ED) | - |
| Staphylococcal enterotoxin | + (*seh*) | + (*sei, sem, seo, seu*) |
| Antiseptic resistance genes (*qac*A, *qac*B, *qac*C, *qac*D) | - | + (*qac*C, *qac*D) |

# Discussion

The present study provides an insight into the distribution of MRSA isolates of various *spa* types in a tertiary hospital in Greece. Most of the isolates were collected from infection sites and few from colonization sites; however, colonization usually precedes infection (Love et al. 2020). It was shown that most isolates were resistant to several antimicrobial categories. The high resistance to fusidic acid (46.2%) exceeds by far that observed among MRSA globally (2.6%) (Hajikhani et al. 2021). The resistance rate to mupirocin (15.4%) was higher than the 3.1% revealed in a previous multicenter surveillance study (Kresken et al. 2004); this is of crucial importance since resistance to mupirocin could potentially diminish the efficacy of MRSA decolonizing strategies (Bes et al. 2021). For both antimicrobials (fusidic acid and mupirocin) further studies are needed to elucidate the genetic background of the increased resistance (whether it is due to mutation(s) and/or acquired resistance genes). In contrast, resistance to levofloxacin was lower than that reported in a recently published study (17.9% versus 87.9%) (Antonelli et al. 2019).; further analysis is needed. The resistance rates of vancomycin and teicoplanin were low; although infections caused by vancomycin-resistant MRSA have been described (Cong et al. 2020), resistance to vancomycin and teicoplanin are rarely identified in MRSA strains, and they remain active against MRSA causing severe infections (Beibei et al. 2010; Chen et al. 2018).

In total, 22 different *spa* types were identified, and most of them (16/22, 72.7%) were represented as singletons, suggesting a non-clonal MRSA distribution (Table 1, Figure 1). An exception was the t1994 predominance in the paediatric ward during a four-months' time period; however, it seems that it was not associated with intra-hospital infection since the time of sampling was <48h from the admission to the hospital. The high prevalence of t002 and t003 *spa* types seen in the present study, has been reported in recent studies (Engelthaler et al. 2013; Tkadlec et al. 2021), while the rare t422 type has been previously reported also in another hospital in Thessaloniki (Kachrimanidou et al. 2014).

Since t127 type is usually associated with LA-MRSA, and it has been recently detected in Greek workers in the dairy production chain (Karampatakis et al. 2021b), two t127 MRSA isolates (IM-MRSA and PD-MRSA) of the present study were selected for analysis of their whole genome. The two isolates differed each other, as IM-MRSA was assigned to ST9-XII type, while PD-MRSA to ST1-IVa type as was the case in a previous study (Karampatakis et al. 2021b). The two t127 MRSA isolates transferred the γ-hemolysin genes *hlg*A, *hlg*B, *hlg*C and aureolysin *aur* gene, which have been previously described in both ST1-IVa and ST9-XII MRSA (Cortes et al. 2017; Yu et al. 2021). PD-MRSA carried the enterotoxin *seh* gene and the gene encoding leukocidin *luk*ED, as previously described in ST1-IVa MRSA isolates (Shukla et al. 2010; Cortes et al. 2017). IM-MRSA lacked leucocidin components, however it carried the enterotoxin *sei*, *sem*, *seo*, *seu* genes, although enterotoxin genes are usually absent in ST9-XII MRSA isolates (Yu et al. 2021).

LA-MRSA lack the human evasion genes *scn* (staphylococcal complement inhibitor), *chp* (chemotaxis inhibitory protein) and *sak* (staphylokinase), due to loss of the related φSa3 phage (Price et al. 2012). IM-MRSA lacked these three genes, while PD-MRSA lacked only the *chp* gene. IM-MRSA was isolated from wound infection and was assigned to ST9-XII, which, due to loss of *scn*, *chp* and *sak* genes, is considered to have been evolved from human to animal host (Yu et al. 2021). ST9-XII is rarely reported as cause of infection (Jin et al. 2020). *Spa*-type t127 has not been reported for *S. aureus* ST9 so far. Since generation of this *spa*-type from the repeat spa sequences observed in this clonal lineage would need several genetic events (mutations, deletion(s), insertion), the most probable mechanism seems to be the acquisition of the chromosomal sequence which contains *spa* from a ST9 donor; there are several examples for nontypical *spa* types for particular STs based on this genetic event, e.g. *spa* type t030 in MRSA ST239 (Monecke et al. 2018). Comparative studies are needed to confirm

which mechanism(s) took place. The second t127 isolate, PD-MRSA, was isolated from an axillary abscess and belonged to ST1-IVa-t127, which is originally considered a human-associated lineage. ST1-t127 isolates have been reported as one of the most frequent types isolated from human samples (Monaco et al. 2013).

## Conclusions

The current study provides an insight into the *spa* types of MRSA in a tertiary hospital in Greece and together with the information of time interval between admission and sampling suggests polyclonal introductions in the various wards. The extensive resistance to most antimicrobial classes needs further attention since it is associated with high antimicrobial consumption. A more detailed genetic characterization is gained when WGS is applied, as shown by the results of the analysis of the two t127 MRSA isolates, which showed that although they belonged to the same *spa* type, they were different strains. Molecular surveillance studies are of high priority in the hospitals since they can lead the design of guidelines and infection control measures that must be applied to reduce and prevent the spread of MRSA.

## Genome Sequences

The whole genome sequences of IM-MRSA and PD-MRSA were submitted to European Nucleotide Archive (ENA) under the study PRJEB47007 and received the Accession numbers ERS7262952 and ERS7262953, respectively.

## Declarations

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# Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

# Availability of data and material

Available upon request

# Code availability

Not applicable

# Ethics approval

Not applicable

# Authors’ contribution

All authors have equally contributed to the study and preparation of the manuscript.

# Consent to participate

Not applicable

# Consent for publication

Not applicable

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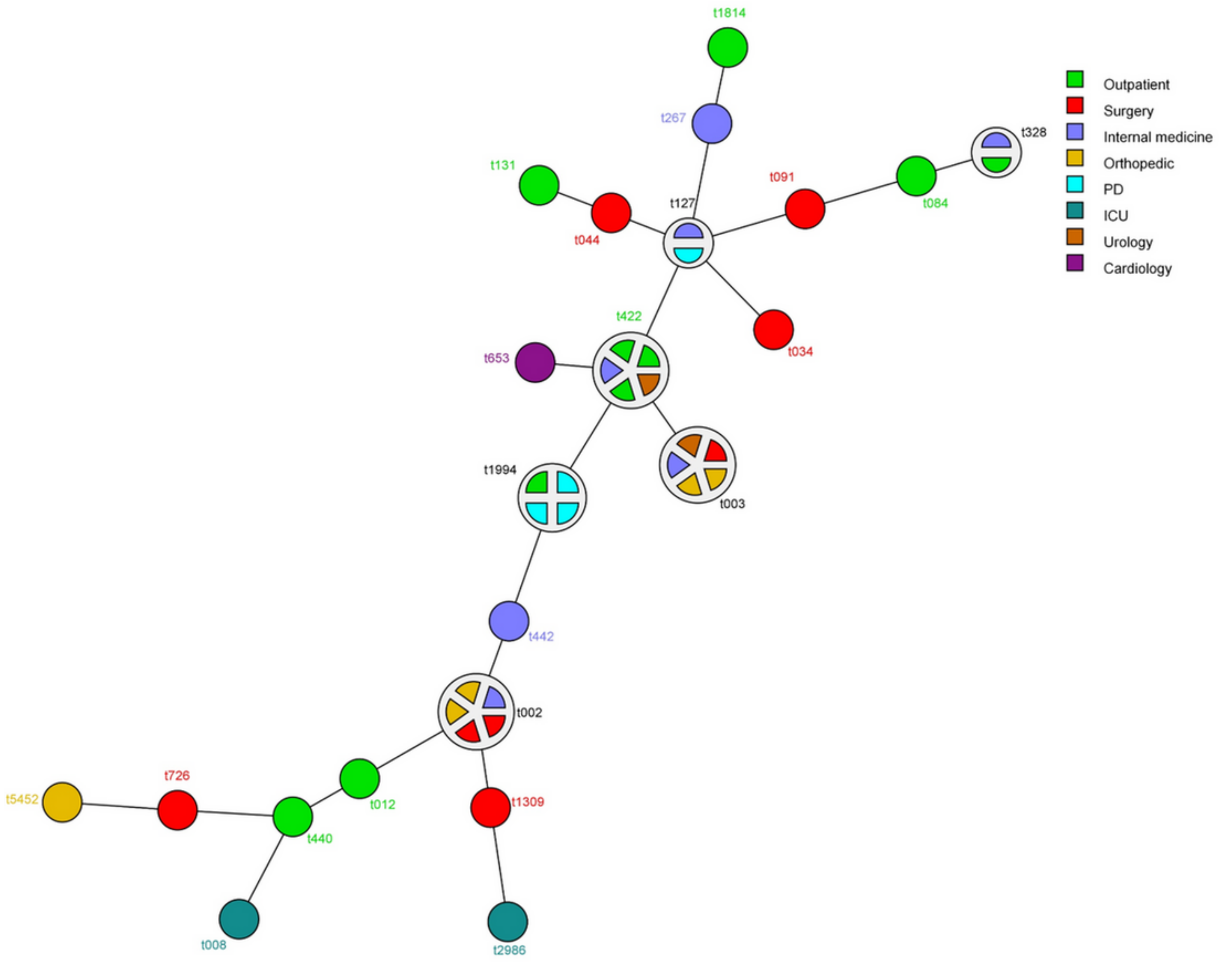
# Figures




## Figure 1

Minimum spanning tree based on spa-typing results of MRSA strains depending on the department of isolation. Each spa type is represented by a single node. The size of the node depends proportionally on the number of strains within the spa type. The colored sections represent a different ward. The distance between nodes represents the genetic diversity of the isolates.