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Nasal carriage of *Staphylococcus aureus* in healthy humans with different level of contact with animals in Tunisia: genetic lineages, methicillin-resistance and virulence factors.

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Abstract

Nasal swabs of 423 healthy-humans who showed different level of contact with animals (frequent, 168; sporadic, 94; no contact, 161) were obtained in Tunisia (2008-2009), and 99 of them presented other associated risk factors. Methicillin-resistant *S. aureus* (MRSA) was detected in one of these 423 samples (0.24%), retrieved from a veterinarian. MRSA isolate was *mecA*-positive, typed as ST80-t203-SCC*mecIVc*-agrIII, and contained *tet(K)*, *ant(6)-Ia* and *aph(3')-IIIa* genes encoding tetracycline, streptomycin and kanamycin resistance, respectively. This MRSA isolate also contained the *lukF/lukS* virulence gene encoding Panton-Valentine leukocidin. Fifty-four (12.8%) additional nasal samples contained methicillin-susceptible *S. aureus* and one isolate/sample was characterized. A high diversity of *spa*-types (n=43; 4 new) and PFGE-types (n=37) was detected among the 55 recovered *S. aureus*. Percentages of antimicrobial resistance/detected resistant genes were as follows: tetracycline [22%/*tet(K)*-*tet(L)*-*tet(M)*], erythromycin [5%/*msrA*], ciprofloxacin [14.5%], trimethoprim-sulfamethoxazole [2%/*dfrA*], streptomycin [11%/*ant(6)-Ia*], kanamycin [7%/*aph(3')-IIIa*], amikacin [5%], and chloramphenicol [2%]. Four and two isolates carried the *lukF/lukS* and *eta* and/or *etb* genes, respectively, and always in individuals with contact with animals. Eleven isolates carried the *tst* gene and were recovered from individuals with different level of contact with animals.

Introduction

Nosocomial infections caused by *Staphylococcus aureus* constitute an important health problem worldwide. This microorganism is usually associated with skin and soft tissue infections. However, it is also able to cause serious diseases such as pneumonia, meningitis or septicemia. Additionally, the pathogenicity of *S. aureus* infections is facilitated by the expression of several virulence factors which include cell wall-associated adhesins and several toxin groups [1,2].

Of particular concern is methicillin-resistant *S. aureus* (MRSA), which has become a major threat due to an increasing incidence since its first report in 1961. First MRSA infections were facilitated by several predisposing factors including recent hospitalisation, admission to an Intensive Care Unit (ICU), prolonged antibacterial therapy or surgery. MRSA strains acquired in these environments are known as hospital acquired MRSA (HA-MRSA) [3]. The presence of multiple antimicrobial resistance in *S. aureus* isolates, common trait among HA-MRSA strains, is an important clinical problem limiting therapeutic options [4].

The first cases of MRSA in the community setting were reported in the early 1990s from patients without the aforementioned risk factors. These strains are well known as community acquired MRSA (CA-MRSA) [5]. Currently, CA-MRSA infections occur among healthy persons without risk factors for HA-MRSA acquisition, being reported at an increasing frequency having a worldwide distribution. These infections seem to be caused by isolates that differ from typical HA-MRSA by their genetic background, epidemiology, clinical spectrum, antibacterial resistance and by the presence of determined toxin genes [6]. For example, CA-MRSA isolates are more likely than HA-MRSA isolates to encode putative virulence factors, such as Panton-Valentine leukocidin (PVL), a cytotoxic virulence factor that has been associated with severe pneumonia and with skin and soft tissue infections [6-8]. In the last years, a livestock-associated genetic lineage of MRSA of the sequence type ST398

has emerged in farm animals, especially in pigs, and human infections due to this specific type of MRSA have also been reported [9-11].

So far, few data do exist about the prevalence of MRSA in nasal samples of healthy population or about the genetic lineages of *S. aureus* that colonize the nares of healthy humans. A previous study carried out in USA reported a MRSA carriage rate in healthy humans of about 0.84% [12]. Another study carried out in the Netherlands among healthcare workers with and without exposition to livestock, detected MRSA carriage rates of 1.7% and 0.15%, respectively [13]. It is known that nasal carriage of *S. aureus* has been identified as a risk factor for the development of infections at the hospital level, and if the microorganism is methicillin-resistant, it can cause serious health problems. Few studies have focused on which individuals are most likely to be colonized with *S. aureus* and which are most likely to specifically have MRSA. Likewise, little is known on the potential influence of animal contact for the acquisition of animal and non-animal associated *S. aureus* strains.

Therefore, the present study was undertaken to determinate the nasal carriage of MRSA and *S. aureus* in healthy individuals in order to identify whether the intensity of animal contact might be a risk factor for the acquisition of MRSA or other specific types of *S. aureus* strains. Likewise, we aimed to determinate the characteristics of the obtained strains using different molecular techniques including its clonal diversity.

Materials and methods

Study design and sampling

Nasal swabs of 423 healthy individuals were obtained in different regions of Tunisia from October 2008 to February 2009. Each of the individuals was asked to provide information about the level of contact with farm or companion animals (frequent, sporadic or no contact) and also about the use of antimicrobials or contact with hospitalized patients during the three months prior to the sample time.

Microbiological isolation

All swabs were incubated in Tryptone Soy Broth (TSB) for 48h and then, broths were subcultured on ORSAB medium (Oxacillin Resistance Screening Agar Base) (Oxoid, UK) for 24-48 hours. *S. aureus* compatible colonies were initially identified by conventional methods [Gram-staining, catalase test, oxidase test and DNase production, and ability to coagulate rabbit plasma (BioRad, France)]. Species identification was then confirmed by species-specific PCR amplification of the *nuc* and *spa* genes [14].

Antimicrobial susceptibility testing

Susceptibility to 17 antimicrobial agents was performed using the disk-diffusion method in accordance with the Clinical and Laboratory Standards Institute recommendations [15]. Antimicrobial agents tested were (charge in µg): oxacillin (1), cefoxitin (30), kanamycin (30), gentamicin (10), tobramycin (10), tetracycline (30), chloramphenicol (30), trimethoprim-sulfamethoxazole (1.25/23.75), erythromycin (15), clindamycin (2), amikacin (30), ciprofloxacin (5), mupirocin (5), vancomycin (30), teicoplanin (30), and penicillin and streptomycin (10 units). Double-disk diffusion test (D-test) with erythromycin and clindamycin discs was implemented in all isolates to detect inducible clindamycin resistance. Minimum inhibitory concentrations (MICs) for vancomycin and teicoplanin were performed by the agar dilution method [15]. *S. aureus* ATCC 25923 and ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

*Detection of *mecA* gene and SCC_{mec}-typing*

The presence of the *mecA* gene was studied by PCR in all oxacillin and/or cefoxitin resistant isolates, as previously described [16]. The SCC_{mec}-typing was performed for *mecA* positive isolates by a multiplex PCR strategy as previously described [17].

Detection of antimicrobial resistance genes

The ribosomal methylases encoded by *erm(A)*, *erm(B)* and *erm(C)* genes, which confer resistance to erythromycin and clindamycin, and the efflux pump encoded by *msr(A)* gene, conferring resistance to erythromycin, were studied by PCR in erythromycin-resistant isolates [18,19]. In addition, *tet(K)*, *tet(M)* and *tet(L)* genes, conferring resistance to tetracycline, and *aac(6')-Ie-aph(2'')-Ia*, *aph(3')-IIIa*, and *ant(6)-Ia* genes, conferring resistance to gentamicin, kanamycin and streptomycin, respectively, were amplified by PCR in all aminoglycoside-resistant *S. aureus* isolates [20,21]. The presence of genes associated with resistance to trimethoprim-sulfamethoxazole (*dfrA* and *dfrD*) and chloramphenicol [*cat*(pC194), *cat*(pC221), *cat*(pC223)] were analysed by PCR as previously reported [22].

Pulsed-Field-Gel-Electrophoresis (PFGE)

All *S. aureus* isolates were characterized by PFGE with *SmaI* restriction enzyme digestion as previously described [23]. The samples were run on 1% agarose gel in 0.5% TBE buffer at 14°C on a CHEF DR-II PFGE system by using an initial switching time of 5s which was increased to 40s during 20 h at 6V/cm. The DNA fingerprints generated by PFGE were analyzed with manual method according to previous proposed criteria [24], and digitalized method by *Gel-Pro* version 3.1 software. The obtained results were submitted to multivariate statistical package (MVSP) software generating dendrogram according to simple matching coefficient and unweighted pair group method with arithmetic mean (UPGMA) algorithm.

Other molecular typing methods of S. aureus isolates

Spa-typing was performed in all *S. aureus* isolates as elsewhere described [25]. The X region of *spa* gene was amplified by PCR, and sequences were analyzed using Ridom Staph-Type software version 1.5.21 (Ridom GmbH), which automatically detects *spa* repeats and assigns a *spa*-type according to <http://spaserver.ridom.de/>.

Identification of *agr* allele group (I–IV) was determined by multiplex PCR. The nucleotide sequence of each primer and the amplification conditions were as earlier described [26].

Multilocus-sequence-typing (MLST) was performed in selected *S. aureus* isolates (those containing the genes of the Panton-Valentine **Leukocidin** (PVL), and those presenting new *spa*-types) as **described previously** [27]. The allelic profile of each strain was obtained by sequencing internal fragments of seven unlinked housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*). Alleles of the 7 genes defined the allelic profile, which corresponded to a sequence type (ST) assigned by the MLST database (<http://www.mlst.net>).

Detection of staphylococcal toxin genes

All isolates were tested by PCR for the presence of four staphylococcal **toxin genes** [28]. The targeted genes were the following ones: *lukS-lukF* encoding the PVL toxin, *eta* and *etb* encoding exfoliative **toxins ETA** and ETB, **respectively**, and *tst* encoding the toxic shock syndrome toxin (TSST).

Results

Table 1 shows the characteristics of the 423 individuals tested in relation with the level of contact with animals and other risk factors. Individuals included in the study were classified into four groups: 1) frequent contact with farm animals (veterinarians, veterinary students, farmers and abattoir-workers) (n=111); 2) high contact with companion animals (n=57); 3) sporadic contact with animals (n=94); 4) no contact with animals (n=161). Ninety-nine of these individuals had other risk factors as previous contact with hospitalised patients or use of antibiotic in the three months prior sampling (Table 1).

Carriage of MRSA and typing of isolates

The nasal swabs of all 423 individuals tested were analysed for MRSA recovery **and only one** contained a *mecA*-positive MRSA isolate, representing 0.24% **of the total number of** tested samples. This MRSA isolate (C2444) was recovered from a **veterinarian** and showed a phenotype of resistance that included, **i**n addition to beta-lactams, resistance to tetracycline,

streptomycin and kanamycin, and harboured the *tet(K)*, *ant(6)-Ia*, and *aph(3')-IIIa* genes. MRSA C2444 was typed as SCC*mec* type IVc, and was ascribed to the *agr*-type III, to the *spa*-type t203 and to the MLST type ST80. It is interesting to remark that the genes encoding the PVL toxin were identified in this strain, but not the other tested toxin genes (*tst*, *eta* and *etb*).

Detection of S. aureus and characterization of antimicrobial resistance mechanisms and virulence genes

S. aureus was recovered in 55 of the 423 nasal samples tested and one isolate per sample was maintained for further studies. The origin of the 55 *S. aureus* isolates and their antimicrobial resistance patterns are shown in Table 2. All isolates exhibited penicillin resistance, although only one of them showed oxacillin and cefoxitin resistance by disk-diffusion test and the *mecA* gene was identified in this isolate (MRSA C2444, recovered from the veterinarian previously indicated). Seventeen additional *S. aureus* isolates exhibited resistance to oxacillin but not to cefoxitin and all of them were negative for *mecA* gene. The remaining 37 *S. aureus* isolates showed oxacillin-susceptibility even though they were recovered in ORSAB medium. All 55 *S. aureus* isolates showed susceptibility to vancomycin, teicoplanin, gentamicin, tobramycin, clindamycin and mupirocin, showing MICs for vancomycin and teicoplanin of $\leq 1\mu\text{g/ml}$. Variable resistance rates were observed to tetracycline (21.8%), ciprofloxacin (16.4%), streptomycin (10.9%), kanamycin (7.3%), erythromycin and amikacin (5.4% each one), and trimethoprim-sulfamethoxazole and chloramphenicol (1.8% each one). No inducible clindamycin resistance was identified in the three erythromycin-resistant *S. aureus* isolates when the double-disk erythromycin-clindamycin screening test was used.

The *tst* gene, encoding the toxic shock toxin, was detected in 11 of 55 isolates (20%). Other toxin genes detected included the *lukS-lukF* genes encoding the Panton-Valentin-leukocidin toxin detected in 4 of 55 studied *S. aureus* isolates (7.3%), and the *eta* and *etb* genes,

encoding the exfoliative ETA and ETB toxins, respectively, harboured by two isolates. All four *S. aureus* isolates carrying the genes of PVL toxin were obtained from individuals with high contact with animals (n=3) or with sporadic contact with animals (n=1), but no PVL-positive isolate was identified in the group of individuals with no contact with animals. It is important to note that isolate C2431 is characterized by a high virulence potential, since it produced both exfoliative toxin ETA and ETB, and it was recovered from an individual that had taken antimicrobial agents in the previous months and had also contact with hospitalised patients and with animals.

No correlation was found between the presence of virulence genes and antimicrobial resistance. It is important to note that in our study, almost half of *S. aureus* obtained from individuals that referred high contact with farm or companion animals harboured genes encoding virulence factors but only 13% of isolates of individuals with no contact with animals presented this characteristic.

Molecular typing of S. aureus isolates and detection of virulence genes

All 55 *S. aureus* isolates were submitted to molecular typing by *spa*, *agr* and PFGE techniques, and some selected *S. aureus* isolates were also submitted to MLST typing, and results are shown in Table 2.

Analysis of *Sma*I macrorestriction profiles revealed a high clonal diversity with 37 unique PFGE patterns (Figure 1). These patterns were classified from S1 to S37, each defining a clone in accordance to the previous reported interpretive criteria [24]. The pulsotype S34 was the most frequently encountered one (7.3% of isolates, 4 isolates). Two isolates with S6 pulsotype produced exfoliative toxins (ETA and/or ETB). Four of 11 isolates harboured the *tst* gene and belonged to pulsotype S2. The four PVL producing *S. aureus* isolates were ascribed to four different pulsotypes (S4, S19, S23 and S25).

Among the high diverse *spa* gene types (n=43), four not earlier described types were identified (t6222, t6223, t6224, and t6225). Spa-typing analysis results confirmed those previously found in the analysis by PFGE: 1) isolates C2410 and C2425 belonging to pulsotype S35 presented the same *spa*-type t166; 2) two isolates which shared the same t024 *spa*-type belonged to pulsotype S5 (C2447 and C2442); 3) two isolates with identical pulsotype S7 (C2440 and C2432) shared the common *spa*-type t084. There was no clear association between the *spa* type and the antimicrobial resistance pattern.

Amplification of the hypervariable region of the *agr* locus showed that *agr* group I was predominant (detected in 21 of 55 *S. aureus* isolates, 38.2%), followed by *agr* groups II and III (20% each), and *agr* group IV (1.8%). However, 11 isolates were untypable (20%).

All 4 PVL-positive *S. aureus* isolates and the four isolates presenting new *spa*-types were analysed by MLST and they were compared with the *S. aureus* MLST database. All eight *S. aureus* isolates belonged to 7 different already known sequence types, and a new sequence type ST1704 was also identified for *S. aureus* C2459 that presented the new *spa*-type t6225 (Table 1).

PFGE revealed remarkable clonality among isolates producing the virulence factor TSST-1, and also among those producing the exfoliatin A and B. However, no clonal relationship has been detected among the four isolates in which the genes of the PVL toxin have been demonstrated (Figure 1).

Discussion

The rate of MRSA nasal carriage seems to be very low in healthy individuals in Tunisia, as only one person of 423 tested was positive for MRSA (0.24%), although the initial number of positive cultures on the selective ORSAB medium suggested a much higher prevalence. It is interesting to remark that the MRSA-positive person was a veterinarian who worked with farm animals, and if we consider only the group of individuals with high contact with farm

animals, the rate of nasal carriage is a little higher (1 out of 113, 0.9%). Studies performed in other countries also show low nasal carriage of MRSA among the healthy population [29], although this prevalence seems to be higher among persons in contact with farm animals [30,31]. It is interesting to point out that the MRSA strain recovered in our study presented a multiresistant antimicrobial phenotype that included not only beta-lactams, but also aminoglycosides and tetracycline. Molecular typing revealed that MRSA strain C2444 belonged to ST80 and was characterized by cassette SCCmec type IVc, *agr* type III and with PVL toxin production. All these characteristics are in line with those of the major PVL-positive CA-MRSA clone detected in many European countries. Today, the ST80 PVL-positive CA-MRSA clone is disseminated in Asia, Europe and the Middle-East [32]. However, the *spa*-type related to the MRSA isolate in our study was t203, which remains different from that of the European clone ST80 which is normally of *spa*-type t044 [32, 33]. Nevertheless, both *spa*-types are very similar, the *spa*-type t203 presenting an extra repeat (r12). The *agr* III/ST80 lineage detected in our study in MRSA strain C2444 has also been reported as one of the predominant lineages of PVL-positive MSSA [34,35]. PVL production is considered as a common trait among CA-MRSA but it is important to recognize that PVL-negative strains can also occur.

In this study it has been detected that all the PVL-positive *S. aureus* isolates were recovered in individuals that had contact with animals, but not among those without contact with animals. It is interesting to remark that these strains (the MRSA strain above mentioned as well as the three MSSA ones) were not clonally related and they presented different *spa*, PFGE and MLST types, indicating no clonal relationship among them. The association of PVL-production with ST30, ST121 or ST22 lineages in three of our MSSA strains has been previously identified as some of the most prevalent lineages of PVL-positive MSSA in different countries [34, 35, 36]. Moreover, sequence type ST30 constitute one of the predominant CA-MRSA clones in Australian and South American isolates [37]. Nevertheless,

the ST121 lineage may have a global distribution and it is more frequently associated to MSSA [36], although it has also been recently detected in cases of CA-MRSA [38].

PVL-encoding genes had been detected in strains representing the majority of *agr* groups (I, III and IV) and belonging to widely distributed STs [34]. The observed diversity of PVL-positive strains might be attributed to the fact that PVL genes are localised on phages, which facilitate spread of PVL genes through *S. aureus* populations [39].

Remarkably, the chromogenic medium intended for selective MRSA recovery also allowed the recovery of oxacillin-susceptible *S. aureus* isolates in some of the nasal samples. It is of interest to remark the low specificity of this media for MRSA recovery as previously reported by other authors [40], as well as the importance of further characterizing isolates grown in this media with the appearance of MRSA.

A high clonal diversity was identified among the 55 *S. aureus* isolates obtained in this study (54 MSSA and one MRSA) reflected by 43 different *spa*-types and 37 different PFGE patterns. In addition, 8 different sequence-types were identified by MLST among 8 selected *S. aureus* isolates (4 isolates PVL-positive and 4 isolates with new *spa*-types). Seventeen *S. aureus* isolates among the 55 recovered in this study (30.9%) showed oxacillin-resistance but cefoxitin-susceptibility and did not carry the *mecA* gene; the presence of other mechanisms of resistance can not be discarded in these *mecA*-negative strains, as it is the case of hyperproduction of penicillinase or modification of other PBP's [41].

Besides the PVL encoding genes, three other toxin genes were detected among *S. aureus* strains: *tst* (11 isolates), *eta* (2 isolates) and *etb* (1 isolate). This finding might have implications in human health.

Previous investigations reported that *agr* groups of *S. aureus* were associated with the patterns of *S. aureus* diseases [28]. They reported that isolates of *agr* group I and II caused preferentially diseases mediated by enterotoxin and that *agr* group III isolates were involved in TSST-1 mediated diseases. In our study, a strong relationship was observed between *agr*

group III and toxic shock toxin TSST-1. Similarly, we also noted that 2 isolates among the four harbouring *lukS-PV* and *lukF-PV* genes belonged to the same *agr* group III.

This study is the first one that reports both, the rate of nasal carriage by MRSA and the nasal carriage of PVL-positive or TSST-positive *S. aureus* in healthy humans, not only in Tunisia but also in the African continent. In this study, MRSA and PVL-positive *S. aureus* isolates (both MRSA and MSSA) were detected mostly among individuals in contact with animals. More studies should be performed in the future, including more individuals with different risk factors, in order to assess the risk of contact with animals in relation with the possibility of being a nasal carrier of this specific microorganism, as well as the risk for acquisition of staphylococcal infections.

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Table 1. Characteristics of the 423 healthy individuals in relation with specific risk factors and characteristics of recovered *S. aureus* isolates.

Characteristics of individuals tested		No. individuals tested	No. individuals with <i>S. aureus</i>	No. (%) individuals with MRSA	Individuals with toxigenic <i>S. aureus</i> ^b (No.)
Contact with animals	Other risk factors ^a				
High contact with farm animals: veterinarian students or staff; farmers; abattoir workers	No	83	16	1 (1.2 %)	PVL (3) TSST-1 (2)
	Yes	28	5	0	ETA + ETB (1)
High contact with companion animals	No	36	8	0	TSST-1 (4) ETA (1)
	Yes	21	1	0	
Sporadic contact with animals	No	73	2	0	PVL(1)
	Yes	21	1	0	
No contact with animal	No	132	15	0	TSST-1 (2)
	Yes	29	7	0	TSST-1 (3)
Total		423	55	1 (0.2%)	(17)

^a Use of antibiotics or contact with hospitalised patients in the previous three months of sampling

^bPVL: Pantón-Valentine leukocidin; ETA and ETB: exfoliative toxins A and B, respectively;

TSST: toxic shock syndrome toxin.

Table 2. Phenotypic and genotypic characteristics of the 55 *S. aureus* isolates recovered from healthy individuals in relation with their specific risk factors

<i>S. aureus</i>	Spa-type	MLST	PFGE profile	<i>agr</i> type	Toxins ^a	Phenotype of antibiotic resistance ^b	Resistance genes detected
Frequent contact with farm animals:							
C2444	t203	ST80	S23	III	PVL	PE-OX-FX-TE-ST-KA	<i>mecA</i> , <i>tetK</i> , <i>ant(6)-Ia</i> , <i>aph(3')-IIIa</i>
C2435	t318	ST30	S19	III	PVL	PE-TE	<i>tetK</i>
C2448	t645	ST121	S25	IV	PVL	PE-OX	
C2431 ^c	t159		S6	NT	ETA, ETB	PE-TE-ST	<i>tetK</i>
C2436	t046		S2b	III	TSST-1	PE-TE	<i>tetK</i>
C2438	t6222	ST46	S8a	I	TSST-1	PE-OX	
C2441	t6223	ST72	S21	I			
C2445 ^d	t6224	ST101	S24	II		PE-OX-TE-K	<i>tetK</i> , <i>aph(3')-IIIa</i>
C2430	t254		S17	NT		PE-TE-ST-KA	<i>aph(3')-IIIa</i>
C2442	t024		S5	I		PE	
C2447	t024		S5	I		PE-CP	
C2423	t018		S2a	NT		PE-OX	
C2425	t166		S35	III		PE	
C2428	t711		S16	I		PE-ST	
C2433	t084		S7	II		PE-TE	
C2440	t084		S7	II		PE-OX	<i>tetK</i>
C2434	t377		S18	I		PE	
C2437	t346		S34	NT		PE	
C2439 ^c	t164		S20	I		PE-TE-SX-ER	<i>dfrA</i>
C2443 ^c	t1743		S22	I		PE-OX	
Frequent contact with companion animals							
C2420	t254		S6	NT	ETA	PE-OX-ST-TE-KA-ER	<i>tetK</i> , <i>ant(6)-Ia</i> , <i>aph(3')-IIIa</i>
C2455	t012		S3	III	TSST-1	PE-CP	
C2456	t012		S2b	III	TSST-1	PE-OX	
C2451	t018		S3	III	TSST-1	PE-CP	
C2421	t725		S14	NT	TSST-1	PE-TE	
C2412	t040		S8b	I		PE	
C2415	t127		S13	III		PE-CL	
C2408	t537		S10	I		PE	
C2409 ^c	t254		S11	II		PE	
C2426 ^c	t1268		S36	I		PE	
Sporadic contact with animals							
C2450	t3638	ST22	S4a	I	PVL	PE	
C2454	t701		S29	I		PE-CP	
C2452 ^d	t224		S27	I		PE-OX	
No contact with animals							
C2411	t012		S2a	NT	TSST-1	PE-OX	
C2460	t5598		S33	NT	TSST-1	PE	
C2449 ^d	t223		S26	I	TSST-1	PE-CP	
C2459 ^d	t6225	ST1704	S32	III	TSST-1	PE	
C2419	t674		S34	NT	TSST-1	PE-OX ST	<i>ant(6)-Ia</i>
C2429	t002		S1	II		PE	
C2413	t008		S1	I		PE	
C2446	t094		S34	II		PE	
C2416	t279		S34	NT		PE	
C2407	t127		S1	II		PE-OX	
C2427	t230		S8a	I		PE	
C2424	t701		S8b	I		PE-OX	
C2453	t311		S28	II		PE-TE-CP	<i>tetL</i> , <i>tetM</i>
C2417	t559		S9	II		PE-OX-TE	
C2457	t587		S30	NT		PE-CP-ER	<i>msrA</i>
C2406	t922		S37	III		PE	
C2414	t2883		S12	I		PE-OX	
C2432 ^c	t095		S8a	I		PE	

C2410	t166	S35	III	PE
C2422 ^d	t166	S15	II	PE-OX
C2458 ^d	t166	S31	II	PE-OX-CP
C2418 ^d	t709	S4b	I	PE-OX-CP

^aPVL: Panton-Valentine leukocidin; ETA and ETB: exfoliative toxins A and B, respectively; TSST: toxic shock syndrome toxin.

^bPE: penicillin, TE: tetracycline, ST: streptomycin, KA: kanamycin, CP: ciprofloxacin, CL: chloramphenicol, OX: oxacillin, FX: cefoxitin, ER: erythromycin, SX: trimethoprim-sulfamethoxazole.

^cThis strain was obtained from individual which had contact with hospitalized patients

^dThis strain was obtained from individual which referred previous use of antibiotics

Figure 1- Dendrogram displaying the grouping of 55 *S. aureus* isolates. Similarities are based on simple matching coefficient and UPGMA algorithm of *Sma*I macrorestriction profiles demonstrated by PFGE.