

Isoenzymatic genotyping of *Staphylococcus aureus* from dairy cattle and human clinical environments reveal evolutionary divergences

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ABSTRACT

Background: The genetic variability of 610 *S. aureus* isolates from the hands of professional dentists (A), dental clinic environment air (B), bovine milk from cows with and without mastitis (C), an insufflator for milking equipment (D) and milking environment air (E) was studied by isoenzyme genotyping and genetic and cluster analysis. **Results:** Monoclonal and polyclonal patterns of *S. aureus* were detected in every bacterial population; however, isolates belonging to the same strain were not found among the populations, suggesting the genetic heterogeneity and the intrapopulation spread of strains. Genetic relationship analysis revealed the co-existence of highly related strains at low frequency among populations. **Conclusion:** The data suggest that some strains can adapt and colonize new epidemiologically unrelated habitats. Consequently, the occurrence of an epidemiological genotypic identity can assume a dynamic character (spread to new habitats), however infrequently. A tendency of microevolutionary and genetic divergences among populations of *S. aureus* from human sources (AB) and bovine milk (DE), and especially the mammary quarter (C), is also suggested. This research can contribute to the knowledge on the distribution and dissemination of strains and the implementation of control measures and eradication of *S. aureus* in important dental clinic environments, as well as animal environments and dairy production.

KEYWORDS: *Staphylococcus aureus*. Genetic diversity. Environment and anatomical site. Propagation dynamics. MLEE.

INTRODUCTION

The dissemination of *S. aureus* is considered a major public health problem because resistant strains can cause serious infections, especially in children and hospitalized patients^{1,2}. Dentists treat a wide variety of patients, a fact that exposes these health professionals to people colonized or infected with resistant microorganisms^{3,4}. The skin, the environment and instruments can be contaminated with saliva, blood or debris during routine dental treatment^{4,5}. Several researchers have noted an increase in the amount of microorganisms present during clinical procedures in dental clinic environments, suggesting contamination from aerosols, especially when high-speed devices or ultrasonic scalers are used^{6,7}. Among the species identified in microbiological studies, *viridans* group *streptococci* (VGS) and *Staphylococcus* spp. are the most prevalent microorganisms found on the surfaces of dental equipments⁶⁻⁸, including methicillin-resistant *Staphylococcus aureus* (MRSA), which has been detected on dental operator surfaces, air-water syringes and recliner chairs⁹.

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Received: 21 April 2017

Accepted: 18 December 2017

In animal science, mastitis is considered as one of the most important infectious diseases to occur in dairy cattle herds, causing significant economic loss in every part of the world. *S. aureus* is an important pathogen associated with bovine mastitis¹⁰, which spreads mainly within and among cattle animals during milking; udder being the main source of infection. Therefore, control measures are primarily designed to improve hygiene and milking routines, such as milking order and the immersion of teats. Other measures include dairy cattle therapy and proper disposal of infected animals. However, such control measures are not always effective in preventing new infections of *S. aureus*¹¹, indicating the complexity of the problem and the possibility of other sources of infection. The probability of udder infections increases if the host has direct contact with reservoirs of pathogens or indirect contact via fomites¹². Several studies investigating potential reservoirs and fomites of *S. aureus* on dairy properties have been performed¹³⁻²⁰.

Phenotypic and genotypic methods have allowed researchers to classify isolates of microorganisms in systematic, taxonomic, evolutionary, phylogenetic and epidemiological studies²¹⁻²³. Among these methods, multilocus enzyme electrophoresis (MLEE) has been used for several decades as a standard method to study eukaryotic population genetics²⁴ and systematics²⁵ as well as large-scale studies to estimate the genetic diversity and structure of natural populations of a variety of bacterial species²⁶⁻²⁹ and fungal species including yeasts³⁰⁻³³. Results of different studies, with some exceptions³⁴, suggest that relatively few clones of *S. aureus* are responsible for the majority of intramammary cattle infections with broad geographical distribution^{23,35-37}. Interestingly, the most prevalent clones of bovine mastitis are rarely isolated from humans, suggesting the host specificity of *S. aureus* clones^{16,35}. However, Mørk *et al.*³⁷ observed that common genotypes caused mastitis in cows, goats and dairy sheep, suggesting specificity of anatomical sites rather than host specificity. The distribution of *S. aureus* clones associated with human infections³⁸⁻⁴¹ and with bovine mastitis^{42,43} has been characterized and investigated using molecular techniques such as multilocus sequence typing (MLST). Important clonal complexes (CCs) associated with infections by *S. aureus* in human beings (CC1, CC5 and CC30) were also found in isolates from dairy cattle in geographically distinct herds²³. These observations have suggested the possibility of the transmission of strains between humans and cattle/dairy products, as previously reported^{42,43}. Further investigation demonstrated that those CCs (CC1, CC5 and CC30) were often isolated from asymptomatic carriers or patients with invasive disease^{38,40,41}; moreover, it has been suggested that the genetic background of these CCs allows greater

capacity to spread among humans⁴⁰. In addition, some of these CCs (CC5 and CC30), corresponding to some of the main epidemic strains of MRSA, are responsible for hospital-acquired MRSA (HA-MRSA) and/or community-acquired MRSA (CA-MRSA)⁴⁴.

The current study evaluated the genetic structure of natural populations of *S. aureus* of environmental, human and bovine origin. The genetic variability within and among populations of *S. aureus* from epidemiologically (i.e., professional dentists, dental clinic environment, bovine milk, insufflator and milking environment) and geographically (i.e., Alfenas and Passos cities, MG, Brazil) related and unrelated strains, the frequency of occurrence of strains and operational taxonomic groups (*taxon* and cluster) and possible epidemiological correlations were investigated by using isoenzymatic markers (MLEE), as well as genetic and grouping analysis.

MATERIALS AND METHODS

Microbiological sampling

This research was conducted in the Laboratory of Pharmacogenetics and Molecular Biology, Faculty of Medical Sciences and Center for Research in Animal Science, University of Alfenas, Alfenas city, MG, Brazil. A total of 610 bacterial isolates of oxacillin-resistant (ORSA) and oxacillin-sensitive *S. aureus* (OSSA), belonging to the bacteria collection of the laboratory, were kindly provided and used for the present study. These samples were previously isolated (culture medium – Mannitol salt phenol red agar) from the (i) hands of professional dentists (Population A: $n = 132$ ORSA and OSSA; Dental clinics, School of Dentistry, University of Alfenas, Alfenas city, MG, Brazil), (ii) dental clinic environment air (Population B: $n = 272$ ORSA and OSSA; Dental clinics, School of Dentistry, University of Alfenas, Alfenas city, MG, Brazil), (iii) bovine milk from cows with and without mastitis (Population C: $n = 31$ OSSA, being 16 sampling coming from cows with mastitis; Dairy Farm, Passos city, MG, Brazil), (iv) an insufflator for milking equipment (Population D: $n = 24$ OSSA; Dairy Farm, Passos city, MG, Brazil) and (v) milking environment air (Population E: $n = 151$ OSSA; Dairy Farm, Passos city, MG, Brazil), and characterized by microbiological methods of identification (Gram staining, growth on chromogenic medium CHROMagar *Staphylococcus aureus*[®], biochemical tests such as catalase test, coagulase test [Coagu-Plasma, Laborclin Produtos para Laboratórios Ltda.], clumping factor A test [Staphy Test, Probac do Brasil Produtos Bacteriológicos Ltda., Marnes La Coquette, France], mannitol fermentation test, DNase test and Voges-Proskauer

test)^{6,45} and antimicrobial susceptibility testing (by disk diffusion method)^{46,47} and confirmatory screening for resistance to oxacillin⁴⁸.

Enzyme extraction

Bacterial cultures were grown in flasks containing 200 mL of BHI (Brain Heart Infusion) at 35 °C for 24 h under constant shaking at 150 rpm (Shaker Incubator mod. NT 712, Nova Técnica Instrumentos e Equipamentos de Laboratório Ltda.). After growth, cells were centrifuged at 5,000 × *g* for 3 min and washed twice in sterile 40 mM PBS (pH 7.5), submitting each wash to the same centrifugal force. Pellets (~250 µL) were transferred to 2-mL microtubes (Biospec Products, Inc.) containing cold PBS (approximately 8 °C) and glass beads (0.45–0.55 mm) (1:1:1). These mixtures were kept on ice (4 °C) for 5 min and agitated 4 times in a BeadBeater machine (Biospec Products, Inc., Bartlesville, OK, USA) at 4,200 rpm for 30 s, at one-minute intervals. Cell fragments were centrifuged at 5,000 × *g* at 4 °C for 5 min (Eppendorf 5403, rotor 16F24-11). The resulting upper aqueous phase was applied to Whatman n3 filter papers (wicks), 12 × 5 mm in size, and maintained at -70 °C until time of use^{21,27,31-33}.

Electrophoresis and specific enzyme staining

The enzymes were separated in 13% (wt/vol) starch gels (dimensions 200 × 120 × 10 mm; Penetrose 30[®]; Refinações de Milho Brasil Ltda.) in Tris-citrate buffer pH 8.0 (electrode buffer diluted 1:29). The wicks were then immediately soaked in 5 µL [0.02% (wt/vol)] of bromophenol-blue solution and then perpendicularly applied to a longitudinally cut gel (20 mm). Electrophoresis was performed in a horizontal and continuous system at 130 volts at 4 °C overnight (bromophenol-blue migration equivalent to 80 mm) using Tris-citrate electrode buffer (pH 8.0). To assure reproducible results (i.e., the percentage of strains that give the same result on repeated testing), *S. aureus* ATCC[®] 29213 enzymes were included in each gel. After electrophoresis, gels were put on an acrylic base and sliced into 1.5-mm sections with the aid of rulers and #15 nylon strings. The sections were carefully placed inside white porcelain containers and stained for 10 enzymes^{21,31-33}. The enzymatic activities that were analyzed included: alcohol dehydrogenase (EC 1.1.1.1), sorbitol dehydrogenase (EC 1.1.1.14), mannitol-1-phosphate dehydrogenase (EC 1.1.1.17), malate dehydrogenase (EC 1.1.1.37), glucose dehydrogenase (EC 1.1.1.47), D-galactose dehydrogenase (EC 1.1.1.48), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), catalase (EC 1.11.1.6), and α- and β-esterase (EC 3.1.1.1.) (Table 1).

Genetic interpretation of the MLEE patterns

Pattern interpretation was performed following the general rules commonly accepted in the deduction of allelic composition for haploid organisms. The bands on the gels were numbered in order of decreasing mobility, and their corresponding alleles were numbered by using the same nomenclature. A lack of demonstrable activity for an enzyme was scored as one null allele at the corresponding gene locus. Each unique combination of alleles over the enzyme loci examined resulted in an electrophoretic type (ET) – subtype or strain. The percentage of polymorphic loci, the average number of alleles per locus and the average number of alleles per polymorphic locus were also determined^{21,31-33}.

Discriminatory power

The discriminatory power of the MLEE method based on genetic interpretation of the electrophoretic patterns was established by the numerical index of discrimination (*D*), according to the probability that two unrelated isolates sampled from the test population will be classified into different types (i.e., strains or ETs)^{31,33}. This probability can be calculated by Simpson's index of diversity, which was developed for the description of species diversity within an ecological habitat⁴⁹. This index can be derived from an elementary probability theory⁵⁰ and is given by the following equation: $D = 1 - \frac{1}{N(N-1)} \sum n_j(n_j - 1)$, where *N* is the total number of isolates in the sample population, *S* is the total number of types (strains) described, and *n_j* is the number of isolates belonging to the *j*th type. This equation was derived as follows. The probability that two isolates sampled consecutively will belong to that type is: $\frac{n_j(n_j - 1)}{N(N - 1)}$. These probabilities can be summed for all the described types to determine the probability that any two consecutively sampled isolates will be the same type. This sum can be subtracted from 1 to obtain the equation above. This equation can be applied both to a direct comparison of the discriminating power of typing methods and to analysis of the discriminating power of combined typing schemes. An index of greater than 0.90 would be desirable if the typing results are to be interpreted with confidence^{51,52}.

Grouping and genetic diversity analysis

The statistical methods of Nei⁵³ were used to estimate the genetic distance among the isolates and/or strains (ETs)

Table 1 - Systems and solutions utilized for the MLEE analyses of the *S. aureus* metabolic enzymes

EC number	Enzyme		Compound for staining				
	Name	Symbol	Substrate	Buffer	Salt	Coenzyme	Dye and Catalyser
1.1.1.1.	alcohol dehydrogenase	ADH	Ethanol (3mL) Isopropanol (2mL)	200 mM Tris-HCl pH 8.0 (q.s.p. 50mL) ^a		NAD 1% (2 mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.17	mannitol-1-phosphate dehydrogenase	M1P	Mannitol 1-phosphate (5mg)	200 mM Tris-HCl pH 8.0 (q.s.p. 50 mL) ^a		NAD 1% (2 mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.37.	malate dehydrogenase	MDH	2M Malic acid (6 mL) ^b	200 mM Tris-HCl pH 8.0 (q.s.p. 50 mL) ^a		NAD 1% (2 mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.47	glucose dehydrogenase	GDH	D-glucose (500 mg)	200 mM Tris-HCl pH 8.0 (q.s.p. 50 mL) ^a		NAD 1% (2 mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.48	D-galactose dehydrogenase	GLDH	Galactose (450mL)	Tris-HCl 100 mM pH 8.4 (q.s.p. 50 mL) ^c		NAD 1% (1 mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.49	glucose-6-phosphate dehydrogenase	G6PDH	Glucose-6-phosphate disodium salt (100 mg)	200 mM Tris-HCl pH 8.0 (q.s.p. 50 mL) ^a	100 mM MgCl ₂ (1 mL) ^d	NADP 1% (1 mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.11.1.6	catalase	CAT ^e					
3.1.1.1.	α- and β-esterase	EST	α- and β-Naphthyl acetate (1% solution in acetone) (1.5ml)	50mM Sodium phosphate pH 6.0 (q.s.p. 50mL) ^f			Fast Blue RR salt (25 mg)

Electrode buffer: Tris-citrate pH 8.0 [83.2 g of C₄H₁₁NO₃ (Tris), 33.09 g of C₆H₈O₇·H₂O (Citric acid), 1 L of H₂O]; Gel buffer: Electrode buffer diluted 1:29. ^a 24.2 g of C₄H₁₁NO₃ (Tris), 1 L of H₂O (pH adjusted with HCl); ^b 26.8 g of C₄H₆O₅ (DL-malic acid) and 16 g of NaOH in 0.1 L of H₂O (caution: potentially explosive reaction); ^c 12.1 g of C₄H₁₁NO₃ (Tris), 1 L of H₂O (pH adjusted with HCl); ^d 2.03 g of MgCl₂·6HCl (Magnesium chloride) in 0.1 L of H₂O; ^e Incubate gel slice for 30 min at 0 °C in 50 mL of 0.1 M sodium phosphate pH 7.0 buffer, then pour off solution, and immerse it in 50 mL of 1.5% potassium iodide solution (KI) for 2 min. Therefore, rinse gel slice with water, and immerse it in 50 mL of 0.03% hydrogen peroxide (H₂O₂) solution. Mix gently and remove stain solution when white zones appear on dark-blue background; ^f Sodium phosphate buffer pH 7.0: mix equal parts of 27.6 g of NaH₂PO₄·H₂O (monobasic) in 1 L of water and 53.6 g of Na₂HPO₄·7H₂O in 1 L of water, then dilute the mixture 1:25 with water.

of each population of oxacillin-resistant and/or oxacillin-

sensitive *S. aureus*: $d_{ij} = -\ln(I)$ or $d_{ij} = -\ln\left[\frac{\sum_k |x_{ki}x_{kj}|}{\sqrt{\sum_k x_{ki}^2x_{kj}^2}}\right]$,

where I is the normalized identity of genes among two populations (ranging from 0 to infinity), a measure of genetic distance based on the identity of genes (frequency of alleles for all loci, including monomorphic loci) among populations. This genetic distance measures the accumulated allelic differences per locus, which can also be estimated from the amino acid sequences of proteins and even for a distantly related species. As a consequence, if enough data are available, the genetic distances among any pair of organisms can be measured in terms of d_{ij} . In addition, this measure can be applied to any kingdom of organism regardless of the level of ploidy or mating scheme. Its interpretation in terms of enzyme loci infers that, on

average, zero to an infinite number of allelic substitutions are detected (by electrophoresis) in every 100 loci from a common ancestral strain^{31-33,53,54}.

Dendrograms were generated using the SAHN grouping method (Sequential, Agglomerative, Hierarchic, Nonoverlapping Clustering Methods) and the UPGMA algorithm (Unweighted Pair-Group Method Using an Arithmetic Average), based on their respective matrix d_{ij} ⁵⁵. Once MLEE provides all levels of relatedness, which must be resolved by DNA fingerprinting (i.e., identifying the same strain in independent isolates, identifying microevolutionary changes in a strain, identifying clusters of moderately related isolates and identifying completely unrelated isolates), a threshold (average value: \bar{d}_{ij}) was established in the dendrograms to identify clusters of identical isolates and highly related isolates ($0 \leq d_{ij} < \bar{d}_{ij}$) and taxa ($d_{ij} \geq \bar{d}_{ij}$)^{31,33,54,55}.

The Pearson product–moment correlation coefficient

$$\text{(ranging from } -1 \text{ to } +1), r_{jk} = \frac{\sum_{i=1}^n (X_{ij} - \bar{X}_j)(X_{ik} - \bar{X}_k)}{\sqrt{\sum_{i=1}^n (X_{ij} - \bar{X}_j)^2 \sum_{i=1}^n (X_{ik} - \bar{X}_k)^2}},$$

[where X_{ij} stands for the character state value of character i in OTU (Operational Taxonomy Unit) j , \bar{X}_j is the mean of all state values for OTU j , and n is the number of characters sampled], was used as a measure of the agreement between the genetic distance values implied by the UPGMA dendrograms and the ones from the original genetic distance matrices d_{ij} . These agreements were interpreted as follows: $0.9 \leq r_{jk}$, very good fit; $0.8 \leq r_{jk} < 0.9$, good fit; $0.7 \leq r_{jk} < 0.8$, poor fit; $r_{jk} < 0.7$, very poor fit. All of these analyses were obtained using the NTSYS-pc 2.1 program⁵⁵.

An additional index was used as a measure of genetic diversity of bacterial isolates, as proposed by Nei⁵⁶, which employs the genic frequencies (allele frequencies). This index allows hierarchical partition of diversity in its components “between” and “within” the experimental units. The unit may be the species or a large population constituted by several subpopulations or demes. Several hierarchical levels can be determined from the following formula: $H_T = 1 - \sum P_i^2$, where H_T and P_i correspond to the total diversity of species, considering a single locus and the average frequency of allele i at a given locus in the species as a whole (averaged over populations). The total diversity (H_T) is constituted by $H_T = H_S + D_{ST}$, where H_S and D_{ST} correspond to the component of diversity “within” populations and the component “between” populations, respectively, within the species (population) considered. In turn, the diversity genetic proportion given to the component “between” populations is estimated by the following equation: $G_{ST} = \frac{D_{ST}}{H_T}$. However, when considering multiple loci, the total diversity (H_T) of species (population) is calculated using the arithmetic average of the values estimated for each locus separately⁵⁶.

RESULTS

Genetic interpretation of the MLEE patterns

The isoenzymatic patterns of each population of oxacillin-resistant and/or oxacillin-sensitive *S. aureus*, including the type strain ATCC® 25923, were reproducible in different gels after three repetitions of each electrophoretic run (i.e., the reproducibility was excellent for the replicate samples: $\geq 95\%$). According to the haploid nature of *S. aureus*, these patterns demonstrated the following characteristics (Supplemental Table 1):

Population A (hands of professional dentists $n = 132$):

- Number of polymorphic loci: 34 (94.4%).
- Average number of alleles per locus: 1.861.
- Average number of alleles per polymorphic locus: 1.750.
- Electrophoretic types (ET): 127 strain (95.4% of total isolates).
- Discriminatory power: 0.99895, that is, 99.895% probability that two unrelated *S. aureus* isolates sampled from the test population will be classified in different strains.
- Clonality patterns: Monoclonal and polyclonal.

Population B (dental clinic environment air $n = 272$):

- Number of polymorphic loci: 43 (90.7%).
- Average number of alleles per locus: 3.00.
- Average number of alleles per polymorphic locus: 2.02.
- Electrophoretic types (ET): 235 strain (86.4% of total isolates).
- Discriminatory power: 0.99688, that is, 99.688% probability that two unrelated *S. aureus* isolates sampled from the test population will be classified in different strains.
- Clonality patterns: Monoclonal and polyclonal.

Population C (bovine milk from cows with and without mastitis $n = 31$):

- Number of polymorphic loci: 36 (100%).
- Average number of alleles per locus: 1.527.
- Average number of alleles per polymorphic locus: 1.527.
- Electrophoretic types (ET): 22 strain (70.9% of total isolates).
- Discriminatory power: 0.96559, that is, 96.559% probability that two unrelated *S. aureus* isolates sampled from the test population will be classified in different strains.
- Clonality patterns: Monoclonal and polyclonal.

Population D (an insufflator for milking equipment $n = 24$):

- Number of polymorphic loci: 23 (100%).
- Average number of alleles per locus: 1.130.
- Average number of alleles per polymorphic locus: 1.130.
- Electrophoretic types (ET): 23 strain (95.8% of total isolates).
- Discriminatory power: 0.99637, that is, 99.637% probability that two unrelated *S. aureus* isolates sampled from the test population will be classified in different strains.
- Clonality patterns: Monoclonal and polyclonal.

Population E (milking environment air $n = 151$):

- Number of polymorphic loci: 47 (92.2%).

- Average number of alleles per locus: 2.274.
- Average number of alleles per polymorphic locus: 1.882.
- Electrophoretic types (ET): 144 strain (95.3% of total isolates).
- Discriminatory power: 0.99947, that is, 99.947% probability that two unrelated *S. aureus* isolates sampled from the test population will be classified in different strains.
- Clonality patterns: Monoclonal and polyclonal.

Total Population ($n = 610$):

- Number of polymorphic loci: 51 (92.7%).
- Average number of alleles per locus: 3.652.
- Average number of alleles per polymorphic locus: 2.200.
- Electrophoretic types (ET): 553 strain (90.3% of total isolates).
- Discriminatory power: 0.999, that is, 99.9% probability that two unrelated *S. aureus* isolates sampled from the test population will be classified in different strains.
- Clonality patterns: Monoclonal and polyclonal.

Grouping and genetic diversity analysis

The genetic diversity of 610 isolates of *S. aureus* from 5 different populations (Populations A $n=132$, B $n=272$, C $n=31$, D $n=24$ and E $n=151$) and different geographic areas (Alfenas and Passos cities, Minas Gerais state, Brazil) was analyzed using Nei's distance⁵³ and an UPGMA dendrogram (Supplemental Figure 1). The genetic diversity of the total bacterial population ranged from 0 to 0.3810; that is, on average, the population of isolates contained from 0 to 38.1 allelic substitutions for each 100 loci, starting from a common ancestor. Taking into consideration the average genetic distance ($\overline{d_{ij}} = 0.0551 \pm 0.0624$) obtained from the total population of bacteria, these analyses also permitted the verification of the coexistence (threshold: $0.0551 > d_{ij} \geq 0$ – identical or highly related isolates/strains) or non-coexistence (thresholds: $0.1175 > d_{ij} \geq 0.0551$ – moderately related isolates/strains; $d_{ij} \geq 0.1175$ – isolates/strains unrelated or genetically related to distance) of strains among the bacterial populations and the inference of possible propagation among them or microevolutionary alterations and better adaptations.

Eighty highly polymorphic *taxa* (of I out LXXX) were identified from the d_{ij} value over 0.1175 (*taxa* genetically related to distance). Then, moderately related isolates/strains or clusters ($0.1175 \geq d_{ij} > 0.0551$) were identified within each of these large *taxa*. A total of 91 clusters ($d_{ij} = 0.0551$) distributed in these *taxa* was also identified. Each cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates

(strains) ($0.0551 > d_{ij} \geq 0$). The interpretation of the UPGMA dendrogram and, consequently, the qualitative distribution (i.e., epidemiological characteristic of each bacterial population) and quantitative distribution of these isolates/strains and clusters of each taxon, are shown in the Supplemental Table 2.

The genetic diversity proposed by Nei⁵⁶, which enables the hierarchical partition of diversity into its components “between” and “within” the experimental units, was also determined for bacterial isolates from each of the 5 populations (A, B, C, D and E). The heterozygosity for each enzyme locus was determined in these populations. The total diversity (H_T) was calculated from the arithmetic averages of the values estimated for the loci existing between paired populations. The values (H_T) ranged between 0.8642 and 0.9565 in ten pairings [i.e., A versus B ($H_T = 0.9319$), A versus C ($H_T = 0.8950$), A versus D ($H_T = 0.8642$), A versus E ($H_T = 0.9499$), B versus C ($H_T = 0.9527$), B versus D ($H_T = 0.9485$), B versus E ($H_T = 0.9547$), C versus D ($H_T = 0.9077$), C versus E ($H_T = 0.9565$) and D versus E ($H_T = 0.9424$)]. The components of diversity “within” (H_S) and “between” (D_{ST}) bacterial populations and the genetic diversity that is assigned to the component “between” populations (G_{ST}) were then determined. These results revealed indices of total genetic variability of 0.1%, 2.5%, 1.8%, 2.5%, 0.6%, 0.3%, 0.1%, 4.1%, 1.1% and 0.0% attributable to differences “between” populations A versus B, A versus C, A versus D, A versus E, B versus C, B versus D, B versus E, C versus D, C versus E and D versus E, respectively, whereas the total genetic variability present “within” these populations was 99.9%, 97.5%, 98.2%, 97.5%, 99.4%, 99.7%, 99.9%, 95.9%, 98.9% and 100%, respectively. Such variability can also be observed in the UPGMA dendrogram generated from the matrix of genetic variability⁵⁶ attributable to differences “between” populations (Figure 1).

DISCUSSION

Inter and intrapopulation clonality of *S. aureus*

In this study, the isoenzymatic electrophoresis profiles of *S. aureus* isolates from five distinct populations (A, B, C, D and E) were reproducible in three replicates of electrophoretic tests ($\geq 95\%$). The high discriminatory power of MLEE (0.965–0.999) proves it to be a powerful and reliable tool for typing *S. aureus* in epidemiological studies (Supplemental Table 1). The reported results for the reproducibility and discriminatory power of MLEE accord with those previously reported in studies of medical relevance^{21,26,27,30}; however, the discriminatory power

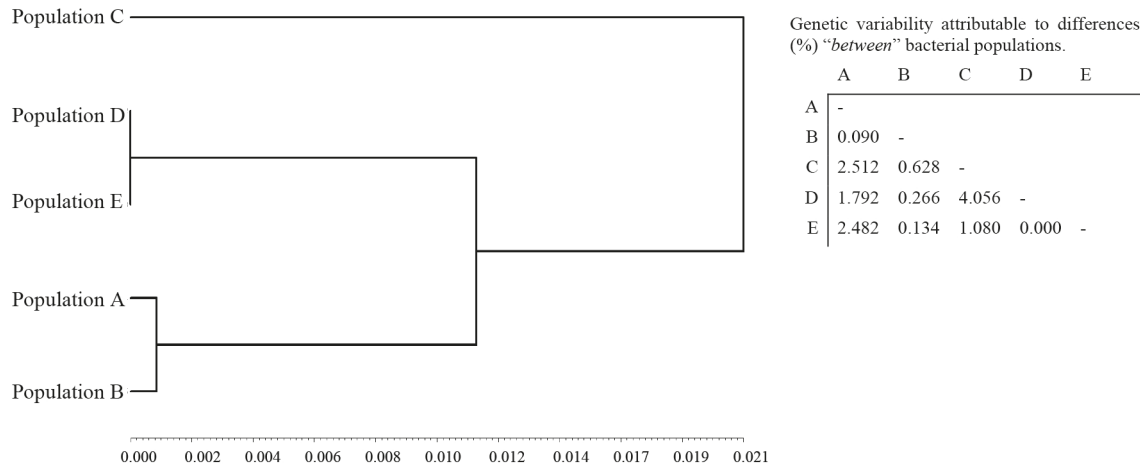


Figure 1 - Total genetic variability attributable to differences "between" bacterial populations of *S. aureus* from the hands of professional dentists (A), from dental clinic environment air (B), from bovine milk from cows with and without mastitis (C), from an insufflator for milking equipment (D) and from milking environment air (E). UPGMA dendrogram generated from the genetic diversity matrix⁵⁶

described in this research was greater than those reported by other groups of researchers^{36,57}.

S. aureus is a heterogeneous bacterium (polymorphic)⁵⁸ with a clonal population structure⁴⁰. Consequently, it is believed that *S. aureus* does not undergo extensive recombination, diversifies in large part by nucleotide mutations and displays a high degree of linkage disequilibrium (non-random associations among genic loci). In this study, qualitative and quantitative variations of polymorphic loci, average number of alleles per locus and average number of polymorphic alleles of *S. aureus* were observed across all 5 populations sampled. These variations have been observed in several studies of genetic diversity of *S. aureus* populations from human and bovine sources^{35,36,57-59}. In addition, the identification of *S. aureus* strains in each population revealed polyclonal and monoclonal patterns within the populations. However, no monoclonal pattern was observed between the populations (A, B, C, D and E). These data suggest a high genetic diversity of *S. aureus* in every bacterial population studied (i.e., intrapopulation genetic heterogeneity) and among them, even without any correlation of interpopulation monoclonality. Specific and limited patterns of spread and transmission of *S. aureus* were also suggested to occur in every bacterial population. The evidence of interpopulation monoclonality and interpopulation polyclonality of *S. aureus* has been reported previously. Strains of *S. aureus* toxin 1 producers (Toxic Shock Syndrome – TSS) from ovine sources were genotypically characterized (*tst* gene) and considered distinct from those clones associated with cases of TSS in humans (approximately 90%), suggesting that allele differences exist (structural *tst* gene) among strains recovered from different host species⁶⁰.

The host specificity among *S. aureus* clones was also demonstrated^{61,62}. An extensive study from 2.077 *S. aureus* isolates from humans, cows and sheep revealed considerable genetic diversity among strains and found that most of the 252 identified clones were preferentially associated with a single species of host, whereas only 6 of 33 clonal strains were shared among cattle and humans. These results suggested that the ability of the bacterium to colonize humans or cows evolved several times during the differentiation of *S. aureus* populations and provided strong evidence for host specificity among clones⁶². The concept of host specialization among *S. aureus* strains is also reinforced by the fact that certain bacterial clones are predominantly associated with certain hosts, in other words, humans or animals (dairy cow), although some clones can be shared among both hosts. This fact indicates that the successful transfer of bacteria between humans and animals (dairy cow) is limited³⁵.

Other evidence of genetic heterogeneity and host specificity comes from an analysis of *S. aureus* strains isolated from bovine teat skin, human skin, milking equipment and bovine milk. The analysis employed the PFGE method of genomic DNA digested by *Sma*I. Twenty-four main types and 17 subtypes among the isolates of 43 herds were identified, and there was still discrimination between isolates of bovine teats and milk. Although small in number, identical pulsotypes were found on human skin and on bovine teat skin, whereas the milking equipment harbored distinct pulsotypes; that is, there were both skin and milk strains. These results suggest that *S. aureus* strains from skin and milk can be transmitted via milking machines; however, there is no relationship with potential sources of intramammary infections caused by *S. aureus* in dairy cows¹⁸.

Genetic relationship of *S. aureus* clusters/taxa

The analysis of the genetic relationship among the *S. aureus* strains was determined satisfactorily using Nei's genetic distance⁵³ and an UPGMA dendrogram⁵⁵, as demonstrated by correlation coefficient based on Pearson product-moment ($r_{jk} = 0.84949$) [i.e., good agreement between d_{ij} (matrix of genetic distance) and elements C_{jk} (matrix of correlation derived from the UPGMA dendrogram)] (Supplemental Figure 1 and Supplemental Table 2). A high degree of genetic polymorphism ($0.000 \leq d_{ij} < 0.381$) was found in the total population of bacterial isolates (i.e., on average, from zero to 38.1 allele substitutions were detected for each 100 existing loci from a common ancestral strain). Such isolates were distributed within 80 taxa (from I to LXXX), which were determined from a genetic distance of $0.1175 < d_{ij} \leq 0.3810$ (i.e., taxa genetically related to distance – strains/isolates not related). The taxon I presented a larger number of isolates, strains and bacterial clusters, followed by taxa IV, L, XXI, X, XXII, LXIV and LVII, LVI, V and II, XXIV, VI, XIII, III, XXIII and XXXVII, XXXVIII and LXVI, LXXIX, XVI, XLII and XVII, XII, XXXV and LXII, LIII, XLI and XXVI, LX, LII and LXVII, LIX, LXV, LXIX, XXIX, XLVI, LXIII and LXXIII, VIII, LI, IX, XI, XXX, XXXII and XXXIII, and others which had only one isolate/ET and no cluster. These results also suggest a high degree of genetic polymorphism because most isolates/strains of *S. aureus* ($n = 365$; 59.8%) can adapt to several population ecological niches (i.e., in two or even five distinct population origins: populations A, B, C, D and E), although allocated within few taxa ($n = 16$: taxa I, IV, V, VI, VIII, X, XI, XIII, XXII, XXIII, XXX, XXXIII, L, LVI, LIX and LXIV). However, a lower frequency of highly polymorphic isolates/bacterial strains ($n = 245$; 40.2%) can be specific to certain population groups best adapted to their ecological niches, genetically divergent and distributed in many taxa ($n = 64$).

The genetic relationship among clusters and between clusters and isolated/strain non-clustered, although allocated within a single taxon, was considered moderately related ($0.0551 < d_{ij} \leq 0.1175$). In turn, each cluster has two or more isolates/strains that are identical or highly related ($0.000 \leq d_{ij} \leq 0.0551$). Highly related strains come from a common ancestor, that is, descendants have undergone microevolution and adaptations as a consequence of recombination (not extensive), nucleotide mutations and non-random associations among genetic loci (linkage disequilibrium)^{40,58}. Therefore, these data suggest the possibility of intrapopulation microevolutionary processes for *S. aureus* (i.e., on average, from zero to 5.5 allele substitutions in 100 existing loci were detected from

a common ancestral strain), as demonstrated in most of the clusters ($n = 75$ 82.4% clusters harboring isolates/strains exclusively of populations A, B, C, D or E) and, consequently, the propagation of these microorganisms. However, epidemiological genotypic identity was suggested, that is, epidemiologically related populations maintain *S. aureus* strains (identical and/or highly related, moderately related, and completely unrelated) that genetically diverge from those epidemiologically unrelated populations. Interestingly, although occurring at a low frequency ($n = 16$ 17.6% clusters), such microevolutionary changes or the co-existence of highly related strains (non-identical) were observed among bacterial populations of different origins [i.e., co-existence of highly related strains in populations A and B (clusters II and XI), in populations B and D (clusters XXX, XLVI and XLVIII), in populations B and E (clusters XVII, XVIII, XX, XXVIII, LX, LXII and LXIX), in populations D and E (cluster XLIV), in populations A, B and D (cluster XXIX), in populations B, D and E (cluster XLV) and in populations A, B, D and E (cluster I)]. These results suggest the hypothesis that some bacterial strains of *S. aureus* can adapt and colonize new habitats by spreading from indirect sources and are not epidemiologically related. Consequently, the occurrence of a genotypic identity can take an epidemiological dynamic character (i.e., spread to new habitat), although this is a low-frequency occurrence. The geographical location of bacterial isolates of *S. aureus* can partially explain this epidemiological genotypic identity. In fact, the habitat can have an important role on the adaptation of genotypically related bacterial groups and the occurrence of that identity, given that the populations A and B or C, D and E were geographically related.

The total genetic variability of populations of bacterial isolates, as proposed by Nei⁵⁶, revealed a low index (<5%) attributable to differences “between” populations and a high index attributable to differences “within” populations (>95%). However, these data suggest a tendency of genetic divergence and microevolution between the *S. aureus* isolates of human origin (populations A and B) and bovine milkmaid origin (populations D and E), especially the mammary quarter animal anatomical site (population) (Figure 1). Another hypothesis suggests that populations from *S. aureus* strains of environmental and animal origin (populations D and E), as well as those of environmental and human origin (populations A and B), contribute little to the colonization (adaptation) and infection of certain animal anatomical sites (i.e., the mammary quarter, population C), regardless of the events of bacterial propagation, as previously described^{18,63,64}. Under this hypothesis, intrinsic factors of the host could be involved in the selection and

adaptation process or genetic convergence of groups of bacterial strains. This hypothesis should also be explored.

The molecular differentiation and clonal relationship (PFGE method and binary typing) of *S. aureus* (MRSA and MSSA) isolated from bovine mammary secretions, geographically unrelated bovines and humans (USA and the Netherlands), and the association of bacterial strains with the clinical observations in herds (clinical symptomatology and somatic cell counts – SCC) were analyzed⁶⁵. Some PFGE (sub)types (A, F, G, B1, B2, E1 and E2) were identified only once, while PFGE types C, D and E were found in two, three, and four herds, respectively, as were 16 binary types. A limited number of prevalent types of *S. aureus* recovered from bovine mammary secretions, as well as the heterogeneity genetic, were found within and among herds⁶⁵, suggesting that certain variants present in the environment may have a predilection for causing intramammary infections^{36,65-67}. This genetic heterogeneity (subclonal) within the herds may be due to temporal evolution (longitudinal research over a year), allowing additional genetic diversification^{68,69}. The cluster analysis (UPGMA method) associated with binary typing indicated clusters of bovine strains ($n = 16$) and human strains ($n = 5$) with 90 to 95% similarity. However, at the highest level of similarity (100%), all the clones were host specific. These results were also consistent with the concept of host specificity among *S. aureus* clones and suggest that the successful transfer of bacteria between humans and animals is not a common event^{35,65}, although possible^{13-14,18,42,61-63,70-73}.

S. aureus isolates ($n = 227$) from several herds of dairy cattle with mastitis, located in the southeastern region of Brazil (i.e., 18 dairy herds distributed among 9 municipal districts of the Rio de Janeiro State) were investigated using PFGE and MLST genotyping and analysis of genetic similarity (Dice coefficient and UPGMA algorithm)²³. The PFGE method identified 60 pulsotypes (strains), which were distributed among 6 clonal complexes (CCs) (i.e., each clonal complex realized pulsotypes with $S_{Dice} > 65\%$) characterized by MLST. The predominance of a limited number of closely related pulsotypes (suggesting common ancestry) responsible for bovine mastitis in distinct herds (different geographical locations) and within the herds suggested that these strains have a greater capacity to propagate and cause intramammary infections. The majority of pulsotypes belonged to CC126 (recovered from 13 herds and 8 municipal districts) and CC97 (recovered from 14 herds and 9 municipal districts), which was represented by 91% of the isolates²³. Observations indicated that CC97 and CC126 were rarely or never detected among bacterial isolates from human population, respectively, suggesting the specificity of intramammary infections to ruminants^{23,40,42}.

In addition, important CCs associated with infections by *S. aureus* in humans (CC1, CC5 and CC30) were found in bacterial isolates from dairy cattle in 6 herds located in 5 municipal districts²³.

A prospective analysis using PFGE typing (bacterial genomic DNA digested with *Sma*I), PCR of virulence genes (hemolysins – *hla* to *hlg*; leukocidins – *lukED* and *lukM*; superantigens – *sea*, *sec*, *sed*, of *seg* to *seo*, *seu* and *tst*; adhesins – *fnbA* and *fnbB*; and resistance to penicillin and methicillin – *blaZ* and *mecA*) and genetic relationship (coefficient of Dice and grouping analyses) of *S. aureus*, collected from intramammary infection sites and other extramammary sources (teat skin of dairy cows, teat channels and skin lesions; milking liners; hands and nostrils of milking staff) from two dairy herds independent establishments (herds I and II) of southern Finland, was performed to study the possible sources and reservoirs of bovine intramammary infections²⁰. In this research, unique predominant bacterial genotypes were found in each herd, with the number of pulsotypes in the herd II (an open herd, including imported heifers from different regions of Finland) higher ($n = 7$) than that from herd I (a closed herd; $n = 3$), corroborating other reports on the high polymorphism (i.e., higher genetic heterogeneity) of isolates obtained from open herds¹⁷. Despite the existence of specific pulsotypes within each herd, the genotypes that were most likely better adapted to extramammary multiplication also caused intramammary infections, suggesting the existence of potential extramammary sources as the means of transfer. No correlation was found between specific genes for virulence and the source of the isolate. Still, identical pulsotypes in different herds were reported as being capable of harboring different virulence genes and resistance. In a herd, some pre-labor heifers harbored *S. aureus* strains in colostrum similar to those of lactating animals, and in both herds, the milking workers also displayed *S. aureus* strains identical to those of the animals, but the origin of colonization was considered uncertain²⁰. Other factors may contribute to the selection and diversity of *S. aureus* strains in herds. The number and diversity of *S. aureus* strains will most likely rise if new strains are introduced via importing cattle, as previously shown^{17,20}. Still, the successful control of infection of mastitis can increase the diversity of strains but decrease the spread of strains within the herd, whereas the failure to control can lead to the spread of only one or a few dominant strains throughout the herd. Pre-parturient heifers can also be an important reservoir of *S. aureus*, and strains originating from their skins were capable of causing intramammary infections in dairy cattle from the same herd¹⁷. However, herds may also harbor different strains of those lactating

cattle¹⁸. Recent reports on epidemiological studies of *S. aureus* strains in humans and animals, including isolates from bovine mastitis, suggest host specificity although a high degree of variation within and among clonal strains of *S. aureus* originating from different host animals⁷⁴⁻⁷⁷.

CONCLUSION

This research investigated the intra- and interpopulational genetic diversity of *S. aureus* from certain environments and anatomical sites of bovines and humans, epidemiologically related and nonrelated [i.e., the hands of professional dentists (population A), dental clinic environment air (population B), bovine milk from cows with and without mastitis (population C), an insufflator for milking equipment (population D) and milking environment air (population E)]. Monoclonal and polyclonal patterns of bacterial strains were detected in each of the populations, but isolates belonging to the same *S. aureus* strain were not found between the populations, which initially suggested genetic heterogeneity and the intrapopulational propagation of *S. aureus* strains.

The genetic relationship analysis revealed the distribution of strains within 80 *taxa*, genetically related to the distance ($d_{ij} \geq 0.1175$), suggesting a high degree of polymorphism in the total population of the *S. aureus* strains. Most of these unrelated strains were distributed in a few *taxa* that could adapt to several population ecological niches (i.e., in two or even five distinct population origins), whereas a lower frequency of unrelated strains was distributed in many *taxa* and can adapt to specific ecological niche populations. The propagation of highly related intrapopulational *S. aureus* strains and the existence of microevolutionary processes were suggested by the observations of the contents of the clusters ($d_{ij} = 0.0551$). Bacterial sampling from epidemiologically related populations showed identical strains and/or strains highly related and diversified in clusters, but distinct from those strains from epidemiologically unrelated populations, suggesting an epidemiological genotypic identity.

In addition, the coexistence of highly related strains was also observed between bacterial populations of different origins, although at low frequency. These data reinforce the hypothesis that some bacterial strains of *S. aureus* can adapt and colonize new habitats from indirect propagation sources and are unrelated epidemiologically. Consequently, the occurrence of a genotypic identity can assume an epidemiological dynamic character (i.e., spread to new habitat), though infrequently. Ultimately, a low index (<5%) attributable to differences “*between*” bacterial populations and a high rate attributable to differences “*within*” them (>95%) were found. The tendency of genetic differences and microevolution between populations of *S. aureus* of human

origin (populations A and B) and dairy cattle (populations D and E) and their environment, especially the mammary quarter (population C), was suggested. Intrinsic factors of the host could be involved in the selection and adaptation process, or genetic convergence, of bacterial strains groups, which should be explored further in the future. This research contributes to the knowledge of the distribution and dissemination of strains and the implementation of measures for the control and eradication of *S. aureus* in important medical and dental clinic environments, as well as in animal environments and dairy production (e.g., good biosafety practices and infection control, use of personal and collective protective equipment, implementation of containment barriers, installation of air purifiers, periodic monitoring and treatment of the carrier state of pathogens).

AUTHORS' CONTRIBUTIONS

MFG Boriollo, RC Bassi and JF Höfling wrote and revised the draft, and MFG Boriollo and RC Bassi aided microbiological and genetic assays. MFG Boriollo and JF Höfling performed statistical analysis. MFG Boriollo and JF Höfling have given final approval of the version to be published. All authors have read and approved the final manuscript.

ACKNOWLEDGEMENTS

This study was supported by the Fundacao de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG process N° APQ-3897-4.03/07) and the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq process N° 157768/2011-2).

We thank Elsevier Language Services for help with English language editing.

CONFLICT OF INTERESTS

The authors declare no conflicts of interests.

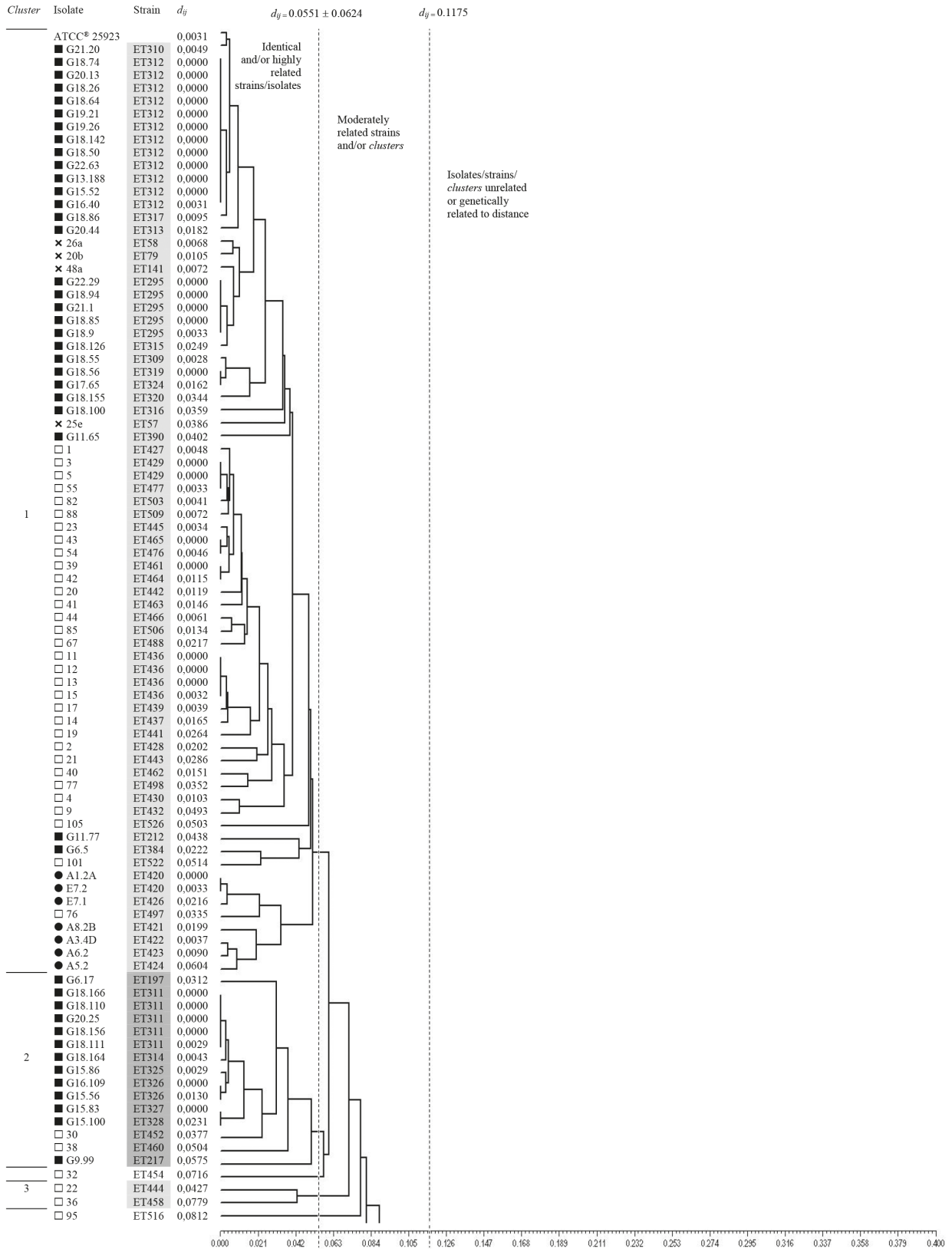
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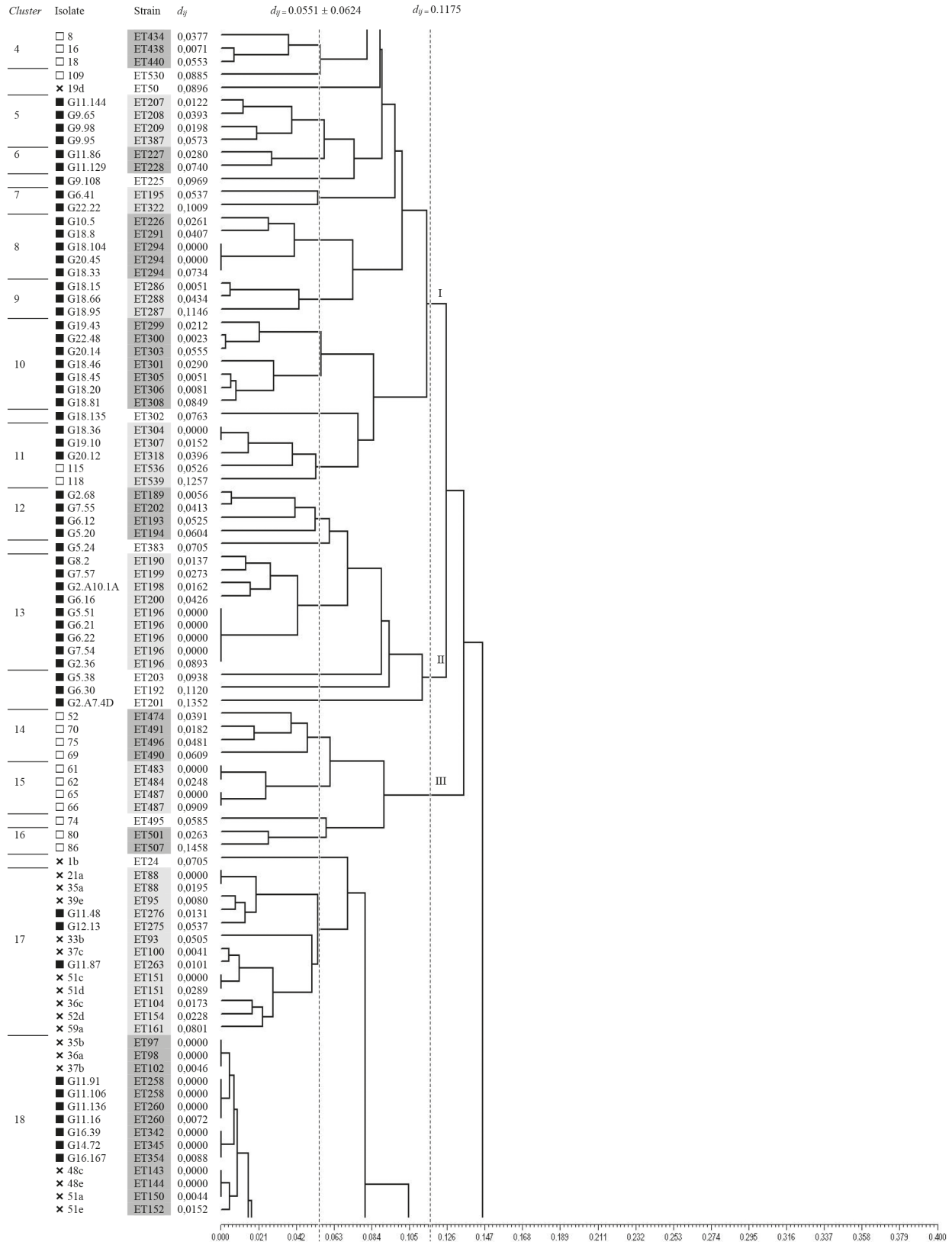
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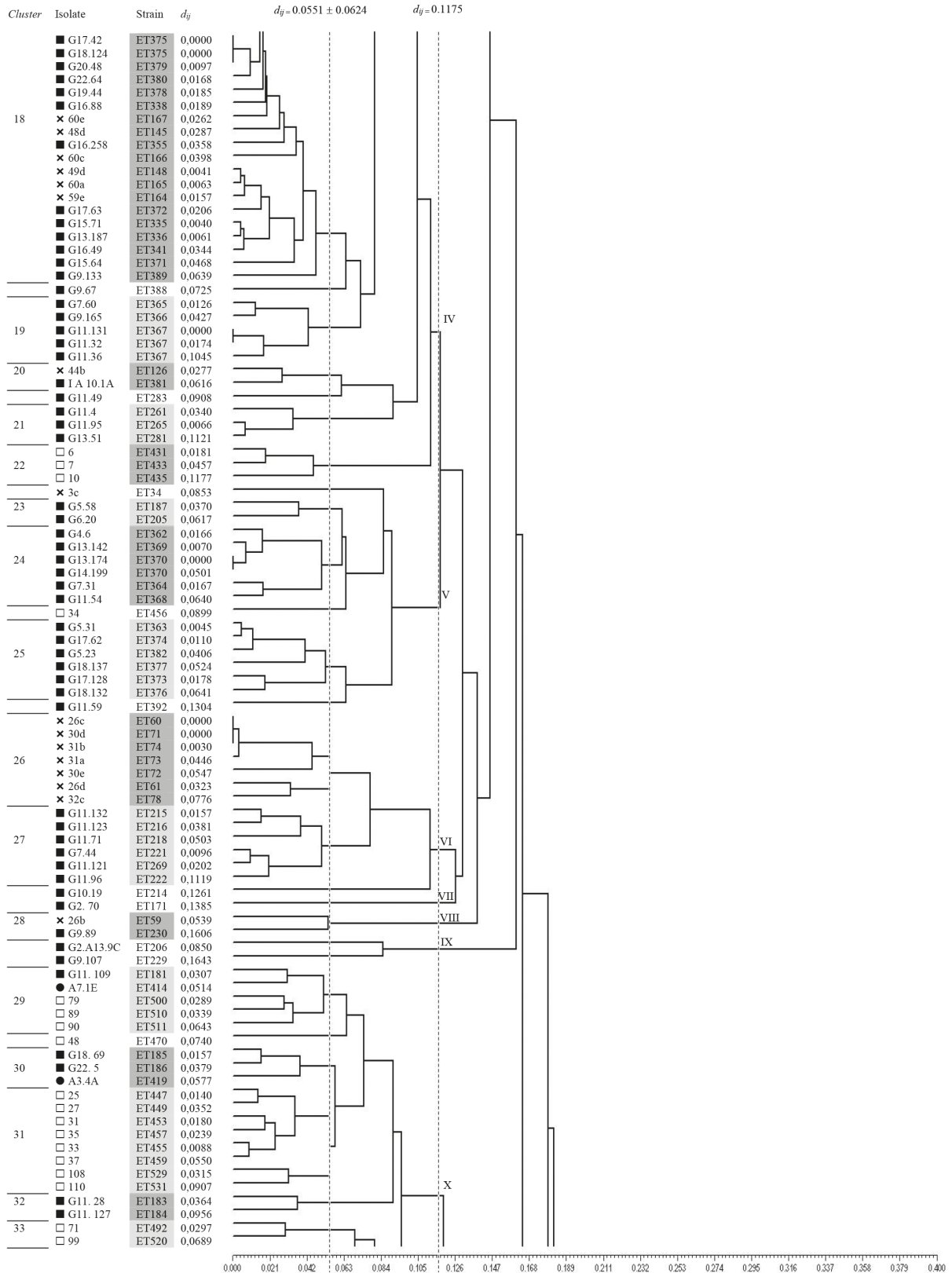
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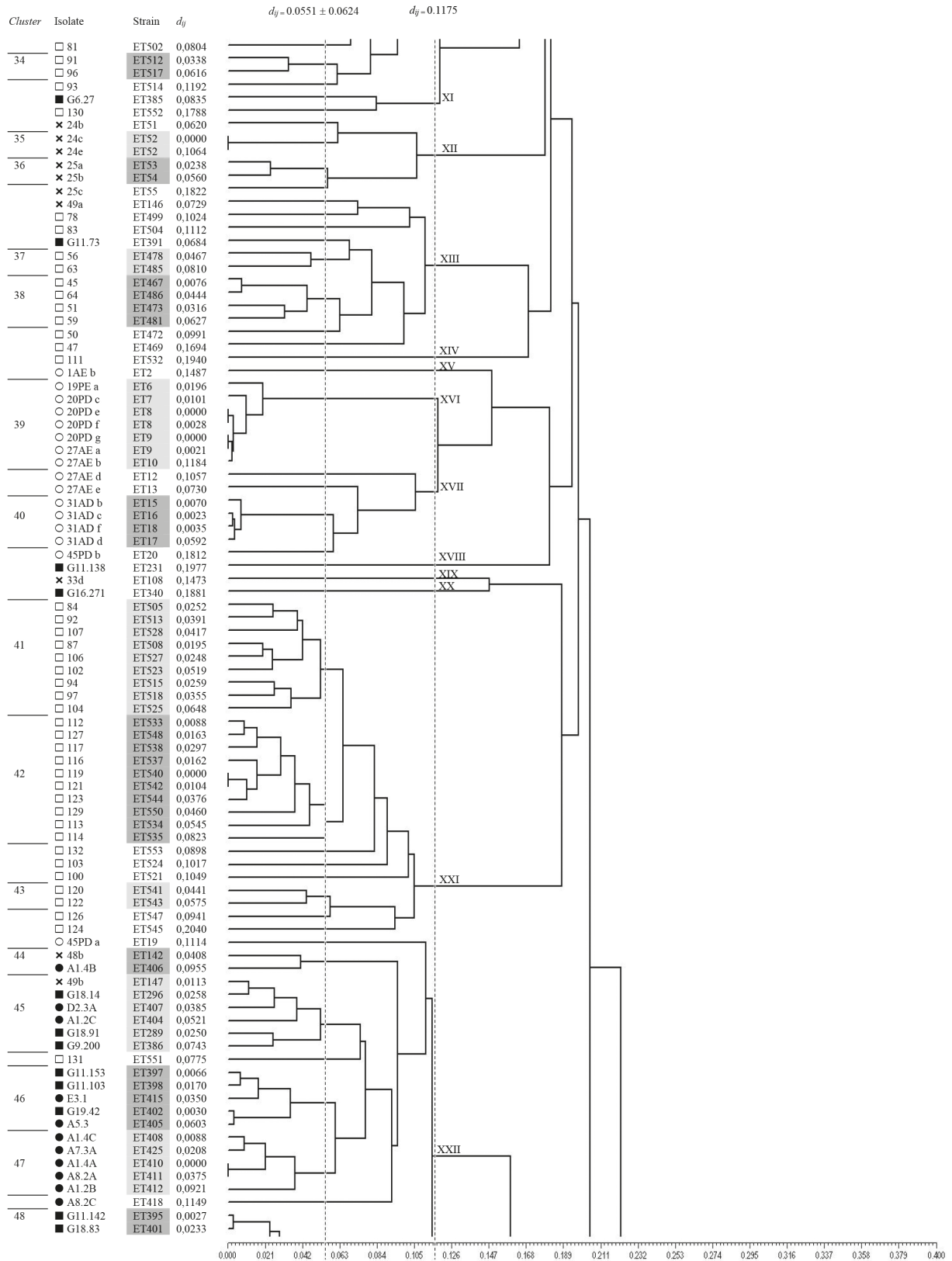
Supplemental Figure 1 - Genetic relationship between 554 strains of *S. aureus*, including the type strain *S. aureus* ATCC® 25923, from five bacterial populations (A[□]: hands of professional dentists; B[■]: dental clinic environment air; C[○]: bovine milk from cows with and without mastitis; D[●]: insufflator for milking equipment; and E^{*}: milking environment air). UPGMA dendrogram ($r_{jk} = 0.84949$: good concordance) generated from the genetic distance matrix⁵³ and the genetic interpretation of the patterns MLEE



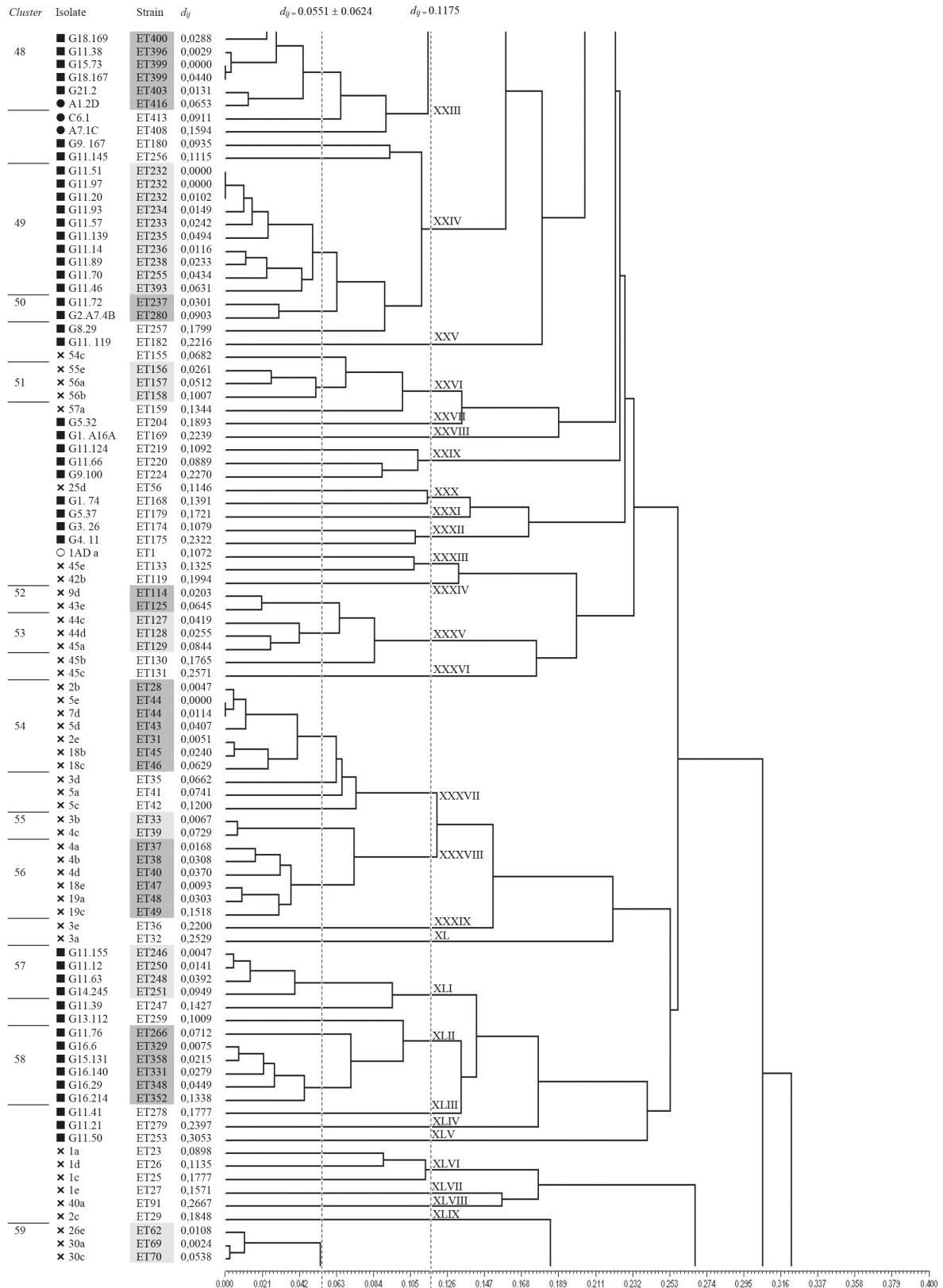
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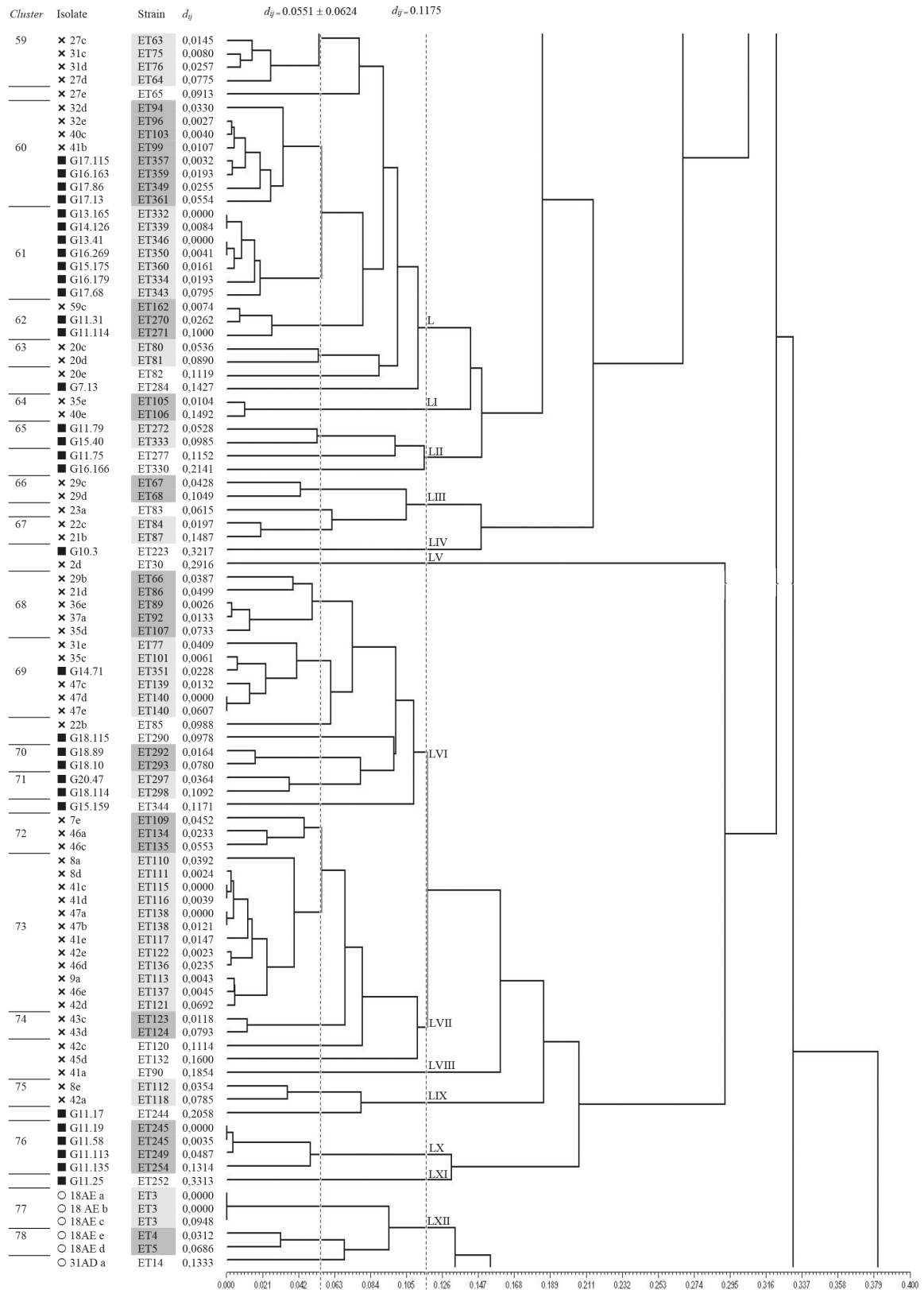
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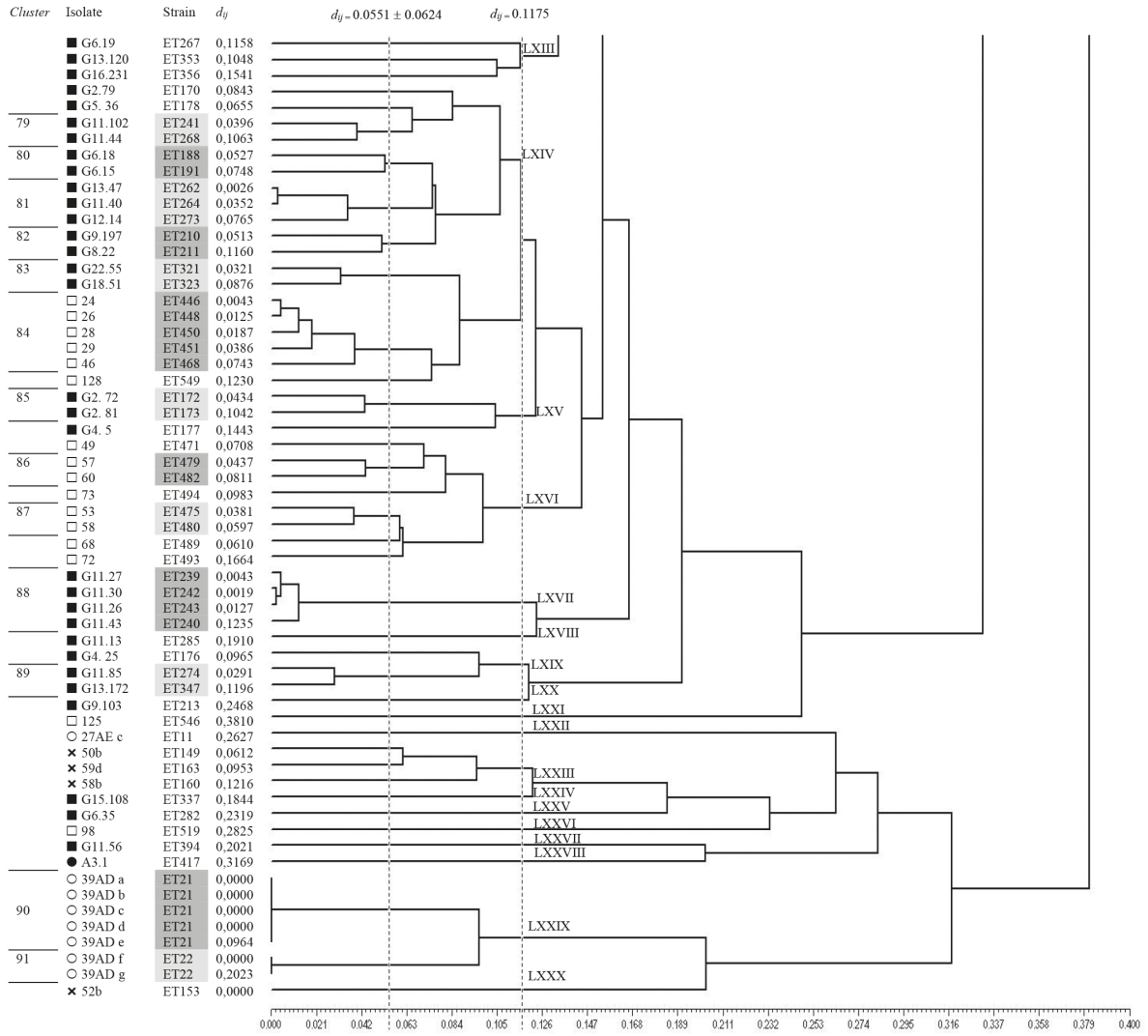
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ET	NI	P	Alleles of 55 loci enzymatic																																	
			Adh			Sdh			M1p			Mdh			Gdh			Gldh			G6pdh			Cat			α-Est			β-Est						
	1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	5	6	7	1	2	3	1	2	3	4	5			
204	1	B	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
205	1	B	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
206	1	B	-	d	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
207	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
208	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
209	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
210	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
211	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
212	1	B	-	b	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
213	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
214	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
215	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
216	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
217	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
218	1	B	-	b	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
219	1	B	-	c	e	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
220	1	B	-	-	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
221	1	B	-	-	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
222	1	B	-	-	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
223	1	B	-	b	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
224	1	B	-	c	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
225	1	B	-	c	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
226	1	B	-	-	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
227	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
228	1	B	-	-	a	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
229	1	B	-	d	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
230	1	B	-	-	-	-	-	c	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
231	1	B	-	d	-	-	-	b	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
232	3	B	-	-	b	a	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
233	1	B	-	-	b	a	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
234	1	B	-	-	b	a	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
235	1	B	-	-	b	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	1	B	-	-	b	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
237	1	B	-	-	b	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ET, NI, P correspond to the electrophoretic type (bacterial strain), number of isolates and bacterial populations A (hands of professional dentists), B (odontological environment air), C (bovine milk with and without mastitis), D (an insuflator for milking equipment) and E (milking environment air), respectively. [-] null allele.

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			1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	5		
408	2	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
410	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
411	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
412	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
413	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
414	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
415	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
416	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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424	1	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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435	1	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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437	1	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
438	1	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
439	1	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
440	1	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
441	1	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ET, NI, P correspond to the electrophoretic type (bacterial strain), number of isolates and bacterial populations A (hands of professional dentists), B (odontological environment air), C (bovine milk with and without mastitis), D (an insuflator for milking equipment) and E (milking environment air), respectively. [-] null allele.

Supplemental Table 1 - Allelic profiles of 553 electrophoretic types (ET; strains) of *S. aureus* isolated from the hands of professional dentists (population A), dental clinic environment air (population B), bovine milk from cows with and without mastitis (population C), an insuflator for milking equipment (population D) and milking environment air (population E) (cont.)

ET	NI	P	Alleles of 55 loci enzymatic																															
			Adh			Sdh			M1p			Mdh			Gdh			Gldh			G6pdh			Cat			α-Est			β-Est				
			1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	5	6	7	1	2	3	1	2	3	4
442	1	A	-	-	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
443	1	A	-	-	a	a	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
444	1	A	-	-	a	a	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
445	1	A	-	a	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
446	1	A	-	-	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	i	-	-	-	-	a
447	1	A	a	-	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
448	1	A	a	-	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	i	-	-	-	-	a
449	1	A	a	-	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
450	1	A	a	a	b	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	i	-	-	-	-	a
451	1	A	-	a	-	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	i	-	-	-	-	a
452	1	A	-	a	-	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
453	1	A	-	a	-	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
454	1	A	-	a	-	-	-	c	-	-	a	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
455	1	A	-	a	-	b	-	-	c	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
456	1	A	-	-	b	-	-	-	c	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
457	1	A	-	a	-	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
458	1	A	-	a	-	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
459	1	A	-	a	-	b	-	-	c	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
460	1	A	-	a	-	b	-	-	c	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
461	1	A	a	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
462	1	A	a	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
463	1	A	a	a	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
464	1	A	a	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
465	1	A	-	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
466	1	A	-	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
467	1	A	-	d	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
468	1	A	-	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	h	-	-	-	-	a
469	1	A	-	d	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
470	1	A	-	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
471	1	A	-	d	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
472	1	A	-	d	-	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
473	1	A	-	d	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
474	1	A	-	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	b	-	-	-	-	a
475	1	A	-	-	b	-	-	c	-	-	-	-	-	a	-	-	c	-	-	a	-	-	a	-	-	d	-	-	h	-	-	-	-	a

ET, NI, P correspond to the electrophoretic type (bacterial strain), number of isolates and bacterial populations A (hands of professional dentists), B (odontological environment air), C (bovine milk with and without mastitis), D (an insuflator for milking equipment) and E (milking environment air), respectively. [-] null allele.

Supplemental Table 2 - Interpretation of UPGMA dendrogram generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the MLEE patterns of *S. aureus*

Taxon ^a	Cluster ^b or NC ^c	Strain ^d		Isolate ^e		Bacterial populations ^f									
		n	%	n	%	A (n and %)		B (n and %)		C (n and %)		D (n and %)		E (n and %)	
I	Σ	98	17.72	126	20.66	44	33.33	70	25.74	-	-	7	29.17	5	3.31
	Cluster 1	52	9.40	72	11.80	32	24.24	29	10.66	-	-	7	29.17	4	2.65
	Cluster 2	10	1.81	15	2.46	2	1.52	13	4.78	-	-	-	-	-	-
	Cluster 3	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	Cluster 4	3	0.54	3	0.49	3	2.27	-	-	-	-	-	-	-	-
	Cluster 5	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-
	Cluster 6	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 7	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 8	3	0.54	5	0.82	-	-	5	1.84	-	-	-	-	-	-
	Cluster 9	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	Cluster 10	7	1.27	7	1.15	-	-	7	2.57	-	-	-	-	-	-
	Cluster 11	5	0.90	5	0.82	2	1.52	3	1.10	-	-	-	-	-	-
NC	5	0.90	6	0.98	3	2.27	2	0.74	-	-	-	-	1	0.66	
II	Σ	13	2.35	17	2.79	-	-	17	6.25	-	-	-	-	-	-
	Cluster 12	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-
	Cluster 13	5	0.90	9	1.48	-	-	9	3.31	-	-	-	-	-	-
	NC	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-
III	Σ	10	1.81	11	1.80	11	8.33	-	-	-	-	-	-	-	-
	Cluster 14	4	0.72	4	0.66	4	3.03	-	-	-	-	-	-	-	-
	Cluster 15	3	0.54	4	0.66	4	3.03	-	-	-	-	-	-	-	-
	Cluster 16	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	NC	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-
IV	Σ	55	9.95	62	10.16	3	2.27	34	12.50	-	-	-	-	25	16.56
	Cluster 17	11	1.99	13	2.13	-	-	3	1.10	-	-	-	-	10	6.62
	Cluster 18	30	5.42	33	5.41	-	-	2	7.35	-	-	-	-	13	8.61
	Cluster 19	3	0.54	5	0.82	-	-	5	1.84	-	-	-	-	-	-
	Cluster 20	2	0.36	2	0.33	-	-	1	0.37	-	-	-	-	1	0.66
	Cluster 21	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	Cluster 22	3	0.54	3	0.49	3	2.27	-	-	-	-	-	-	-	-
	NC	3	0.54	3	0.49	-	-	2	0.74	-	-	-	-	1	0.66
V	Σ	16	2.89	17	2.79	1	0.76	15	5.51	-	-	-	-	1	0.66
	Cluster 23	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 24	5	0.90	6	0.98	-	-	6	2.21	-	-	-	-	-	-
	Cluster 25	6	1.08	6	0.98	-	-	6	2.21	-	-	-	-	-	-
	NC	3	0.54	3	0.49	1	0.76	1	0.37	-	-	-	-	1	0.66
VI	Σ	14	2.53	14	2.30	-	-	7	2.57	-	-	-	-	7	4.64
	Cluster 26	7	1.27	7	1.15	-	-	-	-	-	-	-	-	7	4.64
	Cluster 27	6	1.08	6	0.98	-	-	6	2.21	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
VII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
VIII	Σ	2	0.36	2	0.33	-	-	1	0.37	-	-	-	-	1	0.66
	Cluster 28	2	0.36	2	0.33	-	-	1	0.37	-	-	-	-	1	0.66
IX	Σ	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-

^a Taxa are genetically related to distance ($d_j > 0.1175$) and each taxon displays clustered isolates and/or non-clustered isolates.

^b Cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates/strains ($0.0551 > d_j \geq 0$). ^c NC: non-clustered isolates or strains ($0.0551 < d_j \leq 0.3810$). ^d Number and percentage of strains per Cluster: strain refers to a collection of isolates of the same species that are genetically indistinguishable. ^e Number and percentage of isolates per Cluster: an isolate is defined as a clone collected independently of other isolates. Two independently collected isolates may be completely unrelated (different strains) or genetically indistinguishable (same strain). ^f Number and percentage of isolates per bacterial population (A, B, C, D and E).

Supplemental Table 2 - Interpretation of UPGMA dendrogram generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the MLEE patterns of *S. aureus* (cont.)

Taxon ^a	Cluster ^b or NC ^c	Strain ^d		Isolate ^e		Bacterial populations ^f									
		n	%	n	%	A (n and %)		B (n and %)		C (n and %)		D (n and %)		E (n and %)	
X	Σ	25	4.52	25	4.10	18	13.64	5	1.84	-	-	2	8.33	-	-
	Cluster 29	5	0.90	5	0.82	3	2.27	1	0.37	-	-	1	4.17	-	-
	Cluster 30	3	0.54	3	0.49	-	-	2	0.74	-	-	1	4.17	-	-
	Cluster 31	8	1.45	8	1.31	8	6.06	-	-	-	-	-	-	-	-
	Cluster 32	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 33	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	Cluster 34	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	NC	3	0.54	3	0.49	3	2.27	-	-	-	-	-	-	-	-
XI	Σ	2	0.36	2	0.33	1	0.76	1	0.37	-	-	-	-	-	-
	NC	2	0.36	2	0.33	1	0.76	1	0.37	-	-	-	-	-	-
XII	Σ	5	0.90	6	0.98	-	-	-	-	-	-	-	-	6	3.97
	Cluster 35	1	0.18	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	Cluster 36	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	NC	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
XIII	Σ	12	2.17	12	1.97	10	7.58	1	0.37	-	-	-	-	1	0.66
	Cluster 37	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	Cluster 38	4	0.72	4	0.66	4	3.03	-	-	-	-	-	-	-	-
	NC	6	1.08	6	0.98	4	3.03	1	0.37	-	-	-	-	1	0.66
XIV	Σ	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-
	NC	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-
XV	Σ	1	0.18	1	0.16	-	-	-	-	1	3.23	-	-	-	-
	NC	1	0.18	1	0.16	-	-	-	-	1	3.23	-	-	-	-
XVI	Σ	5	0.90	7	1.15	-	-	-	-	7	22.58	-	-	-	-
	Cluster 39	5	0.90	7	1.15	-	-	-	-	7	22.58	-	-	-	-
XVII	Σ	7	1.27	7	1.15	-	-	-	-	7	22.58	-	-	-	-
	Cluster 40	4	0.72	4	0.66	-	-	-	-	4	12.90	-	-	-	-
	NC	3	0.54	3	0.49	-	-	-	-	3	9.68	-	-	-	-
XVIII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XIX	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XX	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XXI	Σ	26	4.70	26	4.26	26	19.70	-	-	-	-	-	-	-	-
	Cluster 41	9	1.63	9	1.48	9	6.82	-	-	-	-	-	-	-	-
	Cluster 42	10	1.81	10	1.64	10	7.58	-	-	-	-	-	-	-	-
	Cluster 43	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	NC	5	0.90	5	0.82	5	3.79	-	-	-	-	-	-	-	-
	XXII	Σ	21	3.80	21	3.44	1	0.76	6	2.21	1	3.23	10	41.67	2
Cluster 44	2	0.36	2	0.33	-	-	-	-	-	-	-	1	4.17	1	0.66
Cluster 45	6	1.08	6	0.98	-	-	3	1.10	-	-	-	1	4.17	1	0.66
Cluster 46	5	0.90	5	0.82	-	-	3	1.10	-	-	-	2	8.33	-	-
Cluster 47	5	0.90	5	0.82	-	-	-	-	-	-	-	5	20.83	-	-
NC	3	0.54	3	0.49	1	0.76	-	-	1	3.23	1	4.17	-	-	-
XXIII	Σ	9	1.63	10	1.64	-	-	7	2.57	-	-	3	12.50	-	-

^a Taxa are genetically related to distance ($d_{ij} > 0.1175$) and each taxon displays clustered isolates and/or non-clustered isolates. ^b Cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates/strains ($0.0551 > d_{ij} \geq 0$). ^c NC: non-clustered isolates or strains ($0.0551 < d_{ij} \leq 0.3810$). ^d Number and percentage of strains per Cluster: strain refers to a collection of isolates of the same species that are genetically indistinguishable. ^e Number and percentage of isolates per Cluster: an isolate is defined as a clone collected independently of other isolates. Two independently collected isolates may be completely unrelated (different strains) or genetically indistinguishable (same strain). ^f Number and percentage of isolates per bacterial population (A, B, C, D and E).

Supplemental Table 2 - Interpretation of UPGMA dendrogram generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the MLEE patterns of *S. aureus* (cont.)

Taxon ^a	Cluster ^b or NC ^c	Strain ^d		Isolate ^e		Bacterial populations ^f									
		n	%	n	%	A (n and %)	B (n and %)	C (n and %)	D (n and %)	E (n and %)					
	Cluster 48	7	1.27	8	1.31	-	-	7	2.57	-	-	1	4.17	-	-
	NC	2	0.36	2	0.33	-	-	-	-	-	-	2	8.33	-	-
XXIV	Σ	13	2.35	15	2.46	-	-	15	5.51	-	-	-	-	-	-
	Cluster 49	8	1.45	10	1.64	-	-	10	3.68	-	-	-	-	-	-
	Cluster 50	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
XXV	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XXVI	Σ	5	0.90	5	0.82	-	-	-	-	-	-	-	-	5	3.31
	Cluster 51	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
	NC	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
XXVII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XXVIII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XXIX	Σ	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	NC	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
XXX	Σ	2	0.36	2	0.33	-	-	1	0.37	-	-	-	-	1	0.66
	NC	2	0.36	2	0.33	-	-	1	0.37	-	-	-	-	1	0.66
XXXI	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XXXII	Σ	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
XXXIII	Σ	2	0.36	2	0.33	-	-	-	-	1	3.23	-	-	1	0.66
	NC	2	0.36	2	0.33	-	-	-	-	1	3.23	-	-	1	0.66
XXXIV	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XXXV	Σ	6	1.08	6	0.98	-	-	-	-	-	-	-	-	6	3.97
	Cluster 52	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	Cluster 53	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XXXVI	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XXXVII	Σ	9	1.63	10	1.64	-	-	-	-	-	-	-	-	10	6.62
	Cluster 54	6	1.08	7	1.15	-	-	-	-	-	-	-	-	7	4.64
	NC	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
XXXVIII	Σ	8	1.45	8	1.31	-	-	-	-	-	-	-	-	8	5.30
	Cluster 55	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	Cluster 56	6	1.08	6	0.98	-	-	-	-	-	-	-	-	6	3.97
XXXIX	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XL	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XLI	Σ	5	0.90	5	0.82	-	-	5	1.84	-	-	-	-	-	-
	Cluster 57	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-

^a Taxa are genetically related to distance ($d_{ij} > 0.1175$) and each taxon displays clustered isolates and/or non-clustered isolates.

^b Cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates/strains ($0.0551 > d_{ij} \geq 0$). ^c NC: non-clustered isolates or strains ($0.0551 < d_{ij} \leq 0.3810$). ^d Number and percentage of strains per Cluster: strain refers to a collection of isolates of the same species that are genetically indistinguishable. ^e Number and percentage of isolates per Cluster: an isolate is defined as a clone collected independently of other isolates. Two independently collected isolates may be completely unrelated (different strains) or genetically indistinguishable (same strain). ^f Number and percentage of isolates per bacterial population (A, B, C, D and E).

Supplemental Table 2 - Interpretation of UPGMA dendrogram generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the MLEE patterns of *S. aureus* (cont.)

Taxon ^a	Cluster ^b or NC ^c	Strain ^d		Isolate ^e		Bacterial populations ^f									
		n	%	n	%	A (n and %)	B (n and %)	C (n and %)	D (n and %)	E (n and %)					
	Isolate	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XLII	Σ	7	1.27	7	1.15	-	-	7	2.57	-	-	-	-	-	-
	Cluster 58	6	1.08	6	0.98	-	-	6	2.21	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XLIII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XLIV	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XLV	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
XLVI	Σ	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
	NC	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
XLVII	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XLVIII	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
XLIX	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
L	Σ	30	5.42	30	4.92	-	-	16	5.88	-	-	-	-	14	9.27
	Cluster 59	7	1.27	7	1.15	-	-	-	-	-	-	-	-	7	4.64
	Cluster 60	8	1.45	8	1.31	-	-	4	1.47	-	-	-	-	4	2.65
	Cluster 61	7	1.27	7	1.15	-	-	7	2.57	-	-	-	-	-	-
	Cluster 62	3	0.54	3	0.49	-	-	2	0.74	-	-	-	-	1	0.66
	Cluster 63	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	3	0.54	3	0.49	-	-	1	0.37	-	-	-	-	2	1.32
LI	Σ	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	Cluster 64	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
LII	Σ	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-
	Cluster 65	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
LIII	Σ	5	0.90	5	0.82	-	-	-	-	-	-	-	-	5	3.31
	Cluster 66	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	Cluster 67	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
LIV	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LV	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
LVI	Σ	17	3.07	18	2.95	-	-	7	2.57	-	-	-	-	11	7.28
	Cluster 68	5	0.90	5	0.82	-	-	-	-	-	-	-	-	5	3.31
	Cluster 69	5	0.90	6	0.98	-	-	1	0.37	-	-	-	-	5	3.31
	Cluster 70	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 71	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	3	0.54	3	0.49	-	-	2	0.74	-	-	-	-	1	0.66
LVII	Σ	18	3.25	19	3.11	-	-	-	-	-	-	-	-	19	12.58

^a Taxa are genetically related to distance ($d_{ij} > 0.1175$) and each taxon displays clustered isolates and/or non-clustered isolates. ^b Cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates/strains ($0.0551 > d_{ij} \geq 0$). ^c NC: non-clustered isolates or strains ($0.0551 < d_{ij} \leq 0.3810$). ^d Number and percentage of strains per Cluster: strain refers to a collection of isolates of the same species that are genetically indistinguishable. ^e Number and percentage of isolates per Cluster: an isolate is defined as a clone collected independently of other isolates. Two independently collected isolates may be completely unrelated (different strains) or genetically indistinguishable (same strain). ^f Number and percentage of isolates per bacterial population (A, B, C, D and E).

Supplemental Table 2 - Interpretation of UPGMA dendrogram generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the MLEE patterns of *S. aureus* (cont.)

Taxon ^a	Cluster ^b or NC ^c	Strain ^d		Isolate ^e		Bacterial populations ^f									
		n	%	n	%	A (n and %)		B (n and %)		C (n and %)		D (n and %)		E (n and %)	
	Cluster 72	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
	Cluster 73	11	1.99	12	1.97	-	-	-	-	-	-	-	-	12	7.95
	Cluster 74	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	NC	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
LVIII	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
LIX	Σ	3	0.54	3	0.49	-	-	1	0.37	-	-	-	-	2	1.32
	Cluster 75	2	0.36	2	0.33	-	-	-	-	-	-	-	-	2	1.32
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LX	Σ	3	0.54	4	0.66	-	-	4	1.47	-	-	-	-	-	-
	Cluster 76	3	0.54	4	0.66	-	-	4	1.47	-	-	-	-	-	-
LXI	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXII		4	0.72	6	0.98	-	-	-	-	6	19.35	-	-	-	-
	Cluster 77	1	0.18	3	0.49	-	-	-	-	3	9.68	-	-	-	-
	Cluster 78	2	0.36	2	0.33	-	-	-	-	2	6.45	-	-	-	-
	NC	1	0.18	1	0.16	-	-	-	-	1	3.23	-	-	-	-
LXIII	Σ	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	NC	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
LXIV	Σ	19	3.44	19	3.11	6	4.55	13	4.78	-	-	-	-	-	-
	Cluster 79	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 80	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 81	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	Cluster 82	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 83	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	Cluster 84	5	0.90	5	0.82	5	3.79	-	-	-	-	-	-	-	-
	NC	3	0.54	3	0.49	1	0.76	2	0.74	-	-	-	-	-	-
LXV	Σ	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	Cluster 85	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXVI	Σ	8	1.45	8	1.31	8	6.06	-	-	-	-	-	-	-	-
	Cluster 86	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	Cluster 87	2	0.36	2	0.33	2	1.52	-	-	-	-	-	-	-	-
	NC	4	0.72	4	0.66	4	3.03	-	-	-	-	-	-	-	-
LXVII	Σ	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-
	Cluster 88	4	0.72	4	0.66	-	-	4	1.47	-	-	-	-	-	-
LXVIII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXIX	Σ	3	0.54	3	0.49	-	-	3	1.10	-	-	-	-	-	-
	Cluster 89	2	0.36	2	0.33	-	-	2	0.74	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXX	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXXI	Σ	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-
	NC	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-

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^b Cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates/strains ($0.0551 > d_{ij} \geq 0$). ^c NC: non-clustered isolates or strains ($0.0551 < d_{ij} \leq 0.3810$). ^d Number and percentage of strains per Cluster: strain refers to a collection of isolates of the same species that are genetically indistinguishable. ^e Number and percentage of isolates per Cluster: an isolate is defined as a clone collected independently of other isolates. Two independently collected isolates may be completely unrelated (different strains) or genetically indistinguishable (same strain). ^f Number and percentage of isolates per bacterial population (A, B, C, D and E).

Supplemental Table 2 - Interpretation of UPGMA dendrogram generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the MLEE patterns of *S. aureus* (cont.)

Taxon ^a	Cluster ^b or NC ^c	Strain ^d		Isolate ^e		Bacterial populations ^f									
		n	%	n	%	A (n and %)		B (n and %)		C (n and %)		D (n and %)		E (n and %)	
LXXII	Σ	1	0.18	1	0.16	-	-	-	-	1	3.23	-	-	-	-
	NC	1	0.18	1	0.16	-	-	-	-	1	3.23	-	-	-	-
LXXIII	Σ	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
	NC	3	0.54	3	0.49	-	-	-	-	-	-	-	-	3	1.99
LXXIV	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXXV	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXXVI	Σ	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-
	NC	1	0.18	1	0.16	1	0.76	-	-	-	-	-	-	-	-
LXXVII	Σ	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
	NC	1	0.18	1	0.16	-	-	1	0.37	-	-	-	-	-	-
LXXVIII		1	0.18	1	0.16	-	-	-	-	-	-	1	4.17	-	-
	NC	1	0.18	1	0.16	-	-	-	-	-	-	1	4.17	-	-
LXXIX	Σ	2	0.36	7	1.15	-	-	-	-	7	22.58	-	-	-	-
	Cluster 90	1	0.18	5	0.82	-	-	-	-	5	16.13	-	-	-	-
	Cluster 91	1	0.18	2	0.33	-	-	-	-	2	6.45	-	-	-	-
LXXX	Σ	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66
	NC	1	0.18	1	0.16	-	-	-	-	-	-	-	-	1	0.66

^a Taxa are genetically related to distance ($d_{ij} > 0.1175$) and each taxon displays clustered isolates and/or non-clustered isolates. ^b Cluster comprised two or more bacterial isolates interpreted as identical or highly related isolates/strains ($0.0551 > d_{ij} \geq 0$). ^c NC: non-clustered isolates or strains ($0.0551 < d_{ij} \leq 0.3810$). ^d Number and percentage of strains per Cluster: strain refers to a collection of isolates of the same species that are genetically indistinguishable. ^e Number and percentage of isolates per Cluster: an isolate is defined as a clone collected independently of other isolates. Two independently collected isolates may be completely unrelated (different strains) or genetically indistinguishable (same strain). ^f Number and percentage of isolates per bacterial population (A, B, C, D and E).