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Whole genome sequencing and pan-genome analysis of *Staphylococcus/Mammaliicoccus* spp. isolated from diabetic foot ulcers and contralateral healthy skin of Algerian patients

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Abstract

Background Diabetic foot infections (DFIs) are the most common complications of diabetic foot ulcers (DFUs), and a significant cause of lower extremity amputation. In this study we used whole genome sequencing to characterize the clonal composition, virulence and resistance genetic determinants of 58 *Staphylococcus/Mammaliicoccus* spp. isolates from contralateral healthy skin and DFU from 44 hospitalized patients.

Results *S. aureus* (n = 32) and *S. epidermidis* (n = 10) isolates were recovered from both DFUs and healthy skin, whereas, *S. haemolyticus* (n = 8), *M. sciuri* (n = 1), *S. hominis* (n = 1) and *S. simulans* (n = 3) were recovered exclusively from healthy skin. In contrast, *S. caprae* (n = 2) and *S. saprophyticus* (n = 1) were recovered only from DFUs. Among *S. aureus* isolates, MRSA were present with high prevalence (27/32, 84.4%), 18 of which (66.7%) were from DFUs and 9 (33.3%) from healthy skin. In contrast, the coagulase-negative *Staphylococcus* (CoNS)/*Mammaliicoccus* isolates (n = 26), in particular *S. epidermidis* and *S. haemolyticus* were more prevalent in healthy skin, (10/26, 38.5%) and (8/26, 30.8%), respectively. MLST, spa and SCC*mec* typing classified the 32 *S. aureus* isolates into 6 STs, ST672, ST80, ST241, ST1, ST97, ST291 and 4 unknown STs (STNF); 8 spa types, t044, t037, t3841, t1247, t127, t639, t937 and t9432 and 2 SCC*mec* types, type IV and type III(A). Among CoNS, the *S. epidermidis* isolates belonged to ST54, ST35 and ST640. *S. haemolyticus* belonged to ST3, ST25, ST29, ST1 and ST56. The sole *M. sciuri* isolate was found to carry an SCC*mec* type III(A). A wide range of virulence genes and antimicrobial resistance genes were found among our isolates, with varying distribution between species or STs. The pan-genome analysis revealed a highly clonal population of *Staphylococcus* isolates, particularly among *S. aureus* isolates. Interestingly, the majority of *S. aureus* isolates including MRSA, recovered from the healthy skin and DFUs of the same patient belonged to the same clone and exhibited similar virulence/resistance genotype.

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Conclusions Our study provides clinically relevant information on the population profile, virulence and antibiotic resistance of *Staphylococcus/Mammaliicoccus* spp. in DFIs, which could serve as a basis for further studies on these as well as other groups of pathogens associated with DFIs.

Keywords *Staphylococcus*, *Mammaliicoccus*, MRSA, Diabetic foot Ulcer Infection

Background

Diabetes is a fast-growing global problem with huge social, health, and economic consequences [1]. The prevalence of diabetes in 2021 was estimated to be 10.5% (536.6 million people), and it is expected to raise to 12.2% (783.2 million) in 2045 [2].

People with diabetes are at increased risk of long-term complications such as coronary heart disease, cerebrovascular and peripheral vascular diseases and diabetic foot ulcers (DFUs) [3]. It was estimated that 15% of diabetics will develop DFUs during their existence [4].

Microbial infections of the DFUs, termed diabetic foot infections (DFIs) are key contributors to the amputation risk [5]. Limb loss associated with DFUs have a significant negative impact on mobility, psychosocial well-being, and quality of life of the patients and increase healthcare costs [6, 7].

Bacterial species belonging to the genus *Staphylococcus* are common colonizers of skin and mucous membranes of humans and animals, but also opportunistic pathogens capable of causing a wide range of infections. The staphylococci can be differentiated into Coagulase-Positive (CoPS) and Coagulase-Negative (CoNS), based on their ability to produce coagulase. Recently, five species among the CoNS (S. *sciuri, S. fleurettii, S. lentus, S. stepanovicii* and *S. vitulinus*), and belonging to *S. sciuri* group, were reclassified into the novel genus *Mammaliicoccus*, with *Mammaliicoccus* (*M.*) *sciuri* as the type species [8].

The CoPS, which are considered as more pathogenic than the CoNS, include the notorious pathogen, *S. aureus*, which is the main causative agent of both community acquired and nosocomial infections in humans as well as in animals [9–12], including DFIs [12, 13]. *S. aureus* can deploy numerous virulence factors which are implicated in DFI and delayed wound healing process [14, 15]. However, the CoNS and the M*ammaliicoccus* are now known to be also frequently associated with clinical infections, including diabetic foot osteomyelitis [16], particularly in immune-compromised and hospitalized patients [17].

Both *Staphylococcus* and *Mammaliicoccus* species are becoming increasingly resistant to several antibiotics, as a result of the acquisition of resistance genes [18], limiting further the therapeutic options against the infections caused by these bacteria, and leading to worse clinical outcomes [19].

Thus, understanding the genetic characteristics of *Staphylococcus* and related bacteria in DFIs can be exploited for both therapeutic and diagnostic purposes.

Apart from one study by Djahmi et al. (2013) [20], data on the staphylococci associated with DFIs in Algeria are lacking. Therefore, in the present study we used whole genome sequencing (WGS) to characterize the genetic diversity, antibiotic resistance and virulence genetic determinants of *Staphylococcus* and *Mammaliicoccus* spp. isolates recovered from DFIs; and to assess the potential association between clones/species and/or virulence/resistance genes with PEDIS grades and the source of isolates.

Results

Species and source of *Staphylococcus/Mammaliicoccus* isolates

Eight *Staphylococcus/Mammaliicoccus* species were identified among the 58 isolates, including *S. aureus* (32/58, 55.2%), *S. epidermidis* (10/58, 17.2%), *S. haemolyticus* (8/58, 13.8%), *S. simulans* (3/58, 5.2%), *S. caprae* (2/58, 3.5%), *S. hominis*, *S. saprophyticus* and *M. sciuri* (1/58 each, 1.7%). The characteristics of the 58 isolates included in this study are shown in Table 1.

Among the 32 *S. aureus* isolates, 23/32 (71.9%) were recovered from DFUs and 9/32 (28.1%) from healthy skin. The 10 *S. epidermidis* isolates were recovered from 8 patients, including 6/10 (60%) from healthy skin and 4/10 (40%) from DFUs. All the *S. haemolyticus* (n=8), *M. sciuri* (n=1), *S. hominis* (n=1) and *S. simulans* (n=3) isolates were recovered exclusively from healthy skin, but *S. caprae* (n=2) and *S. saprophyticus* (n=1) were recovered only from DFUs.

Among the 32 *S. aureus* isolates, 27/32 (84.4%) carried the *mecA* gene, and were therefore MRSA; 18/27 of which (66.7%) were from DFUs and 9/27 (33.3%) from healthy skin.

Statistically, *S. aureus* including MRSA isolates and CoNS were significantly associated with the source of isolation (p-value=0.004).

Molecular typing of the *Staphylococcus/Mammaliicoccus* isolates

Clones of *S. aureus* were characterized based on the combination of MLST, SCC*mec* and spa typing. *In silico* determination of MLST revealed that *S. aureus* isolates belonged to 6 known STs including ST80 (16/32, 50%), ST241 (5/32, 15.6%), ST672 (3/32, 9.4%), ST1 (2/32,

Table 1 Clinical, epidemiological and molecular characteristics of *Staphylococcus/Mammaliicoccus* species recovered from DFUs and Healthy skin (n = 58)

Patient	Age/sex	PEDIS grade	Ulcer location	Antibiotics	Isolates (n)	Origin	species	SCC*mec*	ST-spa
P01	73/M	3	Forefoot	Ciprofloxacin	3	DFU	*S. aureus*	IVd(2B)	ST672-t3841
						HS	*S. aureus*	IVd(2B)	ST672-t3841
						HS	*S. epidermidis*	MSCoNS	ST54
P02	51/F	3	Midfoot	Amoxicillin-clavulanate	2	HS	*S. aureus*	IVc(2B)	ST80-t1247
						HS	*S. aureus*	III(3 A)	STNF-t037
P03	61/M	4	Heel	Metronidazole, Cefotaxime	3	DFU	*S.aureus*	IVc(2B)	ST80-t044
						HS	*S.aureus*	IVc(2B)	ST80-t044
						HS	*S. haemolyticus*	MSCoNS	ST3[§]
P04	62/M	3	Forefoot	Clindamycin, Ciprofloxacin	2	DFU	*S. epidermidis*	MSCoNS	ST54
						HS	*S. epidermidis*	MSCoNS	ST54
P05	60/F	4	Midfoot	Metronidazole, Cefotaxime	3	DFU	*S. epidermidis*	MSCoNS	ST54
						HS	*S. haemolyticus*	MSCoNS	ST29
						HS	*M. sciuri*	III(3 A)	STNF
P06	71/M	3	Forefoot	Ciprofloxacin	2	DFU	*S. epidermidis*	MSCoNS	ST54
						HS	*S. epidermidis*	MSCoNS	ST54
P07	61/M	3	Forefoot	Clindamycin	1	HS	*S. epidermidis*	MSCoNS	STNF
P08	63/F	3	Heel	Metronidazole, Cefotaxime	1	HS	*S. haemolyticus*	MSCoNS	ST25
P09	64/M	4	Forefoot	Cefotaxime	3	DFU	*S. aureus*	IVc(2B)	ST80-t044
						DFU	*S. caprae*	MSCoNS	STNF
						HS	*S. aureus*	IVc(2B)	ST80-t044
P10	73/M	2	Heel	Amoxicillin-clavulanate	2	DFU	*S. aureus*	MSSA	ST291-t937
						HS	*S. hominis*	MSCoNS	STNF
P11	66/M	2	Midfoot	Clindamycin	1	DFU	*S. aureus*	III(3 A)	STNF-t037
P13	54/M	4	Forefoot	Bactrim	1	HS	*S. epidermidis*	MSCoNS	ST35
P14	80/F	2	Forefoot	Imipenem	1	HS	*S. haemolyticus*	MSCoNS	ST3
P15	74/M	2	Heel	Amoxicillin-clavulanate	3	DFU	*S. aureus*	III(3 A)	ST241-t037
						HS	*S. aureus*	III(3 A)	ST241-t037
						HS	*S. epidermidis*	MSCoNS	ST35
P16	61/M	3	Forefoot	Cefazolin	2	DFU	*S. aureus*	III(3 A)	ST241-t037
						HS	*S. haemolyticus*	MSCoNS	ST3
P17	70/M	2	Heel	Bactrim	2	DFU	*S. epidermidis*	MSCoNS	ST640
						HS	*S. simulans*	MSCoNS	STNF
P18	49/M	4	Midfoot	Clindamycin, Cefotaxime	1	HS	*S. simulans*	MSCoNS	STNF
P19	44/M	3	Forefoot	Imipenem	2	DFU	*S. caprae*	MSCoNS	STNF
						HS	*S. simulans*	MSCoNS	STNF
P20	71/M	2	Midfoot	Imipenem	1	HS	*S. haemolyticus*	MSCoNS	ST1
P26	85/M	4	Heel	Clindamycin, Ciprofloxacin	1	DFU	*S. aureus*	III(3 A)	STNF-t037
P27	82/M	4	Forefoot	Metronidazole, Cefotaxime	1	HS	*S. haemolyticus*	MSCoNS	ST56
P28	72/M	4	Midfoot	Metronidazole, Cefotaxime	1	HS	*S. haemolyticus*	MSCoNS	ST25
P29	54/M	3	Heel	Bactrim, Cefotaxime	1	HS	*S. aureus*	IVc(2B)	ST80-t044
P30	59/M	3	Forefoot	Ciprofloxacin	1	HS	*S. aureus*	MSSA	ST97-t9432
P31	64/M	4	Forefoot	Clindamycin, Ciprofloxacin	1	DFU	*S. aureus*	IVd(2B)	ST672-t3841
P32	60/M	3	Heel	Metronidazole, Cefotaxime	2	DFU	*S. aureus*	III(3 A)	ST241-t037
						HS	*S. aureus*	III(3 A)	ST241-t037
S16*	57/F	3	Heel	Cefazolin	1	DFU	*S. aureus*	MSSA	STNF-t037
S17K*	72/M	2	Heel	Amoxicillin-clavulanate	1	DFU	*S. aureus*	IVc(2B)	ST80-t044
S104K*	60/F	2	Midfoot	Imipenem	1	DFU	*S. aureus*	IVc(2B)	ST80-t044
S4K*	59/M	2	Forefoot	Clindamycin, Ciprofloxacin	1	DFU	*S. saprophyticus*	MSCoNS	STNF
S6K*	49/M	3	Midfoot	Bactrim	1	DFU	*S. aureus*	IVc(2B)	ST80-t044
S7K*	58/M	3	Heel	Ciprofloxacin	1	DFU	*S. aureus*	IVc(2B)	ST80-t044
S8K*	60/M	4	Heel	Metronidazole, Cefotaxime	1	DFU	*S. aureus*	IVc(2B)	ST80-t044

Table 1 (continued)

Patient	Age/sex	PEDIS grade	Ulcer location	Antibiotics	Isolates (n)	Origin	species	SCC*mec*	ST-spa
S9K*	52/M	3	Midfoot	Cephalexin, Gentamicin	1	DFU	*S. aureus*	IVc(2B)	ST80-t044
S10K*	60/M	4	Forefoot	Metronidazole, Cefotaxime	1	DFU	*S. aureus*	IVc(2B)	ST80-t044
S11K*	67/M	4	Heel	Clindamycin, Ciprofloxacin	2	DFU	*S. aureus*	IVc(2B)	ST80-t044
			Forefoot			DFU	*S. aureus*	IVc(2B)	ST80-t044
S12K*	85/M	3	Forefoot	Ciprofloxacin	2	DFU	*S. aureus*	IVc(2B)	ST80-t044
			Heel			DFU	*S. aureus*	MSSA	ST1-t127
S14K*	60/F	4	Midfoot	Cefazolin	1	DFU	*S. aureus*	MSSA	ST1-t127

DFU: diabetic foot ulcer, HS: healthy skin; M: Male, F: female; MSSA: methicillin-sensitive S. aureus; MSCoNS: methicillin-sensitive coagulase negative staphylococci; ST: sequence type; STNF: ST not found; SCCmec: Staphylococcal Cassette Chromosome *mec*. *Patients sampled only from DFU (additional strains). § Novel allele, ST may indicate nearest ST. Values are numbers

6.3%), ST97 and ST291 (1/32 each, 3.1%), and 4 STNF (4/32, 12.5%).

Eight spa types were identified among *S. aureus* isolates. The dominant one was t044 (13/32, 40.6%), followed by t037 (9/32, 28.1%), t3841 (3/32, 9.4%), t1247 and t127 (2/32 each, 6.3%), t639, t9432 and t937 (1/32 each, 3.1%).

Two SCC*mec* types were identified among MRSA isolates, type IV (19/27, 70.4%) and type III(A) (8/27, 29.6%). The type IV isolates were assigned to subtype IVc(2B) (16/27, 59.3%) and IVd(2B) (3/27, 11.11%).

Among CoNS/*Mammaliicoccus* isolates, SCC*mec* type III(A) (1/26, 3.8%) was detected in the sole *M. sciuri* isolate.

The dominant MRSA clone (13/27, 48.1%) was ST80- t044- IVc(2B), followed by ST241-t037- III(3 A)) (5/27,18.5%); whereas, ST672- t3841- IVd(2B) and STNF-t037- III(3 A) were each represented by 3/27 (11.1%) isolates. In addition, 2 other spa types were detected among ST80- IVc(2B) isolates, t1247 (2/27, 7.4%) and t639 (1/27, 3.7%).

2/5 (40%) of MSSA isolates belonged to ST1-t127, while ST97-t9432, ST291-t937 and STNF-t037 were each represented by one isolate (1/5, 20%).

Among *S. epidermidis* isolates, 6/10 (60%) belonged to ST54, 2/10 (20%) to ST35 and 1/10 (10%) to ST640. 3/8 (37.5%) *S. haemolyticus* belonged to ST3 and 2/8 (25%) to ST25, while ST29, ST1 and ST56 were each represented by 1/8 (12.5%) isolate.

Virulence genes

The presence and distribution of the virulence genes are summarized in Tables 2, 3 and S2.

A total of 116 virulence genes were detected among *S. aureus* isolates including 42 adhesion genes and a large number of type 8 capsular polysaccharide, immune evasion and exoenzyme genes.

Thirty-five toxin-encoding genes were found among MRSA/MSSA isolates including 6 hemolysins (*hlgA, hlgB, hlgC, hlb, hld and hla/hly)*, 13 staphylococcal enterotoxins (*se*) and staphylococcal enterotoxin-like toxins (*sel*) (*sea, seb, seh, sek, seq, seg, sei, sem, sen, seo, seu selk and selq)*, with different carriage proportions ranging from 3.1 to 100%. ST1 isolates carried the highest number (n=7) of *se/sel* genes, *sea, seb, seh, sek, seq, selk and selq*. In contrast, none of the *se/sel* genes were detected among the ST80 isolates.

Remarkably, none of the *Staphylococcus/Mammaliicoccus* isolates harbored an exfoliatine toxin gene (*eta* or *etb*) or a toxic shock syndrome toxin (*tst*) gene.

The leukocidins genes (*lukD, lukE)* were detected in all *S. aureus* isolates (32/32,100%), while *lukF/lukS-PV* were detected only in ST80 isolates (16/32, 50%). In addition, *edinB* gene encoding epidermal cell differentiation inhibitors was also detected only in ST80 isolates (16/32, 50%) and in ST291- t937 (1/32, 3.1%).

On the other hand, the sole virulence factor found among the 26 CoNS/*Mammaliicoccus* isolates was the arginine catabolic mobile element (*ACME*), which was exclusively present in *S. epidermidis* isolates (9/26, 34.6%).

The association of virulence genes with *S. aureus* ST or CoNS/*Mammaliicoccus* species was statistically significant. In contrast, no statistically significant association was found between the presence/absence of virulence genes and PEDIS grades or the source of isolates (p-value > 0.05).

Resistance genes

S. aureus

The distribution of the genetic determinants of antibiotic resistance among the *S. aureus* is shown in Tables 4 and S2. Genes encoding β-lactam resistance, *mecA* and *blaZ*, were detected among *S. aureus* isolates at frequencies of 84.4% (27/32) and 50% (16/32), respectively.

The genes encoding aminoglycoside-modifying enzymes (*AME*), *aph(3')- III/ aph(3')- IIIa*, were the most prevalent among *S. aureus* isolates (25/32, 78.1%). All ST80 isolates (16/32, 50%) were positive for *ant(6)-Ia* and *aph(3')-III/ aph(3')-IIIa* genes, and all ST241 isolates

Table 2 Virulence gene profiles in *S. aureus* isolates recovered from DFUs and Healthy skin (n = 32)

	ST1-t127 (n = 2) n(%)	ST241-t037 (n = 5) n(%)	ST291-t937 (n = 1) n(%)	ST672-t3841 (n = 3) n(%)	ST80-t044/t1247/t639 (n = 16) n(%)	ST97-t9432 (n = 1) n(%)	STNF-t037 (n = 4) n(%)	Total (n = 32) n(%)	§*p*-value
Adhesion									
clfA	2(100)	5(100)	0(0)	0(0)	0(0)	1(100)	4(100)	12(37.5)	< 0.001
clfB	2(100)	5(100)	0(0)	3(100)	0(0)	1(100)	2(50)	13(40.6)	< 0.001
ebp	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
fnbA	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(6.3)	< 0.001
fnbB	2(100)	0(0)	0(0)	0(0)	1(6.3)	0(0)	0(0)	3(9.4)	0.002
map	0(0)	5(100)	0(0)	3(100)	15(93.8)	1(100)	4(100)	28(87.5)	0.001
capA	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
cap8A	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8B	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8C	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8D	0(0)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	30(93.8)	< 0.001
cap8E	0(0)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	30(93.8)	< 0.001
cap8F	0(0)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	30(93.8)	< 0.001
cap8G	0(0)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	30(93.8)	< 0.001
cap8H	0(0)	5(100)	0(0)	3(100)	16(100)	0(0)	4(100)	28(87.5)	< 0.001
cap8I	2(100)	5(100)	0(0)	3(100)	16(100)	0(0)	4(100)	30(93.8)	< 0.001
cap8J	2(100)	5(100)	0(0)	3(100)	16(100)	0(0)	4(100)	30(93.8)	< 0.001
cap8K	2(100)	5(100)	0(0)	3(100)	16(100)	0(0)	4(100)	30(93.8)	< 0.001
cap8L	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8M	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8N	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8O	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
cap8P	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
capN	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
srtB	2(100)	5(100)	1(100)	3(100)	15(93.8)	1(100)	4(100)	31(96.9)	0.984
sdrC	2(100)	5(100)	0(0)	1(33.3)	12(75)	1(100)	4(100)	25(78.1)	0.104
sdrD	2(100)	5(100)	0(0)	3(100)	15(93.8)	1(100)	4(100)	30(93.8)	0.014
sdrE	1(50)	3(60)	0(0)	0(0)	15(93.8)	1(100)	3(75)	23(71.9)	0.018
icaA/B/C/D/R	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdA	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdB	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdC	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdD	2(100)	5(100)	1(100)	3(100)	15(93.8)	1(100)	4(100)	31(96.9)	0.984
isdE	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdF	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdG	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
isdI	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
harA	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
Toxins									
Haemolysins									
hly/hla	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
hlb	1(50)	0(0)	1(100)	1(33.3)	5(31.3)	1(100)	0(0)	9(28.1)	0.161
hld	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
hlgA/B/C	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
Enterotoxins and Enterotoxin-like									
sea	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	4(100)	6(18.8)	< 0.001
seb	2(100)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	3(9.4)	< 0.001
seh	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(6.3)	< 0.001
*egc cluster**	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)	0(0)	3(9.4)	< 0.001
sek	2(100)	5(100)	0(0)	0(0)	0(0)	0(0)	4(100)	11(34.4)	< 0.001

Table 2 (continued)

	ST1-t127 (n = 2) n(%)	ST241-t037 (n = 5) n(%)	ST291-t937 (n = 1) n(%)	ST672-t3841 (n = 3) n(%)	ST80-t044/t1247/t639 (n = 16) n(%)	ST97-t9432 (n = 1) n(%)	STNF-t037 (n = 4) n(%)	Total (n = 32) n(%)	§*p*-value
seq	2(100)	5(100)	0(0)	0(0)	0(0)	0(0)	4(100)	11(34.4)	< 0.001
selk	2(100)	5(100)	0(0)	0(0)	0(0)	0(0)	4(100)	11(34.4)	< 0.001
selq	2(100)	5(100)	0(0)	0(0)	0(0)	0(0)	4(100)	11(34.4)	< 0.001
Staphylococcal exotoxin-like									
set16	1(50)	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	2(6.3)	0.060
set17	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set18	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set19	0(0)	0(0)	0(0)	1(33.3)	0(0)	1(100)	0(0)	2(6.3)	0.002
set20	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set21	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.1)	0.017
set22	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set23	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set24	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set25	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
set26	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.1)	0.017
Other toxins									
edinB	0(0)	0(0)	1(100)	0(0)	16(100)	0(0)	0(0)	17(53.1)	< 0.001
lukS-PV/lukF-PV	0(0)	0(0)	0(0)	0(0)	16(100)	0(0)	0(0)	16(50)	< 0.001
lukD/E	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
Type VII secretion system									
esaA	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
esaB	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
esaD	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
esaE	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
esaG	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
esaG1	1(50)	5(100)	1(100)	0(0)	0(0)	0(0)	4(100)	11(34.4)	< 0.001
esaG2	1(50)	0(0)	0(0)	2(66.7)	13(81.3)	0(0)	0(0)	16(50)	0.007
esaG3	1(50)	0(0)	0(0)	2(66.7)	0(0)	0(0)	0(0)	3(9.4)	0.006
esaG4	1(50)	5(100)	0(0)	2(66.7)	0(0)	0(0)	4(100)	12(37.5)	< 0.001
esaG5	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.1)	0.017
esaG6	0(0)	0(0)	1(100)	2(66.7)	0(0)	0(0)	0(0)	3(9.4)	< 0.001
esaG7	1(50)	0(0)	0(0)	2(66.7)	0(0)	0(0)	0(0)	3(9.4)	0.006
esaG8	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.1)	0.017
esaG9	1(50)	5(100)	0(0)	2(66.7)	13(81.3)	0(0)	4(100)	25(78.1)	0.091
essA	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
essB	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
essC	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
esxA	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
esxB	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
esxC	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
esxD	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
Degrading enzyme									
Lipases									
geh	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
lip	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
Proteases									
sspA	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
sspB	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	0(0)	28(87.5)	< 0.001
sspC	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
splA	2(100)	5(100)	1(100)	0(0)	16(100)	1(100)	4(100)	29(90.6)	< 0.001
splB	2(100)	5(100)	0(0)	0(0)	16(100)	1(100)	4(100)	28(87.5)	< 0.001

Table 2 (continued)

	ST1-t127 (n = 2) n(%)	ST241-t037 (n = 5) n(%)	ST291-t937 (n = 1) n(%)	ST672-t3841 (n = 3) n(%)	ST80-t044/t1247/t639 (n = 16) n(%)	ST97-t9432 (n = 1) n(%)	STNF-t037 (n = 4) n(%)	Total (n = 32) n(%)	§*p*-value
splE	2(100)	5(100)	1(100)	0(0)	0(0)	1(100)	4(100)	13(40.6)	< 0.001
Hyaluronidases									
hysA	2(100)	5(100)	1(100)	0(0)	16(100)	0(0)	4(100)	28(87.5)	< 0.001
Coagulases									
coa	2(100)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	3(9.4)	< 0.001
vWbp	2(100)	5(100)	0(0)	0(0)	0(0)	0(0)	4(100)	11(34.4)	< 0.001
Other enzymes									
aur	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
Immune evasion									
adsA	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N
chp	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	5(15.6)	< 0.001
sak	2(100)	5(100)	0(0)	3(100)	14(87.5)	1(100)	4(100)	29(90.6)	0.077
scn	2(100)	5(100)	0(0)	3(100)	14(87.5)	1(100)	4(100)	29(90.6)	0.077
spa	2(100)	0(0)	1(100)	3(100)	16(100)	1(100)	0(0)	23(71.9)	< 0.001
sbi	2(100)	5(100)	1(100)	3(100)	16(100)	1(100)	4(100)	32(100)	N

§*Chi-squared test was used to calculate p-values for describing the association of each gene and STs, p-values < 0.05 were considered statistically significant*

**egc cluster corresponds to seg, sei, sem, sen, seo and seu genes*

Values are numbers and percentages in brackets

Table 3 Virulence gene profiles in CoNS/*M. sciuri* isolates recovered from DFUs and Healthy skin (n = 26)

	M.sciuri (n = 1) n(%)	*S.caprae* (n = 2) n(%)	*S.epidermidis* (n = 10) n(%)	*S.haemolyticus* (n = 8) n(%)	*S.hominis* (n = 1) n(%)	*S.saprophyticus* (n = 1) n(%)	*S.simulans* (n = 3) n(%)	Total (n = 26) n(%)	**p*-value
ACME	0(0)	0(0)	9(90)	0(0)	0(0)	0(0)	0(0)	9(34.6)	0.001

*Chi-squared test was used to calculate *p*-values for describing the association between genes and species, *p*-values < 0.05 were considered statistically significant

Values are numbers and percentages in brackets

(5/32, 15.6%) were positive for 3 *AME* genes, *ant(9)-Ia, aac(6')- aph(2")* and *aph(3')- III.*

Four genes encoding resistance to macrolide-lincosamide-streptogramin B (MLSB) were detected in *S. aureus* isolates. The *msr(A)* and *mph(C)* genes were detected in all the ST672 isolates (3/32, 9.4%), and the *erm(A)* in all the ST241 isolates (5/32, 15.6%).

Three genes encoding resistance to tetracycline were detected among *S. aureus* isolates, *tet(M)* (9/32, 28.1%), *tet(K)* (2/32, 6.3%) and *tet(38)* (6/32, 18.8%). Remarkably, only isolates belonging to t037 and harboring *SCCmec*-III (ST241 and STNF) carried *tet(M)*.

The *fusB* and *fusC* genes coding for fusidic acid resistance were detected in all the ST80-t1247 (2/32, 6.3%) and ST1-t127 (2/32, 6.3%), respectively. The *dfrG* gene coding for trimethoprim-sulfamethoxazole resistance was detected only in ST241 isolates (5/32, 15.6%).

CoNS/ M. sciuri

As presented in Table 5, various resistance genes were also identified among the twenty-six CoNS/ *M. sciuri* isolates. 18/26 (69.2%) carried both *mecA* and *blaZ* genes. Remarkably, the single *M. sciuri* isolate harbored both *mecA* and *mecA1* (1/1, 100%). 9/10 (90%) *S. epidermidis* isolates carried both *fusB* and *fosB* genes. In addition, *msr(A)* and *mph(C)* were detected in *S. epidermidis* (2/10, 20%) and *S. haemolyticus* (8/8,100%) isolates. Moreover, *ermC* was found in *S. haemolyticus* (5/8, 62.5%) and *S. epidermidis* (1/10, 10%) isolates. *aac(6')- aph(2")*/ aac(*6')-Ie/aph(2")-Ia* genes were detected in *S .haemolyticus* (7/8, 87.5%) and *M. sciuri* (1/1, 100%).

The *aph(3')*-III*/ aph(3')-IIIa* (6/8, 75%) and *tet(K)* (7/10, 70%) genes were found exclusively in *S. haemolyticus* and in *S. epidermidis*, respectively. Genes conferring resistance to streptogramin (*vat(B), vat(C)* and *vgb(B)*), macrolides (*vga(B)* and *vga(A)LC*), kanamycin/neomycin (*aadD), tetracycline* (*tet(L)*), streptomycin (*str*) and to quaternary ammonium compounds *(qacB*) were detected only in *S. haemolyticus* isolates, at a frequency of one gene per isolate(1/8, 12.5%).

Pan-genome analysis

The pan-genome of each species was determined and phylogenetic trees were built based on gene presence/

Table 4 Resistance gene profiles in *S. aureus* isolates recovered from DFUs and Healthy skin (n = 32)

	ST1-t127 (n = 2) n(%)	ST241-t037 (n = 5) n(%)	ST291-t937 (n = 1) n(%)	ST672-t3841 (n = 3) n(%)	ST80-t044/t1247/t639 (n = 16) n(%)	ST97-t9432 (n = 1) n(%)	STNF-t037 (n = 4) n(%)	Total (n = 32) n(%)	*p-value
β-lactams									
mecA	0(0)	5(100)	0(0)	3(100)	16(100)	0(0)	3(75)	27(84.4)	< 0.001
blaZ	0(0)	5(100)	1(100)	3(100)	2(12.5)	1(100)	4(100)	16(50)	< 0.001
blaI	0(0)	0(0)	0(0)	1(33.3)	0(0)	1(100)	0(0)	2(6.3)	0.002
blaR1	0(0)	0(0)	0(0)	1(33.3)	0(0)	1(100)	0(0)	2(6.3)	0.002
Aminoglycoside									
ant(6)-Ia	0(0)	0(0)	0(0)	0(0)	16(100)	1(100)	0(0)	17(53.1)	< 0.001
ant(9)-Ia	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	5(15.6)	< 0.001
aac(6')- aph(2")	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	3(75)	8(25)	< 0.001
aph(3')-III	0(0)	5(100)	0(0)	2(66.7)	13(81.3)	0(0)	0(0)	20(62.5)	0.005
aph(3')-IIIa	0(0)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	5(15.6)	0.207
sat4	0(0)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	5(15.6)	0.207
Macrolides									
mph(C)	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)	0(0)	3(9.4)	< 0.001
msr(A)	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)	0(0)	3(9.4)	< 0.001
ermA	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	5(15.6)	< 0.001
ermC	0(0)	0(0)	0(0)	0(0)	1(6.3)	1(100)	0(0)	2(6.3)	0.014
Tetracycline									
tet(K)	0(0)	0(0)	0(0)	0(0)	2(12.5)	0(0)	0(0)	2(6.3)	0.907
tet(M)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	4(100)	9(28.1)	< 0.001
tet(38)	1(50)	0(0)	0(0)	1(33.3)	3(18.8)	1(100)	0(0)	6(18.8)	0.214
Fusidic acid									
fusB	0(0)	0(0)	0(0)	0(0)	2(12.5)	0(0)	0(0)	2(6.3)	0.907
fusC	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(6.3)	< 0.001
Trimethoprim-sulfamethoxazole									
dfrG	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	5(15.6)	< 0.001
Fosfomycin									
fosB	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	1(3.1)	0.126

*Chi-squared test was used to calculate *p*-values for describing the association between genes and STs, *p*-values < 0.05 were considered statistically significant

Values are numbers and percentages in brackets

absence matrix. Interestingly, isolates from DFUs are intermingled among the healthy skin isolates throughout the trees. A high number of accessory genes was observed, particularly, in *S. epidermidis* (4383, 72.3%), *S. aureus (*3997, 67.1%), *S. haemolyticus* (3874, 65.9%) and *M. sciuri* (3813, 65.7%) (Fig. 1).

The pan-genome analysis separated the ST80 *S. aureus* isolates into three subgroups (Fig. 2), one comprised of 10 closely related t044 isolates, the second comprised of 2 t1247 and 1 t639 related isolates, and the third contained 3 t044 isolates, which were more distantly related to ST80 strains from the other countries. *S. aureus* ST80, ST1, ST672, ST241 and STNF were found to be closely related to each other and were more distantly related to the reference strains.

The phylogenetic tree of *S. epidermidis* revealed two major clusters, the first included ST54 isolates, which were distinct from the reference *S. epidermidis* strains, and the second included the reference ST54 strains (Fig. 3). The ST35 isolates clustered together, but separate from the ST35 strains from other countries.

The phylogenetic tree of *S. haemolyticus* revealed a clear distinction between our isolates and the reference strains (Fig. 4).

Similarly, *S. simulans* and *S. caprae* isolates (Figs. 5 and 6) from our study were closely related and more distantly related to the clinical isolates from China (CJ16) and Japan (JMUB145, JMUB590 and JMUB898), respectively.

S. saprophyticus and *S. hominis* isolates clustered with clinical isolates from India and the Netherlands, respectively (Figs. 7 and 8).

However, our single *M. sciuri* isolate formed an outgroup, which was distinct from the rest of the reference strains (Fig. 9).

Table 5 Resistance gene profiles in CoNS species and *M. sciuri* recovered from DFUs and Healthy skin (n = 26)

	M.sciuri **(n = 1) n(%)**	***S. caprae*** **(n = 2) n(%)**	***S. epidermidis*** **(n = 10) n(%)**	***S.haemolyticus*** **(n = 8) n(%)**	***S.hominis*** **(n = 1) n(%)**	***S.saprophyticus*** **(n = 1) n(%)**	***S. simulans*** **(n = 3) n(%)**	**Total (n = 26) n(%)**	***p*-value**
Resistance genes									
β-lactams									
blaZ	0(0)	2(100)	10(100)	8(100)	1(100)	0(0)	0(0)	21(80.8)	< 0.001
blaI	0(0)	2(100)	1(10)	2(25)	0(0)	0(0)	0(0)	5(19.2)	0.103
blaR1	0(0)	1(50)	1(10)	2(25)	0(0)	0(0)	0(0)	4(15.4)	0.714
mecA	1(100)	0(0)	9(90)	8(100)	1(100)	0(0)	0(0)	19(73.1)	0.002
mecA1	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.8)	< 0.001
mecI	1(100)	0(0)	1(10)	0(0)	0(0)	0(0)	0(0)	2(7.7)	0.038
mecR1	1(100)	0(0)	1(10)	0(0)	0(0)	0(0)	0(0)	2(7.7)	0.038
Aminoglycoside									
aac(6')- aph(2")	0(0)	0(0)	0(0)	5(62.5)	0(0)	0(0)	0(0)	5(19.2)	0.030
aac(6')-Ie/aph(2")-Ia	1(100)	0(0)	0(0)	2(25)	0(0)	0(0)	0(0)	3(11.5)	0.079
aph(3')-III	0(0)	0(0)	0(0)	4(50)	0(0)	0(0)	0(0)	4(15.4)	0.100
aph(3')-IIIa	0(0)	0(0)	0(0)	2(25)	0(0)	0(0)	0(0)	2(7.7)	0.560
sat4	0(0)	0(0)	0(0)	2(25)	0(0)	0(0)	0(0)	2(7.7)	0.560
Streptogramin									
vat(B)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
vat(C)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
vgb(B)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
Macrolides									
mph (C)	0(0)	0(0)	2(20)	8(100)	0(0)	0(0)	0(0)	10(38.5)	0.004
msr(A)	0(0)	0(0)	2(20)	8(100)	1(100)	0(0)	0(0)	11(42.3)	0.003
ermC	0(0)	0(0)	1(10)	5(62.5)	0(0)	0(0)	0(0)	6(23.1)	0.110
vga(A)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	3(100)	4(15.4)	0.004
vga(B)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
vga(A)LC	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
sal(A)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.8)	< 0.001
Tetracycline									
tet(L)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
tet(K)	0(0)	7(70)	0(0)	0(0)	0(0)	0(0)	0(0)	7(26.9)	0.018
Fusidic acid									
fusB	0(0)	1(50)	9(90)	3(37.5)	0(0)	0(0)	0(0)	13(50)	0.045
fusC	0(0)	0(0)	1(10)	1(12.5)	0(0)	0(0)	0(0)	2(7.7)	0.986
Trimethoprim-sulfamethoxazole									
dfrG	0(0)	0(0)	0(0)	5(62.5)	0(0)	0(0)	0(0)	5(19.2)	0.030
dfrS1	0(0)	0(0)	1(10)	0(0)	0(0)	0(0)	0(0)	1(3.8)	0.948
Fosfomycin									
fosB	0(0)	0(0)	10(100)	0(0)	0(0)	0(0)	0(0)	10(38.5)	< 0.001
Streptomycin									
str	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
kanamycin and neomycin									
aadD	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886
Quaternary ammonium compounds									
qacB	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	1(3.8)	0.886

**Chi-squared test was used to calculate p-values for describing the association between genes and species, p-values < 0.05 were considered statistically significant*

Values are numbers and percentages in brackets

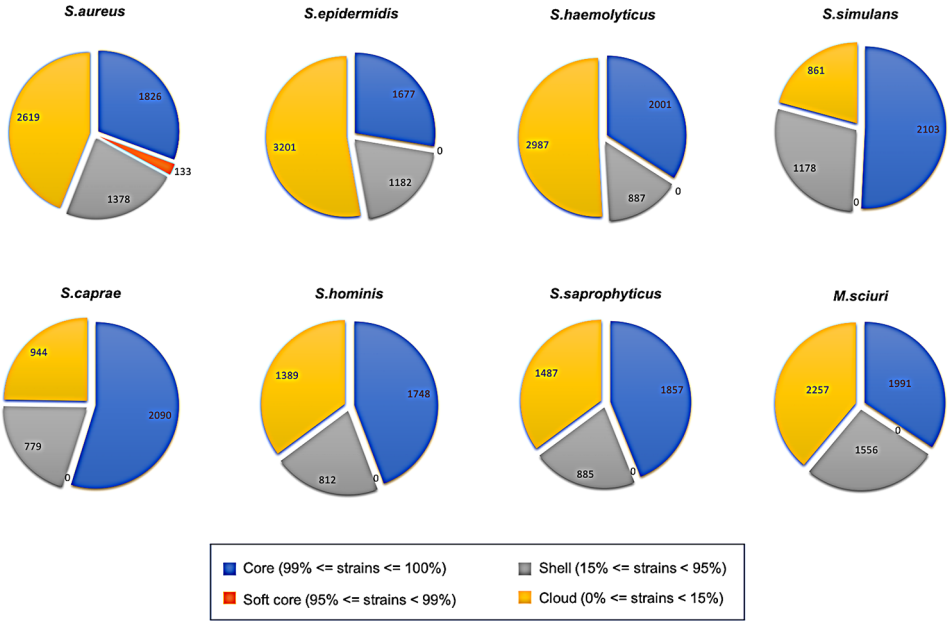

Fig. 1 Pan-genome representation of *Staphylococcus/Mammaliicoccus* spp. The pan-genome pie charts show gene content of *Staphylococcus/Mammaliicoccus* spp., determined by the Roary software. The pan-genome can be classified into core genes (the combination of core and soft core genes) and accessory genes (the combination of shell and cloud genes)

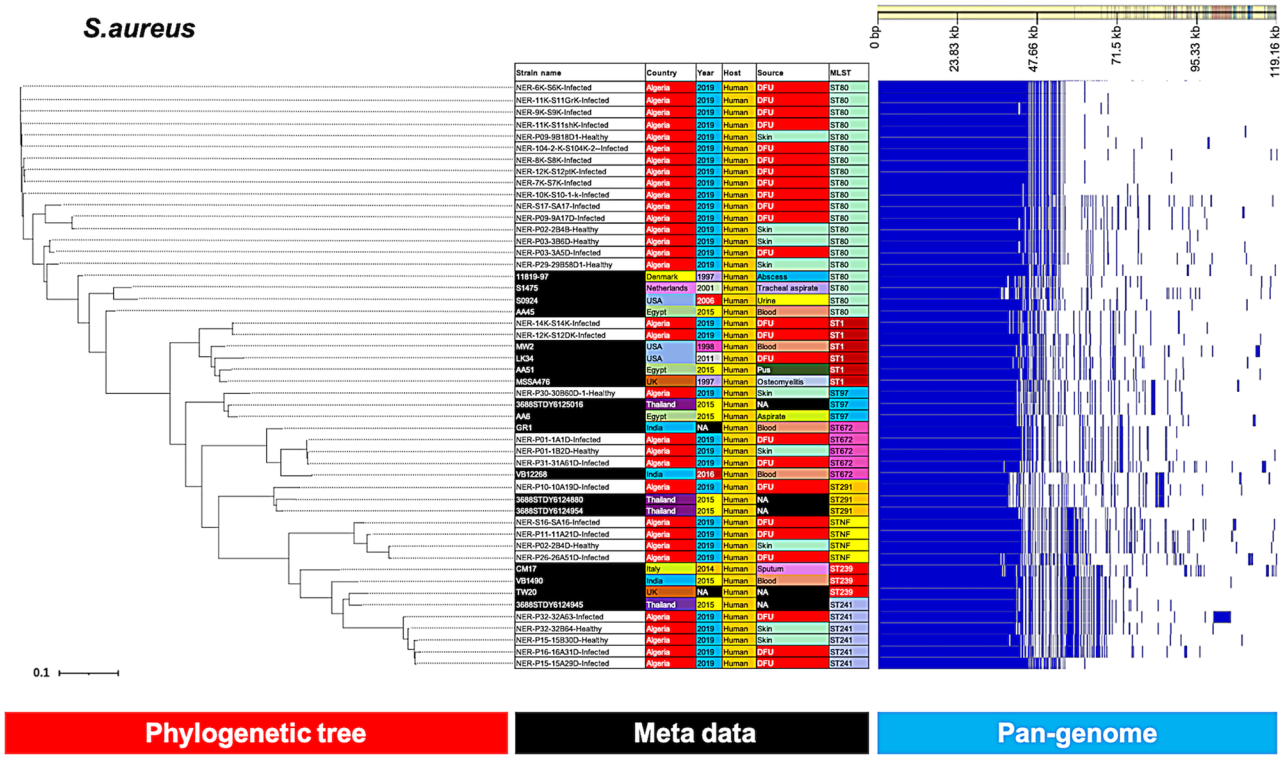

Fig. 2 Phylogenetic analysis of *S. aureus* based on the pan-genomes with Roary. On the right, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. The phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree file in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (11819-97, S1475, S0924, S0924, AA45, MW2, LK34, AA51, MSSA476, 3688STDY6125016, AA6, GR1, VB12268, 3688STDY6124880, 3688STDY6124954, CM17, TW20 VB1490, 3688STDY6124945) were highlighted by black in the strain name

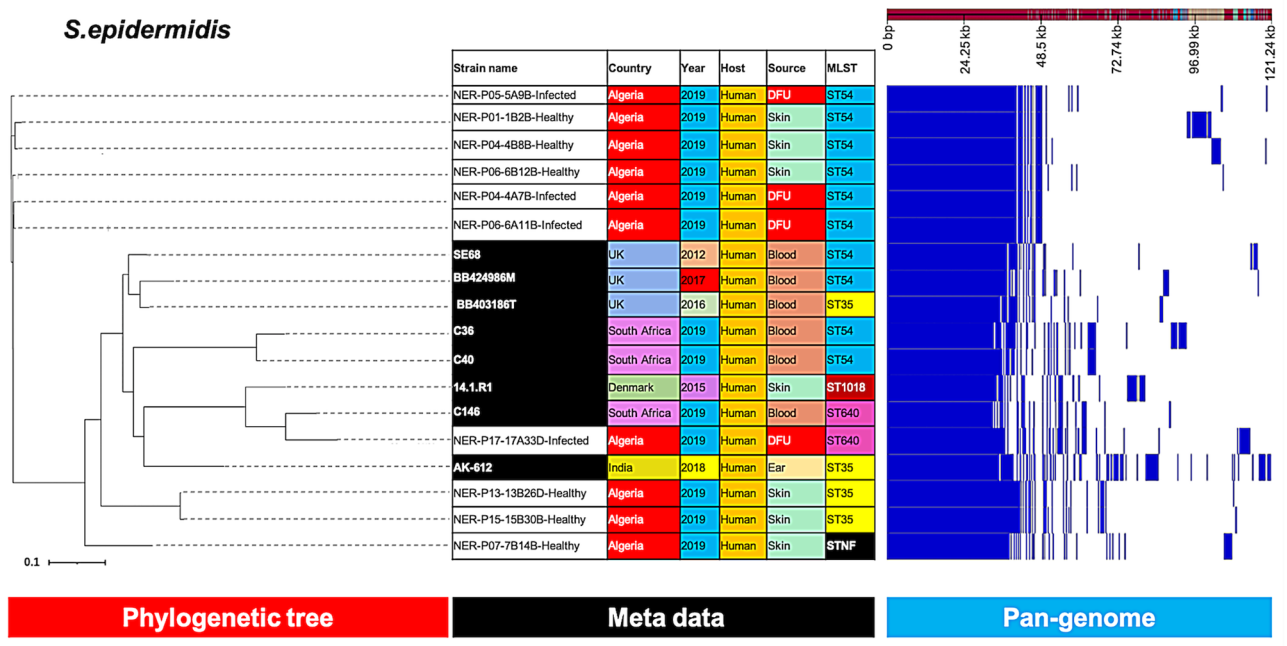

Fig. 3 Phylogenetic analysis of *S. epidermidis* based on the pan-genomes with Roary. On the right of each panel, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. Phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree files in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (SE68, BB424986M, BB403186T, C36, C40, 14.1.R1, C146, AK-612) were highlighted by black in the strain name

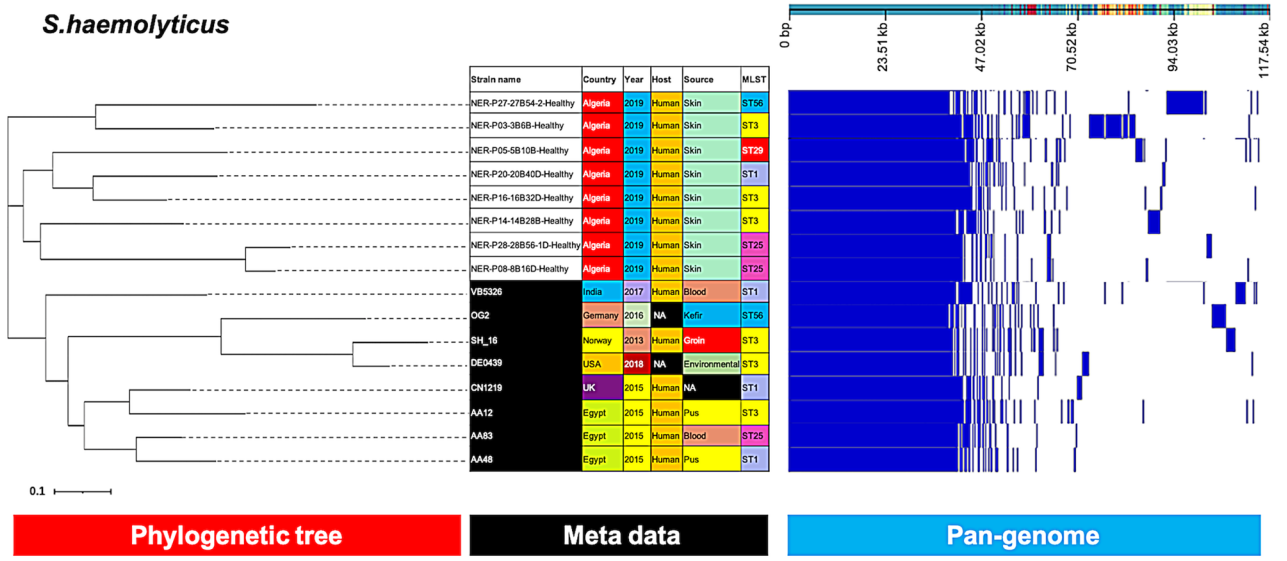

Fig. 4 Phylogenetic analysis of *S. haemolyticus* based on the pan-genomes with Roary. On the right of each panel, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. Phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree files in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (VB5326, OG2, SH_16, DE0439, CN1219, AA12, AA83, AA48) were highlighted by black in the strain name

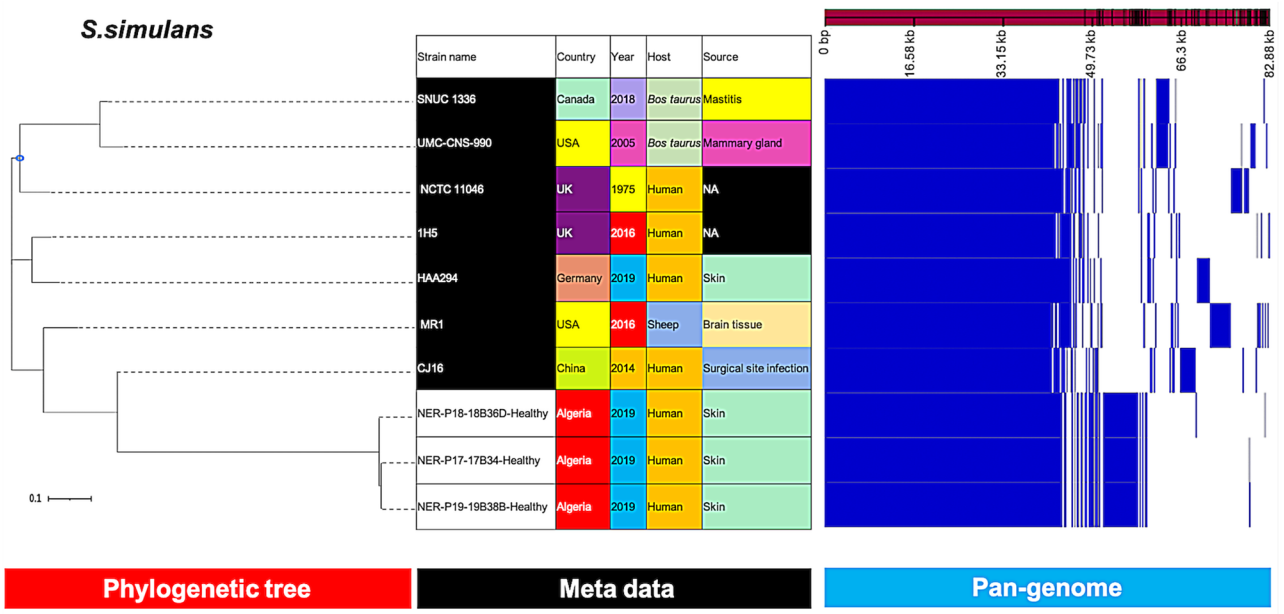

Fig. 5 Phylogenetic analysis of *S. simulans* based on the pan-genomes with Roary. On the right of each panel, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. Phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree files in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (SNUC 1336, UMC-CNS-990, NCTC 11,046, 1H5, HAA294, MR1, CJ16) were highlighted by black in the strain name

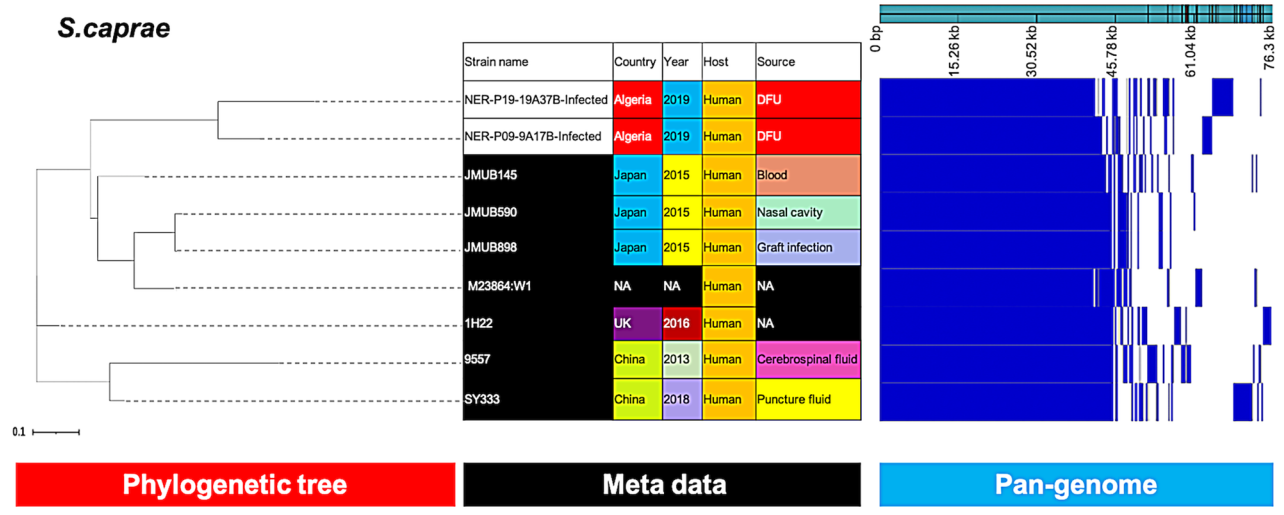

Fig. 6 Phylogenetic analysis of *S. caprae* based on the pan-genomes with Roary. On the right of each panel, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. Phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree files in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (JMUB145, JMUB590, JMUB898, M23864:W1, 1H22, 9557, SY333) were highlighted by black in the strain name

Discussion

In this study, we investigated clonal composition, virulence and resistance determinants of *Staphylococcus/Mammaliicoccus* species isolated from DFUs and healthy skin. *S. aureus* was recovered from the same sampling site alone or in combination with other CoNS/*Mammaliicoccus* species.

S. aureus isolates, including MRSA recovered from the healthy skin and DFUs of each patient, belonged mostly to the same clone and had similar genotype (P1, P3, P9, P15 and P32). This is consistent with earlier findings that

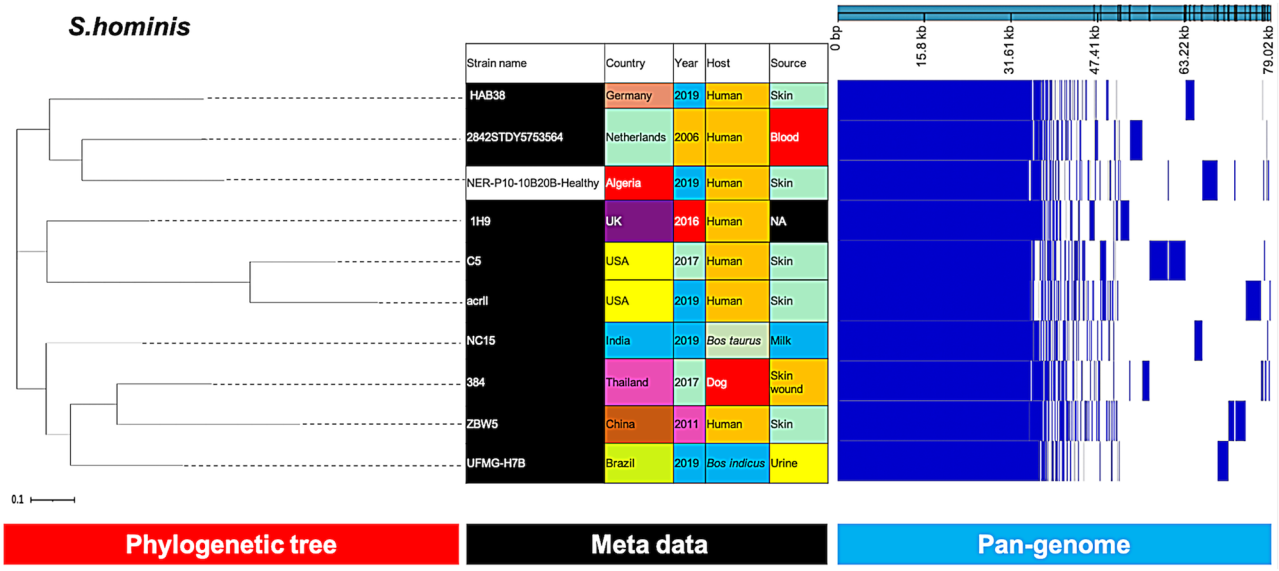

Fig. 7 Phylogenetic analysis of *S. hominis* based on the pan-genomes with Roary. On the right of each panel, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. Phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree files in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (HAB38, 2842STDY5753564, 1H9, C5, acrII, NC15, 384, ZBW5, UFMG-H7B) were highlighted by black in the strain name

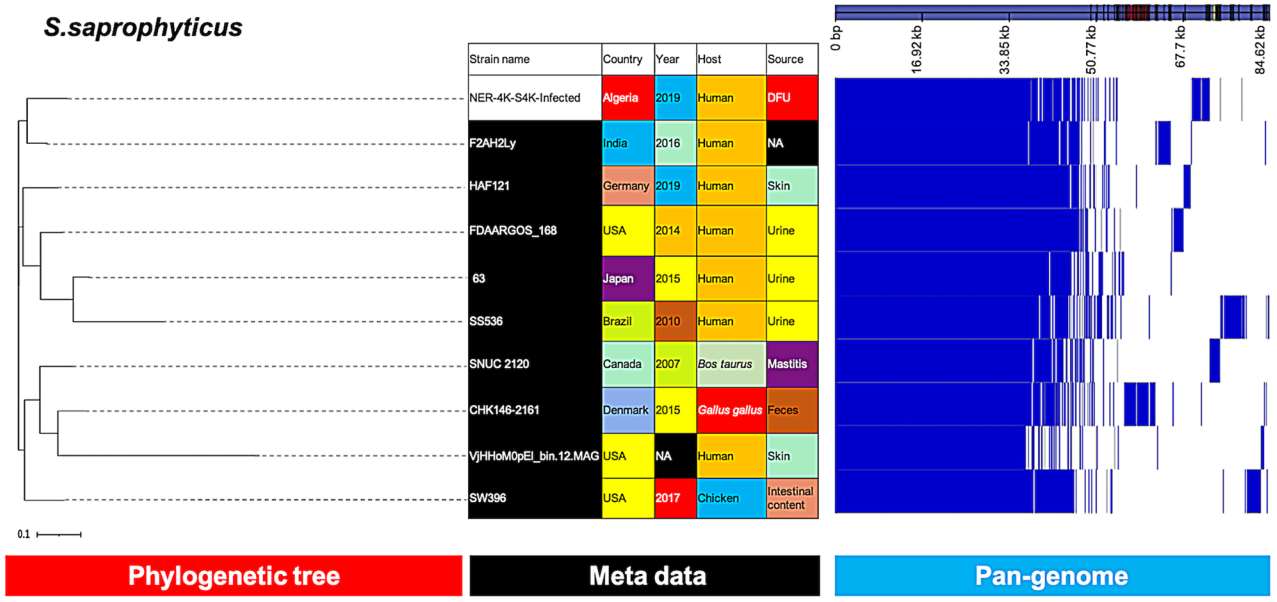

Fig. 8 Phylogenetic analysis of *S. saprophyticus* based on the pan-genomes with Roary. On the right of each panel, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. Phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree files in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (F2AH2Ly, HAF121, FDAARGOS_168, 63, SS536, SNUC 2120, CHK146-2161, VjHHoM0pEl_bin.12.MAG, SW396) were highlighted by black in the strain name

S. aureus isolates recovered from 4 distinct anatomical sites (oro-nasal cavity, periodontal pockets, skin and ulcer) of patients with type 2 diabetes were highly related in the same patient [21]. However, MRSA isolates belonging to different clones were also recovered from the same sampling site (P2).

S. aureus isolates, in particular MRSA, were more prevalent in DFIs than the healthy skin. This finding led to suggest that *S. aureus*, especially MRSA play a significant

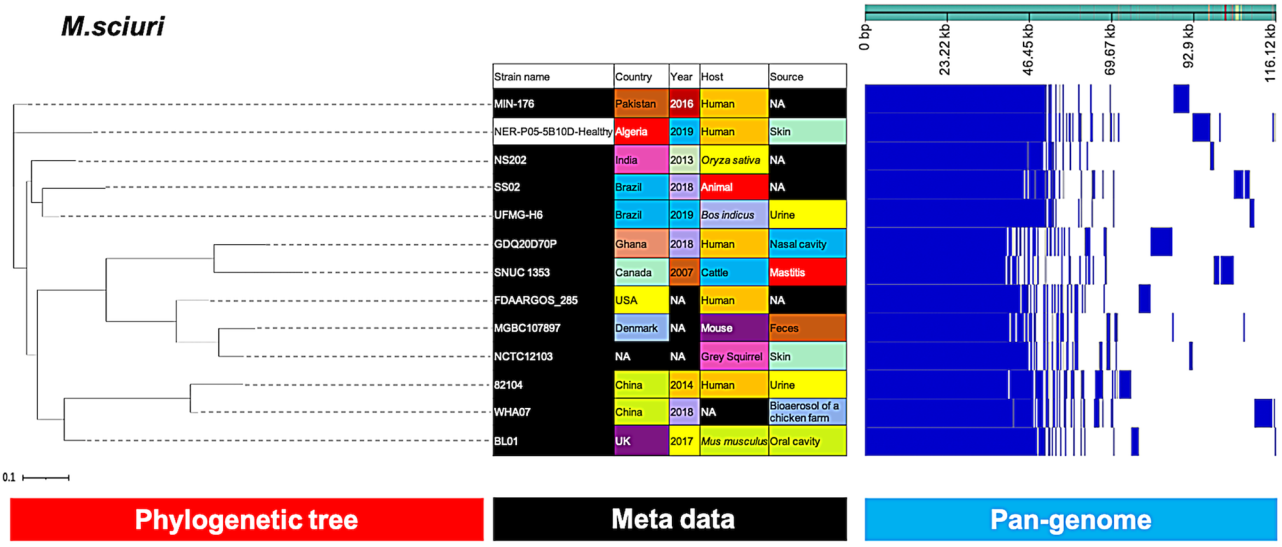

Fig. 9 Phylogenetic analysis of *M. sciuri* based on the pan-genomes with Roary. On the right, the heatmap was generated using the presence and absence of core and accessory genes produced by Roary, genes are represented by white and blue bar for absence and presence, respectively. The phylogenetic tree was visualized in the online interactive viewer Phandango using the absence and presence matrix of genes and the tree file in the standard Newick tree format generated by Roary. Meta data were shown in the middle, reference strains (MIN-176, NS202, SS02, UFMG-H6, GDQ20D70P, SNUC 1353, FDAARGOS_285, MGBC107897, NCTC12103, 82,104, WHA07, BL01) were highlighted by black in the strain name

role in the development and the chronicity of DFIs as described in previous studies [22, 23].

In contrast, CoNS species were mostly recovered from healthy skin, with the exception of *S. caprae* and *S. saprophyticus* which were isolated only from DFIs.

Diabetic patients are thought to be major vehicles for clonal dissemination of staphylococci between hospitals and the community [24]; this could explain the relatively high genetic diversity of our staphylococci. Indeed, our MRSA isolates carried SCC*mec* III or IV, which are commonly associated with healthcare- and community-acquired infections, respectively.

This study revealed that the dominant MRSA clone (13/27, 48.1%) had the characteristics of the European clone (ST80- t044- IVc(2B)) [25]. Only isolates belonging to this clone carried *lukF/lukS-PV.*

Despite the known community origin of *PVL*-positive ST80- IV [26], this clone was highly prevalent in our investigation, confirming the results of other studies from Algeria, which reported the widespread occurrence of *PVL*-positive ST80- IV in Algerian hospitals [27, 28]. Similarly, several studies have also reported that ST80 CA-MRSA was spreading in healthcare settings in Tunisia [29], Jordan [30] and Kuwait [31, 32].

In addition to *PVL*, isolates belonging to the European clone (ST80- t044- IVc(2B)) carried *edinB* and SCC*mec* type IV considered as stable genetic markers for CA-MRSA [26, 33].These properties suggest the community origin of this clone.

The EDIN coding genes are powerful molecular markers associated with poor wound outcomes, that could differentiate colonization from infection in DFUs [34, 35]. In our study, *edinB*-positive ST80- IVc(2B) isolates were recovered from different grades of severity (2–4), from both DFUs and the healthy skin, which indicates the lack of association between these virulence markers and the severity of DFUs. These results contrast with data obtained in France [34], where *edin* were found to be highly prevalent in *S. aureus* isolates from high-grade foot ulcers.

The Brazilian clone (ST241-t037- III(3 A)) was the second most prevalent clone in our study. This clone includes ST239, ST240 and ST241 harboring SCC*mec*-III(A), which differ in mutations in *pta* or *yqiL* genes [36].

Given that isolates belonging to the Brazilian clone (ST241-t037- III(3 A)) carried genes that confer resistance to several classes of antibiotics, corroborates a recent Algerian study performed in the province of Constantine that reported a high prevalence (72.5%) of a worrisome emerging multidrug resistant Brazilian clone (*PVL*-negative ST239/241 SCC*mec*-III mercury) [37]. Furthermore, this clone has been reported to be the major HA-MRSA clone in hospitals in another Algerian province [38].

Interestingly, another study from Algeria reported that 82.2% of the MRSA isolated from DFIs belonged to ST239 [20]. Furthermore, studies from India suggest that the Brazilian clone has been found to be associated with high biofilm production in DFUs, and positive for *luk-DE* and *icaA-B* [39].

ST672-t3841-IVd(2B) is another MRSA clone found in this study, it was detected among healthy skin and DFU

isolates. ST672 is an emerging MRSA clone in India and Australia [40, 41] and commonly associated with CA-MRSA [42]. In addition, this clone has been reported in DFU patients in India [39]. To the best of our knowledge, this is the first report of this clone in Algeria.

The 5 MSSA isolates belonged to 4 different clones, ST1-t127 (2/5, 40%), ST97-t9432, ST291-t937 and STNF-t037 (1/5 each, 20%). The finding of ST1 among MSSA isolates was consistent with previous European studies [26, 43]. However, in the USA, this clone was reported as CA-MRSA, and was also found associated with DFIs across all healing categories [44].

Interestingly, two of our MSSA clones, ST291-t937 and ST97-t9432, were previously identified as livestock-associated [45]. ST291 was also reported in DFU patients in India [39]. We noted that the ST291-t937 isolate lacked the human innate immune evasion cluster (*IEC)* (*sak, chp, scn* and *sea)* which confirms its animal origin [46]. In contrast, the ST97-t9432 carried the *IEC*, harboring the *sak* and *scn* genes, which could suggest a human origin of this clone [47].

The phylogenetic analysis revealed that *Staphylococcus/Mammaliicoccus* spp. carried a high number of accessory genes which have features characteristic of transferred elements (presence of mobility genes) and may provide selective advantages under particular conditions such as antibiotic resistance, adaptation, colonization and pathogenicity [48].

Despite that certain strains clustered with refence strains, there was a clear distinction between our isolates and those from other countries. The phylogenetic comparison of ST80 with the European (11819-97 and S1475), Egyptian (AA45) and the USA (S0924) strains revealed that the Algerian ST80 strains were quite diverse from all the reference strains and mostly clonal, indicated by the extremely short branches at the tip of each clonal branch.

Both the MRSA- and MSSA-STNF exhibited a close clonality and slight variation in gene content, suggesting that the STNF-MRSA clone emerged following the acquisition of SCC*mec* [26]. In addition, the pan-genome analysis confirmed also that the STNF shared a common ancestor with ST241 and ST239.

S. haemolyticus isolates belonging to the same ST were not clustered together throughout the phylogenetic tree. Hence, STs that are intermingled with another may be a result of recent divergence or recombination of the MLST genes [49].

No statistically significant association was found between the presence of virulence genetic determinants and the severity of DFUs. This result contrasts with the findings of a previous report that suggested that infected DFU markers *sea, sei, lukE and hlgγ*, were strongly associated with strains from grades 2–4 DFUs, and that *cap8* was associated with strains from grade 1 ulcers and MSSA strains [50].

The higher frequency of adhesin-encoding genes among our *S. aureus* isolates suggests that they have a potential to form biofilms, which could contribute to their persistence and chronicity in DFU [39, 51].

Likewise, the higher prevalence of γ-hemolysins, *lukE-lukD* and *cap8* cluster genes among our *S. aureus* isolates was consistent with a previous study which reported high frequency of γ -hemolysin genes in MRSA isolates recovered from DFU specimens and patients nares [30].

The absence of *se/sel* genes in the ST80 clone, is in agreement with previous studies which found that ST80 CA-MRSA did not harbor any enterotoxin genes [29, 52]. However, a study in Kuwait hospitals reported that PVL-positive ST80 CA-MRSA carried *sed, sei, seg, seb, seh and sea*, suggesting that ST80 isolates arose from SE negative isolates due to the acquisition of SE-carrying bacteriophages [32].

In contrast, a high number of *se/sel* genes was found among ST672 and ST1 isolates. The production of a large number of superantigen exotoxins (Sag) might contribute to the worsening of DFUs by the activation of T cells and the production of proinflammatory cytokines [53].

The *ACME* was detected only in *S. epidermidis* isolates, which is in agreement with a previous study that reported a higher prevalence of *ACME* amongst *S. epidermidis* [54]. *ACME* contributes to the success of bacteria in acidic environments as the acid environment on the skin [55]. In fact, the extensive success of certain *S. aureus* strains, such as USA300 (ST8-MRSA-IVa), the US epidemic CA-MRSA strain, has been partially attributed to the presence of *ACME* which is thought to originate from *S. epidermidis* [56].

We have identified a wide range of antimicrobial resistance genes among our isolates, with varying distribution between species or ST. Genes coffering resistance to tetracycline, fusidic acid and fosfomycin were prevalent only in *S. epidermidis*. The macrolide resistance genes *erm(A)* and *erm(C)* were predominant in *S. aureus* ST241 clone and *S. haemolyticus*, respectively. Aouati et al. (2021) reported that *ermA* and *ermC* were responsible for erythromycin-resistance in multidrug resistant HA-MRSA ST239/241 strains in Algeria [37], which is in perfect agreement with our finding. Noteworthily, the *erm(A)* gene was previously reported as the most prevalent gene in MRSA strains in DFIs in Algeria [20].

Carriage of *AME* genes was mostly associated with *S. haemolyticus* and *S. aureus*, particularly ST80 and ST241 isolates, which proves that the monotherapy with AME fails to eradicate DFIs due to these bacteria.

Our data suggest a widespread distribution of resistant genes among *S. epidermidis* and *S. haemolyticus* isolates, the opportunistic pathogens which form part of the

normal commensal flora of humans, whilst it is difficult to eradicate because of their resistance to multiple antibiotics [17]. In addition, *S. epidermidis* and other CoNS can provide a reservoir of genes facilitating MRSA infection such as antibiotic resistance determinants [11]. Thus, it has been suggested that *S. epidermidis* may play an essential role in DFI etiology [21].

The *tet(K)* and *fusB* genes were less abundant among ST80 isolates, which was in contrast with previous studies that demonstrated that Algerian PVL-positive ST80- IV strains were resistant to multiple antibiotics, in particular to these drugs [57–59].

Although our study provided some important information on the population and genetic profile of *Staphylococcus/Mammaliicoccus* spp. isolated from DFIs, it suffers from few limitations; (i) small sample size; (ii) lack of phenotypic antibiotic resistance data; and (iii) non-inclusion of a control group of patients who had not received antibiotics.

Conclusions

In conclusion, our pan-genome analysis demonstrated that the Algerian *S. aureus* and CoNS/*Mammaliicoccus* isolates were closely related to each other, and presented novel genetic features, with a widespread distribution of virulence factors and antibiotic resistance genes, rendering this pathology more difficult to manage. The detection of the same *S. aureus/*CoNS clone in both DFIs and the healthy skin suggests that the autochthonous skin staphylococci can act as a reservoir for DFIs. To the best of our knowledge, this study represents the first investigation in Algeria, employing WGS and pan-genome analysis to get an insight on the underlying diversity and pathogenicity of *Staphylococcus/Mammaliicoccus* in DFIs. Most importantly, this study highlights the importance of WGS in disease surveillance and outbreak investigation, as it allows fine typing and detailed gene profiling of bacterial isolates.

Methods

Study group

Patients aged over 18 years who were hospitalized with infected DFUs at the University Regional Military Hospital and the University Hospital Ben Badis, in the province of Constantine, Algeria, from October to December 2019, were included in this study. 32 patients had a single ulcer and were sampled from both healthy foot skin (contralateral site to the chronic wound) and DFU. Patients who underwent surgical procedure including amputations were excluded from the study.

In addition, 14 strains obtained from the routine diagnostic recovered from 12 hospitalized DFU patients were added, including 2 patients who presented with 2 ulcers on the same foot.

DFUs were classified by clinicians using PEDIS classification (grade 2–4) proposed by the Diseases Society of America (IDSA) and the International Working Group on the Diabetic Foot Classifications of Diabetic Foot Infection (IWGDF) [60]. Patient demographics including age, gender, PEDIS grade, ulcer location and antibiotics taken during the 15 previous days were recorded.

Sample collection

After wound debridement and cleansing with sterile saline solution, pus samples were collected in deep wounds from infected tissues. The healthy skin samples were obtained by swabbing of an intact skin area measuring 50 cm^2. Swabs were immediately transported to the laboratory of microbiology in 1 ml of sterile saline 0.9% for culture. The strains were isolated on mannitol salt agar after incubation at 37 °C for 24 h.

Whole genome sequencing analysis

Genomic DNA was extracted from 58 non duplicate *Staphylococcus/Mammaliicoccus* isolates by lytic treatment using achromopeptidase (Wako Pure Chemical Industries, Osaka, Japan) and then Sodium Dodecyl Sulfate (10%). DNA was purified using Zymo Research kit (Zymoresearch, Irvine, Ca, USA), according to the manufacturer's instructions.

A DNA sequencing library (insert size, 300 to 500 bp) was prepared using a QIAseq FX DNA Library Kits (Qiagen, Germany). WGS was performed using the Illumina NextSeq 500 platform with the 300-cycle NextSeq 500 paired-end read sequencing (2×150-mer).

Bioinformatic analysis

Annotation of the genomes was performed with Prokka [61]. Putative bacterial species were determined using Krona [62].

To characterize isolates, sequencing reads were analyzed *in silico* by multi locus sequence typing (MLST) [63]. spa types and SCC*mec* were identified *in silico* with the online tools spaTyper and SCC*mec*Finder [64]. Antimicrobial resistance genes were identified by homology searching against the ResFinder database [65].

For pan-genome analysis, 58 isolates from this study and publicly available sequences either at draft or complete genome sequences (a total of 78 strains) [see Additional file 3] was performed using Roary [66]. Tree construction was performed using FastTree and visualized in the online interactive viewer Phandango [67] using the absence and presence matrix of genes and the tree file in the standard Newick tree format generated by Roary.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS; ver. 26.0). Contingency tables were constructed and Chi-squared tests were used to calculate p-value for describing possible associations between species/ST and virulence/resistance genes with PEDIS grades and the source of isolates. p-values < 0.05 were considered statistically significant.

Abbreviations

CA-MRSA	Community-acquired MRSA
CoNS	Coagulase-negative *Staphylococcus*
CoPS	Coagulase-positive *Staphylococcus*
DFI	Diabetic foot infection
DFU	Diabetic foot ulcer
HA-MRSA	Healthcare-acquired MRSA
MRSA	Methicillin-resistant *S. aureus*
MSSA	Methicillin-sensitive *S. aureus*
MSCoNS	Methicillin-sensitive coagulase negative staphylococci
WGS	Whole genome sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-023-03087-2.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

MS and MK designed the study and reviewed the manuscript. RT and NF performed the whole genome sequencing. TS and MK performed the bioinformatic analysis. NF isolated bacterial strains, performed *in silico* typing, pan-genome analysis, statistical analysis and wrote the draft manuscript. MS supervised the research and revised the manuscript. All authors read and approved the final manuscript.

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Data Availability

All data generated or analyzed in this study are included within the article and its additional files. The Short Read Archives (SRA) were deposited in NCBI database, [https://www.ncbi.nlm.nih.gov/bioproject?LinkName=sra_bioproject&from_uid=26401144] with the accession number PRJDB13730. All complete sequences in this study are available, as shown in the additional file 1.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations, all rules of confidentiality and ethics as prescribed in the Helsinki Declaration have been respected and all experimental protocols were approved by ethics committee of the University Regional Military Hospital and University Hospital Ben Badis of Constantine, Algeria. Informed consent was obtained from all subjects and/or their legal guardians.

Consent for publication

Not applicable.

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References

1. Kaul K, Tarr JM, Ahmad S, Kohner EM, Chibber R. Introduction to Diabetes Mellitus. In: Ahmad SI, editor. Diabetes. New York, NY: Springer; 2012. pp. 1–11. https://doi.org/10.1007/978-1-4614-5441-0_1.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level Diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119. https://doi.org/10.1016/j.diabres.2021.109119.
3. World Health Organization. Diagnosis and management of type 2 diabetes (HEARTS-D). 2020;1–35.
4. Rodrigues BT, Vangaveti VN, Urkude R, Biros E, Malabu UH. Prevalence and risk factors of lower limb amputations in patients with diabetic foot ulcers: a systematic review and meta-analysis. Diabetes Metab Syndr Clin Res Rev. 2022;16(2):102397. https://doi.org/10.1016/j.dsx.2022.102397.
5. Boulton AJM, Armstrong DG, Kirsner RS, Attinger CE, Lavery LA, Lipsky BA, et al. Diagnosis and management of diabetic foot Complications. ADA Clin Compend. 2018;2018(2). https://doi.org/10.2337/db20182-1.
6. Barshes NR, Sigireddi M, Wrobel JS, Mahankali A, Robbins JM, Kougias P, et al. The system of care for the diabetic foot: objectives, outcomes, and opportunities. Diabet Foot Ankle. 2013;4(1):21847. https://doi.org/10.3402/dfa.v4i0.21847.
7. Jacqueline ES. Eliminating the Health disparity of the Diabetic Foot: a Continuing Public Health Crisis for African americans and latinos. Int J Diabetes Clin Res. 2020;7(2):120. https://doi.org/10.23937/2377-3634/1410120.
8. Madhaiyan M, Wirth JS, Saravanan VS. Phylogenomic analyses of the staphylococcaceae family suggest the reclassification of five species within the genus staphylococcus as heterotypic synonyms, the promotion of five

subspecies to novel species, the taxonomic reassignment of five staphylococcu. Int J Syst Evol Microbiol. 2020;70(11):5926–36.
9. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus Infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–61. https://doi.org/10.1128/CMR.00134-14.
10. Moran GJ, Anusha K, Rachel JG, Gregory EF, Mcdougal LK, Carey RB, et al. Methicillin-Resistant S. Aureus Infections among patients in the Emergency Department. N Engl J Med. 2006;355(7):666–74. https://doi.org/10.1056/NEJMoa055356.
11. Otto M. Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA Infection: staphylococcal commensal species such as Staphylococcus epidermidis are being recognized as important sources of genes promoting MRSA colonization and virulence. BioEssays. 2013;35(1):4–11.
12. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, et al. 2012 infectious Diseases society of America clinical practice guideline for the diagnosis and treatment of diabetic foot Infections. Clin Infect Dis. 2012;54(12):132–73.
13. Spichler A, Hurwitz BL, Armstrong DG, Lipsky BA. Microbiology of diabetic foot Infections: from Louis Pasteur to crime scene investigation. BMC Med. 2015;13(1):1–13. https://doi.org/10.1186/s12916-014-0232-0.
14. Dunyach-Remy C, Essebe CN, Sotto A, Lavigne JP. Staphylococcus aureus toxins and diabetic foot ulcers: role in pathogenesis and interest in diagnosis. Toxins (Basel). 2016;8(7):209.
15. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27(4):870–926.
16. Hartemann-Heurtier A, Senneville E. Diabetic foot osteomyelitis. Diabetes Metab. 2008;34(2):87–95. https://doi.org/10.1016/j.diabet.2007.09.005.
17. Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, et al. Whole-genome sequencing of Staphylococcus haemolyticus uncovers the Extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. J Bacteriol. 2005;187(21):7292–308. https://doi.org/10.1128/JB.187.21.7292-7308.2005%0A.
18. Marincola G, Liong O, Schoen C, Abouelfetouh A, Hamdy A, Wencker FDR, et al. Antimicrobial Resistance profiles of Coagulase-negative Staphylococci in Community-based healthy individuals in Germany. Front Public Heal. 2021;9(June):684456.
19. Ertuğrul B, Uçkay I, Schöni M, Peter-Riesch B, Lipsky BA. Management of diabetic foot Infections in the light of recent literature and new international guidelines. Expert Rev Anti Infect Ther. 2020;18(4):293–305.
20. Djahmi N, Messad N, Nedjai S, Moussaoui A, Mazouz D, Richard JL, et al. Molecular epidemiology of staphylococcus aureus strains isolated from inpatients with infected diabetic foot ulcers in an Algerian University Hospital. Clin Microbiol Infect. 2013;19(9):E398–404. https://doi.org/10.1111/1469-0691.12199.
21. McManus BA, Daly B, Polyzois I, Wilson P, Brennan GI, Fleming TE, et al. Comparative microbiological and whole-genome analysis of Staphylococcus aureus populations in the oro-nasal cavities, skin and Diabetic Foot ulcers of patients with type 2 Diabetes reveals a possible Oro-Nasal Reservoir for Ulcer Infection. Front Microbiol. 2020;11:748.
22. Silva V, Almeida F, Carvalho JA, Castro AP, Ferreira E, Manageiro V, et al. Emergence of community-acquired methicillin-resistant Staphylococcus aureus EMRSA-15 clone as the predominant cause of diabetic foot Ulcer Infections in Portugal. Eur J Clin Microbiol Infect Dis. 2020;39(1):179–86.
23. Cervantes-García E, García-González R, Reséndiz-Albor A, Salazar-Schettino PM. Infections of diabetic foot ulcers with methicillin-resistant staphylococcus aureus. Int J Low Extrem Wounds. 2015;14(1):44–9.
24. Mottola C, Semedo-Lemsaddek T, Mendes JJ, Melo-Cristino J, Tavares L, Cavaco-Silva P, et al. Molecular typing, virulence traits and antimicrobial resistance of diabetic foot staphylococci. J Biomed Sci. 2016;23(1):1–10. https://doi.org/10.1186/s12929-016-0250-7.
25. Rolo J, Miragaia M, Turlej-Rogacka A, Empel J, Bouchami O, Faria NA, et al. High genetic diversity among community-associated staphylococcus aureus in europe: results from a multicenter study. PLoS ONE. 2012;7(4):e34768.
26. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant staphylococcus aureus carrying panton-valentine leukocidin genes: Worldwide emergence. Emerg Infect Dis. 2003;9(8):978–84. https://doi.org/10.3201/eid0908.030089.
27. Bekkhoucha SN, Cady A, Gautier P, Itim F, Donnio PY. A portrait of staphylococcus aureus from the other side of the Mediterranean Sea: molecular characteristics of isolates from western Algeria. Eur J Clin Microbiol Infect Dis. 2009;28(5):553–5. https://doi.org/10.1007/s10096-008-0660-x.
28. Antri K, Rouzic N, Dauwalder O, Boubekri I, Bes M, Lina G, et al. High prevalence of methicillin-resistant Staphylococcus aureus clone ST80-IV in hospital and community settings in Algiers. Clin Microbiol Infect. 2011;17(4):526–32. https://doi.org/10.1111/j.1469-0691.2010.03273.x.
29. Ben Nejma M, Mastouri M, Bel Hadj Jrad B, Nour M. Characterization of ST80 Panton-Valentine leukocidin-positive community-acquired methicillin-resistant Staphylococcus aureus clone in Tunisia. Diagn Microbiol Infect Dis. 2013;77(1):20–4. https://doi.org/10.1016/j.diagmicrobio.2008.02.010.
30. Al-Bakri AG, Bulatova NR, Younes NA, Othman G, Jaber D, Schleimer N, et al. Characterization of staphylococci sampled from diabetic foot Ulcer of Jordanian patients. J Appl Microbiol. 2021;131(5):2552–66. https://doi.org/10.1111/jam.15096.
31. Udo EE, O'Brien FG, Al-Sweih N, Noronha B, Matthew B, Grubb WB. Genetic lineages of community-associated methicillin-resistant Staphylococcus aureus in Kuwait hospitals. J Clin Microbiol. 2008;46(10):3514–6. https://doi.org/10.1128/JCM.00966-08.
32. Udo EE, Sarkhoo E. The dissemination of ST80-SCCmec-IV community-associated methicillin resistant Staphylococcus aureus clone in Kuwait hospitals. Ann Clin Microbiol Antimicrob. 2010;9(1):1–7. https://doi.org/10.1186/1476-0711-9-31.
33. Paul SK, Ghosh S, Kawaguchiya M, Urushibara N, Hossain MA, Ahmed S, et al. Detection and genetic characterization of PVL-positive ST8-MRSA-IVa and exfoliative toxin d-positive European CA-MRSA-Like ST1931 (CC80) MRSA-IVa strains in Bangladesh. Microb Drug Resist. 2014;20(4):325–36. https://doi.org/10.1089/mdr.2013.0153.
34. Messad N, Landraud L, Canivet B, Lina G, Richard JL, Sotto A, et al. Distribution of edin in Staphylococcus aureus isolated from diabetic foot ulcers. Clin Microbiol Infect. 2013;19(9):875–80. https://doi.org/10.1111/1469-0691.12084.
35. Shettigar K, Murali TS. Virulence factors and clonal diversity of Staphylococcus aureus in colonization and wound Infection with emphasis on diabetic foot Infection. Eur J Clin Microbiol Infect Dis. 2020;39(12):2235–46. https://doi.org/10.1007/s10096-020-03984-8.
36. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PLoS ONE. 2011;6(4):e17936. https://doi.org/10.1371/journal.pone.0017936.
37. Aouati H, Hadjadj L, Aouati F, Agabou A, Khedher M, Ben, Bousseboua H, et al. Emergence of methicillin-resistant staphylococcus aureus ST239/241 SCCmec-III mercury in Eastern Algeria. Pathogens. 2021;10(11):1503. https://doi.org/10.3390/pathogens10111503.
38. Alioua MA, Labid A, Amoura K, Bertine M, Gacemi-Kirane D, Dekhil M. Emergence of the European ST80 clone of community-associated methicillin-resistant Staphylococcus aureus as a cause of healthcare-associated Infections in Eastern Algeria. Med Mal Infect. 2014;44(4):180–3. https://doi.org/10.1016/j.medmal.2014.01.006.
39. Shettigar K, Jain S, Bhat DV, Acharya R, Ramachandra L, Satyamoorthy K, et al. Virulence determinants in clinical Staphylococcus aureus from monomicrobial and polymicrobial Infections of diabetic foot ulcers. J Med Microbiol. 2016;65(12):1392–404. https://doi.org/10.1099/jmm.0.000370.
40. Khedkar S, Prabhakara S, Loganathan RM, Chandana S, Gowda M, Arakere G, et al. Draft genome sequence of staphylococcus aureus ST672, an emerging Disease clone from India. J Bacteriol. 2012;194(24):6946–7. https://doi.org/10.1128/JB.01868-12%0A.
41. Shambat S, Nadig S, Prabhakara S, Bes M, Etienne J, Arakere G. Clonal complexes and virulence factors of Staphylococcus aureus from several cities in India. BMC Microbiol. 2012;12(1):1–9. https://doi.org/10.1186/1471-2180-12-64.
42. Mahakalkar C, Padhye D, Nandagawali VR, Takale S, Ahdal J, Jain R. Management of acute bacterial skin and skin structure Infections in India: are we equipped to meet the challenges of the growing menace of methicillin-resistant Staphylococcus aureus? Int Surg J. 2020;7(9):3172–9. https://doi.org/10.18203/2349-2902.isj20203821.
43. Holden MTG, Feil EJ, Lindsay JA, Peacock SJ, Day NPJ, Enright MC, et al. Complete genomes of two clinical Staphylococcus aureus strains: evidence for the evolution of virulence and drug resistance. Proc Natl Acad Sci U S A. 2004;101(26):9786–91. https://doi.org/10.1073/pnas.040252110.
44. Kalan LR, Meisel JS, Loesche MA, Horwinski J, Soaita I, Chen X, et al. Strain- and species-Level Variation in the Microbiome of Diabetic Wounds is Associated with clinical outcomes and therapeutic efficacy. Cell Host Microbe. 2019;25(5):641–655e5. https://doi.org/10.1016/j.chom.2019.03.006.

45. Van Wamel WJB, Rooijakkers SHM, Ruyken M, Van Kessel KPM, Van Strijp JAG. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of Staphylococcus aureus are located on β-hemolysin-converting bacteriophages. J Bacteriol. 2006;188(4):1310–5. https://doi.org/10.1128/JB.188.4.1310-1315.2006%0A.
46. Rohmer C, Wolz C. The role of hlb-converting bacteriophages in Staphylococcus aureus host adaption. Microb Physiol. 2021;31(2):109–22. https://doi.org/10.1159/000516645.
47. Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, Aarestrup FM, et al. Livestock origin for a human pandemic clone of community-associated methicillin-resistant Staphylococcus aureus. MBio. 2013;4(4):e00356–13. https://doi.org/10.1128/mBio.00356-13%0A.
48. Hacker J, Carniel E. Ecological fitness, genomic islands and bacterial pathogenicity. EMBO Rep. 2001;2(5):376–81. https://doi.org/10.1093/embo-reports/kve097.
49. Smith JT, Amador S, McGonagle CJ, Needle D, Gibson R, Andam CP. Population genomics of Staphylococcus pseudintermedius in companion animals in the United States. Commun Biol. 2020;3(1):1–11. https://doi.org/10.1038/s42003-020-1009-y.
50. Sotto A, Lina G, Combescure C, Bourg G, Vidal L, Jourdan N, et al. Virulence potential of Staphylococcus aureus strains isolated from Diabetic Foot Ulcers: a new paradigm. Diabetes Care. 2008;31(12):2318–24. https://doi.org/10.2337/dc08-1010.
51. Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in diabetic foot ulcers: significance and clinical relevance. Microorganisms. 2020;8(10):1580. https://doi.org/10.3390/microorganisms8101580.
52. Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, et al. Global distribution of Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus, 2006. Emerg Infect Dis. 2007;13(4):594–600. https://doi.org/10.3201/eid1304.061316.
53. Vu BG, Stach CS, Salgado-Pabón W, Diekema DJ, Gardner SE, Schlievert PM. Superantigens of Staphylococcus aureus from patients with diabetic foot ulcers. J Infect Dis. 2014;210(12):1920–7. https://doi.org/10.1093/infdis/jiu350.
54. O'Connor AM, McManus BA, Kinnevey PM, Brennan GI, Fleming TE, Cashin PJ, et al. Significant enrichment and diversity of the staphylococcal arginine catabolic mobile element ACME in Staphylococcus epidermidis isolates from subgingival peri-implantitis sites and periodontal pockets. Front Microbiol. 2018;9:1558. https://doi.org/10.3389/fmicb.2018.01558.
55. Shore AC, Rossney AS, Brennan OM, Kinnevey PM, Humphreys H, Sullivan DJ, et al. Characterization of a Novel Arginine Catabolic Mobile element (ACME) and Staphylococcal Chromosomal Cassette Mec Composite Island with significant homology to S. Epidermidis ACME type II in Methicillin-resistant S. aureus genotype ST22-MRSA-IV. Antimicrob Agents Chemother. 2011;55(5):1896–905. https://doi.org/10.1128/AAC.01756-10%0A.
56. Chatterjee SS, Otto M. Improved understanding of factors driving methicillin-resistant Staphylococcus aureus epidemic waves. Clin Epidemiol. 2013;5(1):205–17. https://doi.org/10.2147/CLEP.S37071.
57. Ramdani-Bouguessa N, Bes M, Meugnier H, Forey F, Reverdy ME, Lina G, et al. Detection of methicillin-resistant Staphylococcus aureus strains resistant to multiple antibiotics and carrying the Panton-Valentine leukocidin genes in an Algiers hospital. Antimicrob Agents Chemother. 2006;50(3):1083–5. https://doi.org/10.1128/AAC.50.3.1083-1085.2006.
58. Djoudi F, Bonura C, Benallaoua S, Touati A, Touati D, Aleo A, et al. Panton-Valentine leukocidin positive sequence type 80 methicillin-resistant Staphylococcus aureus carrying a staphylococcal cassette chromosome mec type IVc is dominant in neonates and children in an Algiers hospital. New Microbiol. 2013;36(1):49–56.
59. Ouchenane Z, Smati F, Rolain JM, Raoult D. Molecular characterization of methicillin-resistant Staphylococcusaureus isolates in Algeria. Pathol Biol. 2011;59(6):e129–32. https://doi.org/10.1016/j.patbio.2009.11.004.
60. Lipsky BA, Senneville É, Abbas ZG, Aragón-Sánchez J, Diggle M, Embil JM, et al. Guidelines on the diagnosis and treatment of foot Infection in persons with Diabetes (IWGDF 2019 update). Diabetes Metab Res Rev. 2020;36(Suppl 1):e3280. https://doi.org/10.1002/dmrr.3280.
61. Seemann T, Prokka. Rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9. https://doi.org/10.1093/bioinformatics/btu153.
62. Ondov BD, Bergman NH, Phillippy AM. Interactive metagenomic visualization in a web browser. BMC Bioinformatics. 2011;12(1):1–10. https://doi.org/10.1186/1471-2105-12-385.
63. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 2012;50(4):1355–61. https://doi.org/10.1128/JCM.06094-11.
64. Center for Genomic Epidemiology. Accessed. February 2020. [Internet]. http://www.genomicepidemiology.org.
65. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67(11):2640–4. https://doi.org/10.1093/jac/dks261.
66. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: Rapid large-scale prokaryote pan genome analysis. Bioinformatics. 2015;31(22):3691–3. https://doi.org/10.1093/bioinformatics/btv421.
67. Hadfield J, Croucher NJ, Goater RJ, Abudahab K, Aanensen DM, Harris SR. Phandango: an interactive viewer for bacterial population genomics. Bioinformatics. 2018;34(2):292–3. https://doi.org/10.1093/bioinformatics/btx610.

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