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Evaluation of virulence potential of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolates from a German refugee cohort

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ABSTRACT

Background: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) seem to be highly transmissible, often infect otherwise healthy humans and frequently occur in hospital outbreaks.
Methods: Refugees, living in accommodations in Germany were screened for nasal carriage of *S. aureus*. The isolates were investigated regarding resistance and virulence, phenotypically and by whole genome data analysis.
Results: 5.6% (9/161) of the refugees are carriers of *S. aureus*. 2.5% (4/161) are MRSA carriers. Among the refugees, *spa*-types t021, t084, t304, t991 and t4983 were detected, as well as the new *spa*-types t18794 and t18795. t304 and t991 are assumed to be local *spa*-types from the middle east. The isolates are less resistant and marginal biofilm formers. Each isolate has a remarkable set of virulence genes, although genes, encoding for proteins strongly associated with invasive *S. aureus* infections, like Panton-Valentine leucocidin, were not detected.
Conclusion: The detection of strains from the middle east, supports the assumption that strains co-travel with the refugees and persist despite a transition of the host's living conditions. Whole genome data analysis does not permit to finally evaluate a germ's virulence. Nevertheless, an impression of the virulence potential of the strains, regarding skills in colonization, resistance, immune evasion, and host cell damaging can be pictured.

1. Introduction

Refugees escape from various countries and often transit several states on their way. Refugees who arrived in Germany between 2014 and 2016 mostly came from the Arab language area (especially Syria, Iraq, Afghanistan) and Sub-Saharan Africa. The majority escaped via the Balkan route [1]. Along the route, people transit countries which are heterogeneous in prevalence of methicillin-resistant *S. aureus* (MRSA) infections. In countries without MRSA surveillance and respective research, there is a deficiency in the knowledge about prevalences of MRSA infections and common local MRSA variants. While in most African countries, as well as in Syria, Iraq and Afghanistan no MRSA monitoring exists, the Balkan countries have a surveillance network. Compared to Germany, a higher prevalence of invasive infections with antimicrobial resistant bacteria (AMR), including MRSA, was documented in the Balkans. All countries on the Balkan route (Turkey, Greece, North Macedonia, Serbia, Croatia and Slovenia) have rates of 25–50% nosocomial invasive MRSA infections [2]. Similar to the European trend, the rate of invasive MRSA infections in Germany decreased from 11.1% in 2015 to 7.6% in 2018 [3]. The predominant number (63.5%) of infections occur in health care settings [4]. In 2017/2018, community-acquired MRSA (CA-MRSA) represented about 10% of the positive specimen sent to the National Reference Center in Germany. The most frequent clonal complexes within CA-MRSA isolates

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are CC8, CC5 and CC30 [5,6]. In contrast to healthcare-acquired (HA-) MRSA, CA-MRSA are strongly associated with skin- and soft tissue infections (SSTIs) and frequently infect otherwise healthy young individuals. Thus, they spread rapidly among healthy members of a community [7,8]. Worldwide, several local strain types occur. As reviewed by Tuner and colleagues, sequence types ST2, ST5, ST8 and ST398 predominate in America, ST22, ST30, ST36, ST80, ST239 and ST398 in Europe, clonal complexes CC5 and CC8 and sequence types ST59, ST72, ST239 and ST772 in Asia, ST5, ST22, ST30 and ST80 in Africa and ST93 and CC121 are most prevalent in Australia [9]. Amongst them are CA-MRSA and HA-MRSA strains. Regarding to phenotypical properties, CA-MRSA are often characterized by the expression of toxins, in particular Panton-Valentine Leukocidin (PVL) and by presence of the arginine catabolic mobile element (ACME). Furthermore, among CA-MRSA the prevalence of the smaller SCC*mec* cassette types IV and V is higher. CA-MRSA were long time considered to be commonly susceptible to clindamycin, chloramphenicol and fluoroquinolones, though resistant strains incrementally appear [9–11]. Strommenger et al. defined four genes as genetic markers characteristic for common clonal lineages of CA-MRSA: the PVL gene *lukPV*, the enterotoxin H gene *seh*, the arginine deiminase gene *arcA* (on ACME) and the *etd* gene, encoding for exfoliative toxin D. Each of the genes is highly prevalent in one of the most common CA-MRSA lineages [12]. CA-MRSA are supposed to express a particular set of virulence factors and regulatory systems, different from HA-MRSA, promoting the remarkable virulence and successful transmission in the community. For example, phenol soluble modulins (PSMs) are considerably produced by CA-MRSA, while HA-MRSA produce none or marginal amounts of PSMs [13]. Highly pathogenic CA-MRSA are producing higher amounts of cell wall-associated wall teichoic acids (WTA), resulting in an increased WTA-dependent and T-cell-mediated induction of skin abscesses in a murine animal model [14]. Otsuka et al. demonstrated a strong association of the adhesins bone sialoprotein (Bbp) and collagen adhesin (Cna) with CA-MRSA of ST30 [15]. In USA300 and USA400, exoprotein assessment revealed an increased occurrence of eleven virulence factors in the supernatants, amongst them: alpha-hemolysin (Hla), collagen adhesin (Cna), staphylokinase (Sak), coagulase (Coa), lipase (Lip), enterotoxin C3 (Sec3), enterotoxin Q (Seq), V8 protease (SspA) and cysteine protease (SspB) [16]. These virulence factors include adhesins, toxins and proteins involved in host immune evasion.

The knowledge about the CA-MRSA virulence is based, in many cases, on research on USA300/ST8 and USA400/ST1 strains. Other CA-clones, including MSSA, need to be investigated to develop the overall picture. In the on-hand study, *S. aureus* isolates from a local German refugee population are investigated. This small-sized study aims to give an overview of *spa*-types and sequence types (STs) found in the population and tries to retrace, if the strains co-travelled with the refugees. Moreover, the *S. aureus* isolates were comprehensively analyzed regarding antibiotic resistances, biofilm formation and genetic provision with virulence genes.

2. Materials and methods

2.1. Sampling and questionnaire

Sampling was performed in course of the "FlüGe"- refugee health study (University Bielefeld project) between January and August 2018. All participants are refugees,[1] who arrived in Germany within five years before data acquisition. They lived in communal accommodations in North Rhine Westphalia, Germany. In the course of "FlüGe" – refugee health study, all participants answered a questionnaire in interview format, guided by an instructed interviewer and translator in the participant's language. The questionnaire involves demographic data, information about countries of origin and flight routes, history of hospitalization and medical treatments, as well as further information regarding physical, mental, and social health. Each participant of the study was asked to voluntarily provide a nasal swab. Due to specifications of the ethics committee, the participants swabbed their own nasal cavities after instruction. Within 6 h after swabbing, 1 ml phosphate buffered saline (PBS) was added to each viscose swab (Sarstedt, Nümbrecht - Germany). Swabs were frozen at −20 °C until proceeding.

2.2. Cultivation conditions

Nasal swabs were streaked on tryptic soy broth (TSB; Oxoid, Basingstoke, GB), mannitol salt agar (MSA: 10 g/l peptone, 1 g/l beef extract, 75 g/l NaCl, 10 g/l D-mannitol, 0.025 g/l phenol red, 15 g/l agar, pH 7.4 ± 0.2) and MRSA chromogenic agar (Roth, Karlsruhe, GER) without supplementing antibiotics. Pure cultures of the isolates were cultivated at 37 °C on Müller-Hinton agar with 5% sheep blood (Thermo Scientific, Schwerte, GER), on Tryptic Soy Agar, and in TSB under shaking conditions (100 rpm).

2.3. Identification

Isolate colonies were controlled by coagulase-agglutination test (Pastorex Test, Bio-Rad, Germany) and identified by 16S rDNA sequence analysis. 16S rDNA was amplified with the primer pair 5′-CTACCTTGTTACGACTTCAC-3′ and 5′-CACGGCTAACTACGTGCCA-3'. The amplicons were Sanger-sequenced and matched with RDP database (http://rdp.cme.msu.edu/, [17]). For *spa* typing, the *spa* gene was amplified as described before [18]. The isolates were attributed to *spa* types with Ridom SeqSphere+ Software (https://www.ridom.de/spatype, [19]).

2.4. PCR-based analysis

The *spa* and *mecA* genes were amplified by PCR as described before [18]. By a multiplex-PCR developed by Strommenger et al., isolates were screened for genes characteristic for common CA-MRSA lineages [12]. SCC*mec* cassettes of *mecA*-positive strains were classified as described by Kondo et al. and Boye et al. [20,21].

2.5. Antibiograms

Minimal inhibitory concentrations (MICs) of the antibiotics cefoxitin, ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid, gentamycin, moxifloxacin, mupirocin, oxacillin, penicillin, rifampicin, teicoplanin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole, and vancomycin were determined by broth microdilution, according to EUCAST standards [22] and to ISO standard 20776-1. The results were interpreted applying the EUCAST clinical breakpoint table (https://eucast.org/clinical_breakpoints/, European Committee on Antimicrobial Susceptibility Testing [22]. In addition, strains were cultivated in nutrient broth with 2 μg/ml oxacillin and 8 μg/ml sulbactam, to exclude a β-lactamase-mediated oxacillin resistance.

2.6. Biofilm assays

The ability of the strains to form biofilms was assessed as described by Heilmann et al. [24]. As adaption, 1% (w/v) Glucose was supplemented to the medium. *Staphylococcus carnosus* TM300 was set as negative control and *S. aureus* RN4220 as positive control. Strains are defined as biofilm formers, if they produce significantly (p-value <0.05) more biofilm as the negative control. Biofilm thickness was therefore

[1] The term "refugee" is not used as a political category of migrants. In context of this study, it describes asylum seekers, registered by the German government, and allocated to a district for accommodation. At the time of sampling, the participants application for asylum was in preparation, in process or was closed (with positive or negative result).

equalized with the light absorption by the stained cell layer (semi--quantitative approach).

2.7. Whole genome sequencing (WGS)

Genomic DNA was extracted using the NucleoSpin gDNA Clean-up kit (Macherey Nagel, Düren, GER), according to the manufacturer's guidelines. The protocol was extended by a 30 min lysis step with 40 μg/ml lysostaphin (Abcam, Cambridge, UK). The Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA) was used to measure the DNA concentration. Genomic libraries were prepared using the Nextera DNA Flex library preparation kit (Illumina, San Diego, CA) and, subsequently, sequenced using 2 × 300-bp paired-end library on the Illumina MiSeq platform.

For generation of the draft genome, paired end reads were processed in Galaxy (www.usegalaxy.org). First, reads were quality controlled with FastQC [25], then trimmed with Trim Galore! [26] and Trimmomatic [27]. The reads were rechecked with FastQC, before mapping to the reference genome (N315, NC_002745.2) with BWA [28].

2.8. Whole genome data analysis

Whole genome data analysis was performed in a two phased approach. In the first phase online tools provided by the Center for genomic Epidemiology were used (http://www.genomicepidemiology.org/), including the tools ResFinder 3.2 [29] and Virulence Finder 2.0 [30], as well as the typing tool MLST 2.0 [31]. In the second phase, genes in the draft genome were predicted using the annotation tool Prokka [32] and screened for known virulence genes. A phylogenetic tree was computed from whole genome sequences with the online tool CSI Phylogeny 1.4 [33].

3. Results

From a total of 198 participants (76% male) in the "FlüGe" refugee health study, 161 (81.3%, 78% male) agreed to provide a nasal swab. *S. aureus* was detected in 9 (5.6%, 100% male) of the 161 nasal swabs. Four isolates (2.5%) were MRSA. Several colonies per swab were picked and processed. Only one *S. aureus spa*-type could be detected per participant. The isolates belong to *spa*-types t021, t084, t304, t991 and t4983. In addition, two isolates with by now unknown *spa*-types (t18794 and t18795) were found. All isolates were attributed to multilocus sequence types (STs) and clonal complexes (CCs, see Table 1).

3.1. Sociodemography and health status

The *S. aureus* carriers were all male and came from Iraq, Syria, Iran, Afghanistan, and Bangladesh (see Supplementary A). The carriers have spent an average of nearly 50 weeks for their escape and resided in Germany since 3.25 years on average (>5 years as criterion for exclusion). None of the *S. aureus* carriers (0/9) stated suffering from inflammatory skin irritations (in all participants: 12.6%, n = 20). While 4/9 suffered from chronic diseases, 2/9 had been hospitalized and 1/9 underwent surgery in the previous year. Furthermore, 4/8 *S. aureus* carriers took antibiotics in the past, from whom only one took them in the previous 12 months. Considering the overall small number of nine cases, this data cannot be regarded as representative of the German refugee population.

3.2. Antibiograms

All isolated *S. aureus* strains are resistant against penicillin G (PEN). Four isolates (*spa*-types t304, t021 and t991) are confirmed as MRSA and are resistant against oxacillin (OX) and cefoxitin (FOX), which equates to 44% of all *S. aureus* isolates and 2.5% of all specimens. Each MRSA strain was also cultivable in oxacillin/sulbactam medium. The t021/ST30 isolate is resistant against three antibiotic classes: β-lactams (PEN, OX, FOX), erythromycin (ERY) and clindamycin (CLI). Furthermore, one t084 (2)/ST15 MSSA isolate is resistant against tetracycline. Resistance profiles are displayed in Table 1. Antibiotics to which none of the isolates was resistant are not shown. All MRSA strains possess SCC*mec* element type IV (*ccr* gene complex type 2, class B *mec* gene complex).

3.3. Community-acquired strains

The isolates were screened for marker genes indicating common CA-lineages. While PVL genes (*lukSF-PV)* and enterotoxin H gene *seh* were not detected, *arcA* was amplified from MRSA t021/ST30 and *etd* was

Table 1
Minimal inhibitory concentration (MIC) values of all resistances of the isolated *S. aureus* stains. Resistance (R) is represented by dark boxes; light boxes indicate susceptibility (S). Antibiotics are abbreviated as following: PEN – penicillin, OXA – oxacillin, FOX – cefoxitin, ERY – erythromycin, CLI – clindamycin, TET – tetracycline. Growth in oxacillin/sulbactam medium (OXA/SUL) is notified by a plus (+), while no growth is indicated by a minus symbol (−). In case of MRSA, SCC*mec* cassette types are listed as well. MLST-multilocus sequence type, CC – clonal complex.

spa-type MLST CC		t021 ST30 CC30	t084 ST15 CC15	t084 ST15 CC15	t304 ST6 CC5	t304 ST6 CC5	t991 ST913 CC913	t4983 ST46 CC45	t18794 ST7 -	t18795 ST291 -
carriers' origin		$Iraq_1$	$Syria_1$	$Syria_2$	$Iraq_2$	$Iraq_3$	Syria Lebanon	Iran	Bangla-desh	Afghan-istan
Resistance/ MIC [μg/ml]	PEN	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$	$R_{(MIC>1)}$
	OXA	$R_{(MIC>4)}$	S	S	$R_{(MIC>4)}$	$R_{(MIC>4)}$	$R_{(MIC>4)}$	S	S	S
	FOX	$R_{(MIC>16)}$	S	S	$R_{(MIC>16)}$	$R_{(MIC>16)}$	$R_{(MIC>16)}$	S	S	S
	ERY	$R_{(MIC>8)}$	S	S	S	S	S	S	S	S
	CLI	$R_{(MIC>2)}$	S	S	S	S	S	S	S	S
	TET	S	S	$R_{(MIC>8)}$	S	S	S	S	S	S
OXA/SUL		+	-	-	+	+	+	-	-	-
SCC*mec* type		IV	-	-	IV	IV	IV	-	-	-
legend: S= sensitive, I= intermediate resistant, R= resistant										

Table 2
Short overview on geographic occurrence and frequency of the *spa*-types (or sequence types) found in the "FlüGe" health study.

spa-type/ sequence type	Geographic occurrence and frequency	References
t021/ ST30	- ubiquitarian CA-strains - within ST30 a PVL-positive and CA-lineage arose - in this study t021/ST30 was isolated from an Iraqi and does not express PVL	[8] [68]
t084/ ST15	- one of the most prevalent strains worldwide - Europe: mostly MSSA, frequently isolated from healthy carriers - Middle East: mostly MRSA, causing infections and circulating in hospitals - here, two t084/ST15 MSSA were isolated from participants originating from Syria	[69,70] [34,36,69] [71,72]
t304/ ST6	- relatively new strain type, first occurred in the early 2010s - caused outbreaks in Denmark - occur in European studies, wherein refugees were screened and are associated with refugees from Iraq - t304 (no MLST defined) was detected in 2017/18 for the first time in Iran - in this study, two Iraqis carried *spa* type t304/ST6	[73] [50] [74]
t991/ ST913	- exfoliative toxin-positive and PVL-negative local MRSA clone in the Middle East - Europe: only found in refugee screening studies, carriers stated to originate from Syria or Iraq - here, the t991 was isolated from a participant from Syria - the isolate is PVL-negative but *etD*-positive	[51,72,75] [41,50]
t4983/ ST46	- as far as known, t4983 has not been described in the literature before - ST46 was sporadically mentioned in few clinical studies - CC45 is primarily known for the epidemic "Berlin (E)MRSA" (ST45), from Germany and adjoining countries - the t4983/ST46 isolate, in this study, derived from an Iranian participant	[73,76] [77–79]
t18794/ ST7	- ST7 is most common in China, associated with poultry meat - t18794 was newly detected in this study, in the sample of a refugee from Bangladesh - MSSA	[80,81]
t18795/ ST291	- MSSA/MRSA of ST291 frequently occur in hospitals in Iran - worldwide, it occurs sporadically - here, t18795 (MSSA) was isolated from an Afghan refugee, representing the first report of this *spa* type	[52,53,82,83]

detected in MRSA t991/ST913 and MSSA t18795/ST291. Both genes were detected in other lineages as those, which they are characteristic for (etd – ST80, arcA - ST8). Moreover, *spa* types t084 (ST15), t304 (ST6), t4983 (ST46) and t18794 (ST7) were isolated. Therein, none of the marker genes have been detected by PCR. Due to sampling of *per se* healthy refugees in a non-health care-setting, the entirety of the *S. aureus* isolates are CA-isolates, from the epidemiological perspective.

In Table 2, short reports of literature research on each *spa*-type (or ST) and its geographic occurrence and frequency in *S. aureus* screening studies are given.

3.4. Biofilm formation

Assessment of biofilm formation reveals slight to moderate biofilm formation of 5/9 isolates (t021, t3042, t991, t4983, t18794; see Fig. 1) in comparison to *S. aureus* RN4220 and *Staphylococcus carnosus* TM300.

3.5. Whole genome sequencing and virulence profiles

To assess the genetic makeup of the isolates with virulence determinants, the entire genome was sequenced. Whole genome data are provided in the NCBI BioProject PRJNA658858 (Accession numbers

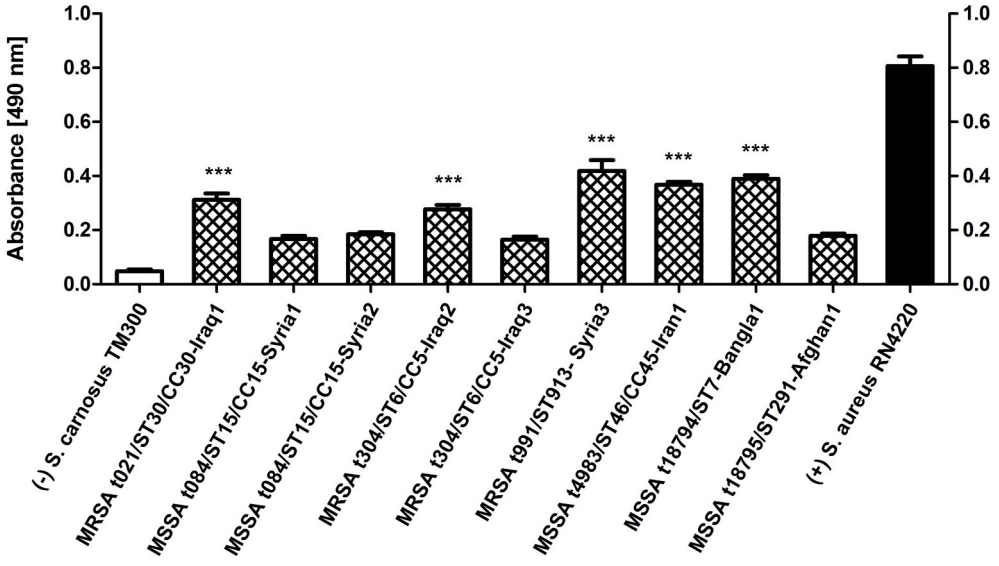

Fig. 1. The isolates capability to form biofilms assayed by a semi-quantitative colorimetric microtiter plate assay. Columns labelled with asterisks (***) are biofilm formers, significantly ($p < 0.05$) producing more biofilm as the negative control (*S. carnosus* TM300). *S. aureus* RN4220 was used as the positive control for biofilm formation.

Table 3
Virulence genes and antimicrobial resistance genes found in *S. aureus* isolates of the refugees by using VirulenceFinder and ResFinder and by screening annotated genes.

major gene function	short term	gene product	t021/ ST30/ CC30	t084(1)/ ST15/ CC15	t084(2)/ ST15/ CC15	t304(2)/ ST6/ CC5	t304(3)/ ST6/ CC5	t991/ ST913	t4983/ ST46/ CC45	t18794/ ST7	t18795/ ST291	
resistance genes	*mecA*	penicillin-binding protein PBP2a, Class B1 PBP	x	o	o	x	x	x	o	o	o	[84]
	mecC	penicillin-binding protein PBP2′, Class B1 PBP	o	o	o	o	o	o	o	o	o	[85]
	pbpD/pbp4	penicillin-binding protein PBP4, Class C PBP	o	o	o	o	o	o	o	o	o	[86]
	mecR	transmembrane sensor protein/mecA-regulator	o	o	o	x	x	o	o	o	o	[84]
	ermA	rRNA adenine N-6-methyltransferase,	x	o	o	o	o	o	o	o	o	[87]
	ermC	rRNA adenine N-6-methyltransferase, ER	o	o	o	o	o	o	o	o	o	
	tet38	MFS tetracycline efflux pump	x	x	x	x	x	x	x	x	x	[88],[89]
	tetA	class B MFS tetracycline efflux pump, TET-RP	o	x	x	x	x	x	o	x	o	
	vanA	low-affinity peptidoglycan precursor synthesis protein	o	o	o	o	o	o	o	o	o	[90]
	aacA/aphD aac (6′)-Ie-aph(2‴)	6′aminoglycoside *N*-acetyltransferase/ 2″aminoglycoside phosphotransferase, AAC (6′)/APH(2‴)	o	o	o	o	o	o	o	o	o	[89][91]
	ant1/aadC ant (9)-Ia	ANT(9)-Ia streptomycin-3″-adenyltransferase	x	o	o	o	o	o	o	o	o	
	aph(3′)-III	aminoglycoside-3′-phosphotransferase-III, APH(3′)-III	o	o	o	o	o	o	o	o	o	
	aadCD/knt/ant (4′)-Ia	kanamycin nucleotidyltransferase, aminoglycoside O-nucleotidyltransferase ANT (4′)-Ia	o	o	x	o	o	o	o	o	o	
	ileS	isoleucyl-tRNA synthetase	o	o	o	o	o	o	o	o	o	[89]
	msrA/B	efflux transporter/peptide methionine sulfoxide reductase	x	x	x	x	x	x	x	x	x	[92]
	mprF	multiple peptide resistance factor	x	x	x	x	x	x	x	x	x	[93]
	norA	multidrug efflux pump NorA	o	o	o	o	o	o	o	o	o	[94],[95]
	norB	multidrug efflux pump NorB	x	x	x	x	x	x	x	x	x	
	norC	multidrug efflux pump NorC	o	o	o	o	o	o	o	o	o	
	sdrM	multidrug efflux pump SdrM	o	x	x	x	x	x	o	x	o	
	mepA	multidrug efflux pump MepA	x	x	x	x	x	x	x	x	x	[89][95]
	sepA	multidrug resistance ABC transporter SepA	o	x	x	x	x	x	o	x	x	
	bmrA	multidrug resistance ABC transporter/ permease protein BmrA	x	x	x	x	x	x	x	x	x	[94](Bacillus subtilis)
	emrB/mdeA	multidrug export protein EmrB/MdeA	o	o	o	o	o	o	x	o	o	[94][96]
	tap	multidrug efflux pump Tap	x	x	x	o	o	o	o	o	o	[97](*Mycobacterium tuberculosis*)
	dltA	D-alanine carrier/transfer protein A	x	x	x	x	x	x	x	x	x	[98]
	dltB	D-alanine carrier/transfer protein B	o	o	o	o	o	o	o	o	o	

(*continued on next page*)

Table 3 (*continued*)

major gene function	short term	gene product	t021/ ST30/ CC30	t084(1)/ ST15/ CC15	t084(2)/ ST15/ CC15	t304(2)/ ST6/ CC5	t304(3)/ ST6/ CC5	t991/ ST913	t4983/ ST46/ CC45	t18794/ ST7	t18795/ ST291	
	dltC	D-alanine carrier/transfer protein C	x	x	x	x	x	x	x	x	x	
	dltD	D-alanine carrier/transfer protein D	x	x	x	x	x	x	x	x	x	
surface determinants	*spa*	staphylococcal surface protein A	x	x	x	x	x	x	x	x	x	[59]
	isdA	iron-regulated surface determinant protein A	o	x	x	x	x	x	x	x	x	
	isdB	iron-regulated surface determinant protein B	x	x	x	x	x	x	x	x	x	
	isdC	iron-regulated surface determinant protein C	x	x	x	x	x	x	x	x	x	
	isdH	iron-regulated surface determinant protein H	x	x	x	x	x	x	x	x	x	
	sasG	*S. aureus* surface protein G	o	o	o	o	o	o	o	o	o	
	pls	plasmin sensitive protein	o	o	o	o	o	o	o	o	o	
	sraP	serine-rich adhesin for platelets	x	x	x	x	x	o	o	o	x	[59][99]
	bap	biofilm-associated protein Bap	o	o	o	o	o	o	o	o	o	[59][100]
	ebpS	elastin-binding protein EbpS	x	x	x	x	x	x	x	x	x	[99]
	sasX	*S. aureus* surface protein	o	o	o	o	o	o	o	o	o	[59][101]
	clfA	clumping factor A	x	x	x	o	x	x	o	o	o	[59][102]
	clfB	clumping factor B	o	o	o	o	o	o	o	o	o	
	bbp	bone sialoprotein-binding protein	x	o	o	x	o	o	o	o	x	
	fnbA	fibronectin-binding protein A	x	x	x	x	x	x	x	x	x	
	fnbB	fibronectin-binding protein B	o	o	o	o	o	o	x	x	x	
	cna	collagen adhesin	o	o	o	o	o	o	o	o	o	
	sdrC	serine-aspartate repeat containing protein C	o	x	o	x	o	x	o	x	o	
	sdrD	serine-aspartate repeat containing protein D	o	x	x	x	x	o	x	x	x	
	sdrE	serine-aspartate repeat containing protein E	o	x	x	x	x	x	x	x	o	
	isaB	immunodominant staphylococcal antigen B	o	x	x	x	x	x	o	x	o	[99][103]
toxins	*hly/hla*	α-toxin/α-hemolysin	o	x	x	x	x	x	x	x	x	[104]
	hlb	β-hemolysin, phospholipase C, sphingomyelinase C	o	x	x	x	x	x	x	x	x	
	hlgA	γ-hemolysin component A	x	x	x	x	x	x	x	x	x	
	hlgB	γ-hemolysin component B	x	x	x	x	x	x	x	x	x	
	hlgC	γ-hemolysin component C	o	x	x	x	x	x	o	x	x	
	etA	exfoliative toxin A, serine protease	o	o	o	o	o	o	o	o	o	
	etB	exfoliative toxin A, serine protease	o	o	o	o	o	o	o	o	o	
	sea/entA	enterotoxin A	o	o	o	o	o	o	o	o	o	[104][105]
	seb	enterotoxin B	o	o	o	o	o	o	o	o	o	
	sec	enterotoxin C	o	o	o	o	o	o	o	o	o	
	sed	enterotoxin D	o	o	o	o	o	o	o	o	o	

(*continued on next page*)

Table 3 (*continued*)

major gene function	short term	gene product	t021/ ST30/ CC30	t084(1)/ ST15/ CC15	t084(2)/ ST15/ CC15	t304(2)/ ST6/ CC5	t304(3)/ ST6/ CC5	t991/ ST913	t4983/ ST46/ CC45	t18794/ ST7	t18795/ ST291	
	see	enterotoxin E	o	o	o	o	o	o	o	o	o	
	seg	enterotoxin G	x	o	o	o	o	o	x	o	o	
	seh	enterotoxin H	o	o	o	o	o	o	o	o	o	
	sei/entE	enterotoxin I	x	o	o	o	o	o	x	o	o	
	sej	enterotoxin-like J	o	o	o	o	o	o	o	o	o	[106]
	sek	enterotoxin-like K	o	o	o	o	o	o	o	o	o	
	sel	enterotoxin-like L	o	o	o	o	o	o	o	o	o	
	sem	enterotoxin-like M	o	o	o	o	o	o	x	o	o	
	sen	enterotoxin-like N	x	o	o	o	o	o	x	o	o	
	seo	enterotoxin-like O	x	o	o	o	o	o	x	o	o	
	sep	enterotoxin-like P	o	o	o	o	o	o	o	x	o	
	seq	enterotoxin-like Q	o	o	o	o	o	o	o	o	o	
	ser	enterotoxin-like R	o	o	o	o	o	o	o	o	o	
	seu/entB	enterotoxin-like U	x	o	o	o	o	o	x	o	o	
	selx	staphylococcal enterotoxin-like X	o	x	x	x	x	x	x	x	x	[104]
	tst	toxic shock syndrome toxin −1	x	o	o	o	o	o	o	o	o	
	lukAB	bicomponent leukocidin A and B	o	o	o	o	o	o	o	o	o	
	lukED	bicomponent leukocidin E and D	o	x	x	x	x	x	o	x	x/o	
	lukS	leukocidin S subunit	o	o	o	o	o	o	x	o	o	
	lukFS-PV	panton-valentine leukocidin (PVL)	o	o	o	o	o	o	o	o	o	
	splA	secreted serine protease-like A	o	x	x	x	x	x	o	x	x	
	splB	secreted serine protease-like B	o	x	x	x	x	x	o	x	x	
	splC	secreted serine protease-like C	o	x	x	x	x	x	o	x	o	
	splD	secreted serine protease-like D	o	x	x	x	x	x	o	o	o	
	splE	secreted serine protease-like E	o	o	o	o	o	o	o	o	o	
	splF	secreted serine protease-like F	x	x	x	x	x	x	o	x	x	
	scpA	staphopain A, cysteine protease	x	x	x	x	x	x	x	x	x	
	sspA	V8 protease, serine protase	o	x	x	x	x	o	x	x	x	
	sspB	staphopain B cysteine protease	x	x	x	x	x	x	x	x	x	
	aur	zinc metalloproteinase aureolysin	x	x	x	x	x	x	x	x	x	
PSMs	*psmβ1*	antibacterial protein 3/β-class PSM 1, PSM-β1	x	x	x	x	x	x	x	x	x	[61][104]
	psmβ2	antibacterial protein 2/β-class PSM 2, PSM-β2	o	o	o	o	o	o	o	o	o	
	psmα1-4	α-class phenol soluble modulins	o	o	o	o	o	o	o	o	o	
	hld	δ-hemolysin (α-class PSM)	o	o	o	o	o	o	o	o	o	
	PSMmec	SCCmec encoded phenol-soluble modulin	o	o	o	o	o	o	o	o	o	

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Table 3 (*continued*)

major gene function	short term	gene product	t021/ ST30/ CC30	t084(1)/ ST15/ CC15	t084(2)/ ST15/ CC15	t304(2)/ ST6/ CC5	t304(3)/ ST6/ CC5	t991/ ST913	t4983/ ST46/ CC45	t18794/ ST7	t18795/ ST291	
biofilm genes	*icaA*	Poly-β-1,6-*N*-acetyl-D-glucosamine (PNAG) synthase	x	x	x	x	x	x	x	x	x	[107]
	icaB	PNAG *N*-deacetylase	x	x	x	x	x	x	x	x	x	
	icaC	putative PNAG export protein	x	x	x	x	x	x	x	x	x	
	icaD	PNAG synthesis protein IcaD	x	x	x	x	x	x	x	x	x	
	icaR	*ica operon, negative transcriptional regulator IcaR*	x	x	x	x	x	x	x	x	x	
regulatory systems	*agrA*	accessory gene regulator protein A, QS-System	x	x	x	x	x	x	x	x	x	[108][109]
	agrB	accessory gene regulator protein B	o	x	x	o	o	x	o	o	o	
	sarA	transcriptional regulator SarA	x	x	x	x	x	x	x	x	x	
	sarS	transcriptional regulator SarS	x	x	x	x	x	x	x	x	x	
	sarX	transcriptional regulator SarX	x	x	x	x	x	x	x	x	x	
	sarR	transcriptional regulator SarR	x	x	x	x	x	x	x	x	x	
	sarV	transcriptional regulator SarV	x	x	x	x	x	x	x	x	x	
	sarT	transcriptional regulator SarT	o	x	x	x	x	x	o	o	o	
	sarU	transcriptional regulator SaU	o	x	x	x	x	x	o	o	o	
	sarZ	transcriptional regulator SarZ	x	x	x	x	x	x	x	x	x	
	luxS	QS-System, *S*-ribosylhomocysteine lyase	x	x	x	x	x	x	x	x	x	[110]
	cvfB	conserved virulence factor B	x	x	x	x	x	x	x	x	x	[111]
host immune evasion genes	*chp*	chemotaxis inhibitory protein	x	x	x	o	o	x	x	o	x	[112][113]
	sak	staphylokinase	x	o	o	x	x	x	x	x	x	
	scn	staphylococcal complement inhibitor	x	x	x	x	x	x	x	x	x	
	flr	FPR-like1 inhibitory protein FLIPr	o	x	x	x	x	o	o	x	o	[113]
	fll	FLIPr-like protein	o	o	o	o	o	o	o	o	o	
	ssl1	staphylococcal superantigen-like protein 1	o	o	o	x	o	o	x	x	o	
	ssl3	staphylococcal superantigen-like protein 3	o	x	x	x	x	x	o	x	o	
	ssl4	staphylococcal superantigen-like protein 4	o	x	x	x	x	x	o	x	o	
	ssl5	staphylococcal superantigen-like protein 5	o	x	x	x	x	x	o	x	x	
	ssl7	staphylococcal superantigen-like protein 7	o	x	x	x	x	x	x	x	o	
	ssl10	staphylococcal superantigen-like protein 10	o	x	x	x	x	x	x	x	x	
	ssl13	staphylococcal superantigen-like protein 13	o	x	x	x	x	x	o	x	o	
	ecb	extracellular complement-/fibrinogen-binding protein	o	o	o	o	o	o	o	o	o	
	sbi	immunoglobulin-binding protein	o	o	x	x	x	o	x	o	x	
	efb/fib	(extracellular) fibrinogen-binding protein	o	x	x	x	x	x	o	x	x	
	eap/map		o	o	o	o	o	o	o	o	o	

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Table 3 (*continued*)

major gene function	short term	gene product	t021/ ST30/ CC30	t084(1)/ ST15/ CC15	t084(2)/ ST15/ CC15	t304(2)/ ST6/ CC5	t304(3)/ ST6/ CC5	t991/ ST913	t4983/ ST46/ CC45	t18794/ ST7	t18795/ ST291	
		extracellular adherence protein/MHC analogous protein										
	coa	staphylocoagulase	o	x	x	x	o	o	x	x	o	[114]
	vWbp/vwb	vonWillebrand factor-binding protein	o	o	o	o	o	o	o	o	o	
	esaC	type IV seretion system accessory protein C	o	o	o	o	o	o	o	o	o	[115]
	esxA	type IV secretion system extracellular protein A	x	x	x	x	x	x	x	x	x	
	essC	type IV secretion system protein EssC	o	o	o	x	x	o	x	x	x	
	nuc	thermonuclease Nuc	x	x	x	x	x	x	x	x	x	[104]
others	*ssaA*	staphylococcal secretory antigen SsaA	x	x	x	x	x	x	x	x	x	[116]
	sodA	superoxid dismutase	x	x	x	x	x	x	x	x	x	[117]
	sodM	superoxid dismutase	x	x	x	x	x	x	x	x	x	
	tarS	β-O-GlcNAc transferase, WTA glycoslytransferase	o	x	x	x	x	x	x	x	x	[118]
	ebh/ebhAB	extracellular matrix-binding protein	x	x	x	x	x	x	x	x	x	[119]
	emp/ssp	extracellular matrix-binding protein	o	x	x	x	x	x	x	x	o	
	fmtA	teichoic acid D-alanine hydrolase	x	x	x	x	x	x	x	x	x	[120]
	femA	aminoacetyltransferase FemA	x	x	x	x	x	x	x	x	x	[121]
	femB	aminoacetyltransferase FemB	x	x	x	x	x	x	x	x	x	
	femX	glycyltransferase FemX	x	x	x	x	x	x	x	x	x	
	spn	staphylococcal peroxidase inhibitor	o	o	o	o	o	o	o	o	o	[113]
	ACME	arginine catabolic mobile element (ACME)	o	o	o	o	o	o	o	o	o	[122]

x: gene detected, o: gene not detectable.

Abbreviations: ABC: ATP-binding cassette superfamily; A+A1:N169CME: arginine catabolic mobile element; AG: aminoglycosides; CLI: clindamycin; ERY: erythromycin; MFS: major facilitator superfamily; MLS: macrolides, lincosamides and streptogarmin; MUP: mupirocin; PBP: penicillin binding protein; PNAG: poly-*N*-acetyl-D-glucosamine; PSM: phenol-soluble modulin; QS: quorum sensing.

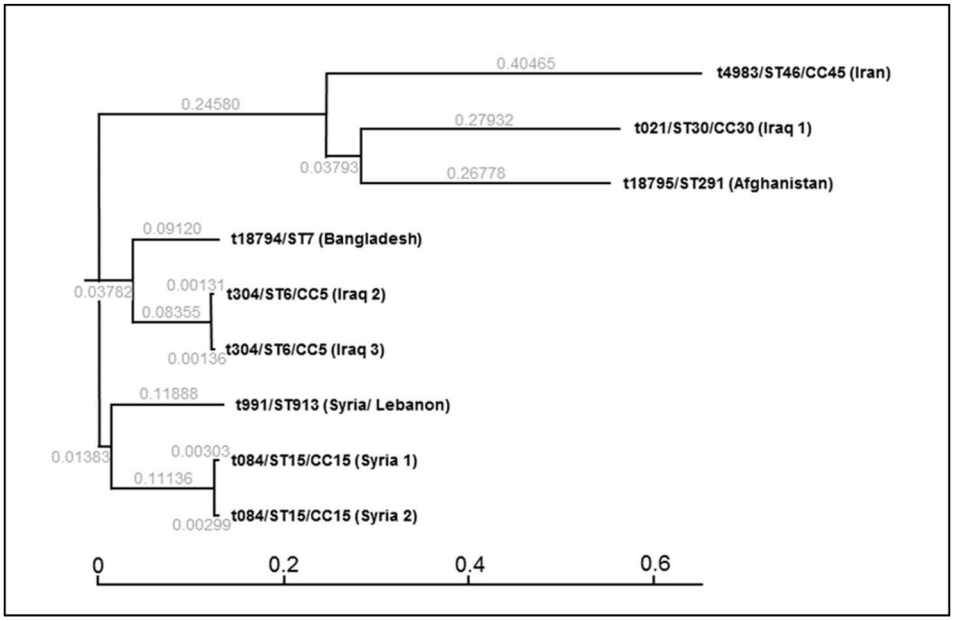

Fig. 2. Phylogenetic relation among the *S. aureus* isolates, illustrated by a rooted phylogenetic tree. Strain USA300 (NC_07793.1) was set as root. The branch length indicates the phylogenetic distances between the strains. Each isolate is represented by only one clone (labelled with *spa*-type, MLST type, MLST clonal complex, and in chambers the origin of the participant from whom the strain was isolated). Isolates belonging to the same *spa*-type cluster together. The phylogenetic tree was computed with CSI Phylogeny based on SNPs in the whole genome sequences.

NZ_CP060596.1 up to NZ_CP060611.1). A coverage of 88.1–93.2% was archieved for the sequences, with GC-contents between 29.4 and 30.8%. Each genome was screened for virulence- and resistance genes with Virulence Finder and ResFinder. Independently, protein annotation was performed with the WGS data. Virulence genes detected with this two-phased approach are listed in Table 3. As expected, the isolates feature genes for the phenotypically shown resistances: t021 harbors *mecA* (β-lactams) and *ermA* (erythromycin, clindamycin), t084(1) has *tetA and norB* (tetracycline), and t991 as well as the t304 isolates feature *mecA* (β-lactams). For t021 and t084(2) a kanamycin, neomycin, amikacin, and tobramycin resistance mediated by the aminoglycoside resistance protein ANT(4′)-Ia can be predicted, although a phenotypic verification of the resistance was not performed. Additionally, the isolates are equipped with a considerable amount of multidrug efflux proteins. Moreover, they feature a lot of genes encoding for surface proteins binding to host structures, like *S. aureus* surface proteins SasG and SasX, fibronectin-binding proteins FnbAB, bone sialoprotein-binding protein Bbp, serine-rich adhesin for platelets SraP, collagen adhesin Cna, Protein A (*spa*), elastin-binding protein EbpS, clumping factors ClfAB, iron-regulated surface determinant IsdA, and serine/aspartate repeat proteins SdrC and SdrD. On an average, 6.6 (5–8) of 14 of these proteins are found. The refugees' isolates are also equipped with toxins. All isolates have α-toxin and β-toxin (*hla* and *hlb*), except for t021/ST30. *hlgA* and *hlgB* are present in all isolates, *hlgC* is only present in 8/9 isolates (t084, t304, t991, t18794, t18795). Furthermore, *luk*ED genes are found in t084, t304, and t18794. t18795 has only *lukE*. *lukS* was found in one isolate (t4983). Neither, the gene locus *lukFS*-PV encoding for PVL, nor exfoliative toxin A and B genes (*etA* and *etB*) were found in any of the isolates. Only, the t021 isolate harbors *tst* encoding for the toxic shock syndrome toxin-1. Staphylococcal enterotoxin (SE) genes are found in three isolates. In the t021 isolate *seg, sei, sen, seo,* and *seu* were detected. In the t4983 isolate *seg, sei, sem, sen, seo* and *seu* were found, as well as *sep* in the t18794 isolate. All isolates harbor the gene for staphylococcal enterotoxin-like X (*sel*X). Moreover, all strains produce serine proteases, except for the t4983 isolate. t021 prossesses only *splF*, while t084, t304, and t991 have *splA-D* and *splF*. *spa*-type t18794 features *splA-C*, and t18795 has only *splA* and *splB*. Regarding regulatory loci, the strains only differ in presence of the accessory gene regulator B (*agrB*) (present in t021, t304, t4983, t18794, t1895) or in presence of *sarU* and *sarT* (both present in t084, t304, t991). Immune evasion genes like *sak*, *scn*, *aur*, *flr*, *chp*, as well as staphylococcal superantigen-like protein genes (*ssl*) are commonly present in all the isolates' genomes. Even though, particularly t021, t4983 and t19795 isolates are lacking many immune evasion genes. The arginine catabolic mobile genetic element (ACME) was not found in any of the isolates.

3.6. Phylogeny

The genome sequences were computed to an USA300-rooted phylogenetic tree comprising all nine isolates (see Fig. 2). Herein *spa*-type t18794 clusters together with the two t304 isolates and t991 clusters together with the t084 isolates. For identical *spa*-types the genetic deviation was calculated using single nucleotide polymorphisms (SNPs). The two isolates of *spa*-type t084 differed in 260 SNPs, the two isolates of *spa*-type t304 differed only in 116 SNPs, respectively. Assembly gaps have been discounted for SNP calculation.

4. Discussion

S. aureus has become a global threat to healthcare, due to its enhanced virulence and increasing resistance. Studies observing *S. aureus* prevalence are still, in most instances, performed within health care context, although in the last decade studies in healthy communities were getting in focus of attention [34–40]. The on-hand study was performed in a non-health care setting, thus the isolates can be categorized as community-acquired. Moreover, the isolates phenotypically appear as expected for CA-strains: All MRSA strains harbor SCC*mec* element type IV, which is most common in CA-MRSA strains. All isolates are susceptible to fluoroquinolones and ciprofloxacin. And, except for t021, the strains are also susceptible to clindamycin. In Germany, the prevalence of *S. aureus* among the healthy population is almost 30%, and the rate of MRSA is about 0.3–0.7% [34,37]. According to the sample size of 161 participants, one could extrapolate about 48 *S. aureus* carriers and one MRSA carrier in the cohort. The quote of nine *S. aureus* carriers in combination with four MRSA carriers among them, reveals a discrepancy between the healthy German general population and the small population of refugees residing in North Rhine Westphalia. Higher rates of MRSA in refugee populations in contrast to the European host countries, have been described before. Both, in health care context (10.3–21.3% [41,42]) and even in generally healthy refugees: In the Netherlands refugees were screened for MRSA in course of registration. Among the refugees the rate of MRSA carriage was calculated to

4.5–13% within the first year after arrival, and to 5.1% among refugees who lived in the Netherlands for more than one year. In contrast, the rate is 1.3% among the Dutch [43]. In Switzerland, a study screened for MRSA in four refugee accommodation centers. The total colonization rate was 15.7%, but with significant differences among the centers, ranging between 2.7 and 25.4% MRSA carriage [44]. These differences suggest an influence of the refugees' accommodation situation on MRSA prevalence, which might also affect the prevalence rates in the refugee population concerned in the on-hand study. The antimicrobial resistance situation in Europe varies depending on the bacterial species, antimicrobial group, and geographical region. In general, lower resistance percentages are reported by countries in the north while higher percentages are reported in the south and east of Europe. Most refugees are from or travelled through countries with a high prevalence of multidrug-resistant organisms (MDRO) in hospital- and community-settings. The Commission for Hospital Hygiene and Infection Prevention at the RKI recommends MRSA-screening at hospital admission for refugees within the first 12 months after arrival. Screening for other MDROs at hospital admission is only recommended if the patient was treated in a hospital abroad in a country with high MDRO prevalence [45].

Due to study design and sampling procedure, some cases of *S. aureus* carriage might remain undetected. Although, *S. aureus* is detectable in the nasal cavity in most cases, there might be a few exceptions in which *S. aureus* only colonizes distinct body sites [46]. Screening of only one body site, like the nasal cavity, might reduce the screening sensitivity for asymptomatic *S. aureus* carriage [47]. Another reason for the discrepancy in *S. aureus* prevalence might be the technically required freezing step between sampling and isolation. *S. aureus* usually survives the procedure, but there might be a reduction in the viable *S. aureus* population. The cross-sectional design of the study also does not allow a distinction of persistent carriers and transient carriers.

In this study, nine cases of asymptomatic carriage of *S. aureus* were intensively investigated. It was shown that the isolates are highly diverse. Diversification in the hostal environment, like the nasal cavity seems to be a strategy of species to enhance the likelihood to succeed in a niche ('insurance hypothesis') and to overcome changing conditions [48,49]. The isolates with the same *spa* types were isolated from carriers from the same country of origin. Those pairs turned out to be the most related. At the time of sampling, the participants who carried the same *spa*-types have not lived in the same accommodation, although it cannot be excluded that they had been in contact. Their genomes differ in 260 SNPs in t084 and 116 SNPs in t304. Coll et al. calculated a cutoff value of 25 SNPs indicating a transmission of MRSA strains in the previous six months [50]. This calculation does not allow an assumption of transmission events that date back several years. Anyhow, a high similarity of the genomes could be an indication that the isolates have a common genetic ancestor in the country of the refugee carriers, especially since both *spa*-types are nowadays common around the world.

The most common CA-lineages in Europe (ST1, ST8, ST80) are not represented in the refugee cohort. Whereas, rare or by know unknown *spa*-types occurred. Presumably, t304 strains originate from Iraq and co-travelled with the participants or the participants got colonized with it by having contact with compatriots in Germany. However, t304 in Germany was only described for Iraqi refugees [51]. Likewise, MRSA t991 appears to be a Middle East local *spa*-type [52], hence it has most likely co-travelled with the refugee. The occurrence of lineage ST291 (new *spa*-type t18795), which is associated with hospitals in Iran [53,54] promotes the idea of a co-travelling, too. Detection of some strains, which are supposed to originate from the middle east, support the assumption that the strains colonizing refugees can co-travel with their carriers and are able to persist in terms of a transition of the host's environment and living conditions. Carriers of t304 and t991 lived in Germany since 3–4.5 years and their escape lasted up to 8 months. Assuming, that the strains co-travelled with the refugees, they must be colonized with the strains since at least 3–5 years. In other studies, a colonization with persistent *S. aureus* strains over several years has been demonstrated [55–58].

In order to evaluate the virulence potential of the isolates collected from the nine refugees, it is reasonable to take a closer look at the functions of the virulence gene products. In contrast to hospital strains, the isolates seem to be faintly resistant against therapeutic antibiotics. Nevertheless, they are equipped with a large amount of multidrug efflux proteins, allowing the efflux of a broad spectrum of potentially cell-damaging molecules, like antibiotics, chemotherapy agents, dyes, antiseptics, disinfectants, organic solvents, and detergents [59]. Furthermore, the strains harbor a remarkable set of genes encoding for cell wall anchored surface proteins, which mediate adhesion or binding to host surfaces and evasion of host defenses. Adhesins play a role in colonization of extra-body tissue (e.g. squames), binding of intra-body ligands (e.g. fibrinogen and collagen), and bacterial cell-cell attachment in order to form biofilms [60]. CA-MRSA are often associated with the production of cytolytic toxins (PVL, α-toxin and PSMs; [61]). PVL genes were not detected among the isolates, while α-toxin and PSM-β2 genes are present. In contrast to PSM-α, PSM-β is a barely cytolytic toxin [62]. In three of the strains, even genes encoding for enterotoxins G, I, M, N, O, P and U were detected. Moreover, a high prevalence of superantigen-like proteins has been found. Superantigens, including enterotoxins are suspected to promote colonization and pathogenicity of the strains [63,64].

The on-hand nine-cases study allows to take a closer look on virulence by detecting the presence or absence of chromosomally encoded genes associated with virulence. Due to sequencing of the chromosomal DNA, genes encoded on plasmids and resistances mediated by gene mutations have been neglected. However, the detection of virulence genes does not give full information about an isolate's virulence property. For a cell, redundant genes mean an enhanced metabolic burden [61,65]. CA-MRSA achieved to balance methicillin resistance with enhanced virulence and fitness. As suspected by Michael Otto, there might be an evolutionary achieved "trade-off" between maintaining sufficient levels of methicillin resistance and obtaining enhanced virulence [61]. Nevertheless, it remains unclear whether community-acquired *S. aureus* are more, or less virulent than hospital-acquired strains. CA-MRSA strains are not more virulent than many MSSA strains [61]. HA- and CA-strains form no genetically related clusters, sharing a directed evolution towards increased virulence. Rather, an HA-strain's combination of virulence and resistance is increasing the likelihood to succeed in infecting humans and surviving therapeutic treatment. In contrast, strains that succeed in the community, in theory, are well adapted for colonization, persistence and transmission. Corresponding with this hypothesis, the nine isolates lack most of those virulence factors, that are strongly associated with disease (like PVL genes, enterotoxin A or exfoliative toxins), but they seem to be well equipped for persistent colonization of the human host. In theory, colonization depends on genes for adhesion and attachment. Persistence requires genes for the evasion of the host immune response (as staphylokinase and coagulase). While invasion of human tissue and causing infections is rather mediated by toxin genes.

Virulence of CA-strains has been disproportionately often investigated only for USA300, which had an unique career as an epidemic CA-strain, causing life threatening infections and circulating in hospitals, primarily on the American continent [66,67]. It remains questionable, if research made on USA300 virulence, can be generalized for other CA-MRSA or CA-MSSA strains. The on-hand study tries to picture an impression of the virulence potential of nine diversified MRSA and MSSA isolates from healthy (non-hospitalized) individuals, who are refugees accommodated in Germany. The examination of the genome enables an outlook on the virulence potential of the isolates regarding skills in colonization, resistance, immune evasion, and host cell damaging. To finally evaluate the effective virulence of the refugees' isolates, a further analysis of gene mutations, gene regulation and expression, as well as *in vivo* assays are required. With the exception of

the very virulent epidemic lineages, such as USA 300, typing and classifying into CA- and HA-strains does not provide any information about the virulence of an isolate. For this to be possible in the future, the various lineages must be compared and examined in more detail, especially those that are not associated with human disease.

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CRediT authorship contribution statement

Ines Creutz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Tobias Busche:** Supervision, Writing – original draft, Writing – review & editing. **Franziska Layer:** Investigation, Methodology, Supervision, Writing – review & editing. **Hanna Bednarz:** Conceptualization, Supervision, Writing – review & editing. **Jörn Kalinowski:** Resources. **Karsten Niehaus:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

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