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Abstract (242 words)

Objectives: Recently, following import by travel and migration, epidemic community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has caused nosocomial outbreaks in Europe, sometimes with a fatal outcome. We describe clinico-epidemiological characteristics of CA-MRSA detected by the European Network for the Surveillance of imported *S. aureus* (www. staphtrav.eu) from May 2011- November 2016.

Methods: Sentinel surveillance at thirteen travel-clinics enrolling patients with travel-associated skin and soft tissue infection (SSTI) and analysing lesion and nose swabs at one central laboratory.

Results: 564 independent case-patients with SSTI were enrolled and had 374 (67%) *S. aureus* positive lesions, of which 14% (n=51/374) were MRSA. The majority of CA-MRSA isolates from SSTI was PVL-positive (n=43/51, 84%). The risk of methicillin-resistance in imported *S. aureus* varied by travel region (P<0.001) and was highest in Latin America (n=16/57, 28%, 95%-CI 17.0-41.5) and lowest in Sub-Saharan Africa (n=4/121, 3%, 0.9-8.3). Major epidemic clones (USA300 / USA300 Latin-American Variant, Bengal Bay, South Pacific) accounted for more than one third (n=19/51, 37%) of CA-MRSA imports. CA-MRSA SSTI in returnees was complicated (n=31/51 multiple lesions, 61%; n=22/50 recurrences, 44%), led to health care contact (n=22/51 surgical drainage, 43%; n=7/50 hospitalisation, 14%), was transmissible (n=13/47 reported similar SSTI in non-travelling contacts, 28%), and associated with *S. aureus* nasal colonisation (n=28 of 51 CA-MRSA cases, 55%; 24 of 28 colonized with identical *spa*-type in nose and lesion, 85%).

Conclusions: Travel-associated CA-MRSA SSTI is a transmissible condition that leads to medical consultations and colonisation of the infected host.

Key words (MeSH): travel medicine; sentinel surveillance; Panton-Valentine leukocidin; Methicillin-Resistant Staphylococcus aureus; staphylococcal skin infections; communicable diseases, emerging; drug resistance, bacterial; molecular epidemiology; communicable disease control; cross-sectional studies

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to cause recurrent skin and soft tissue infections (SSTI) in travellers [1-5] and nosocomial outbreaks [6] with potentially fatal outcomes [7] after introduction into the health care setting from abroad. Hence, systematic research on the clinical, microbiological, and epidemiological characteristics of imported MRSA and associated disease is of importance to travel medicine and public health practice alike.

The global distribution of community-associated methicillin-resistant *S. aureus* (CA-MRSA) clones is heterogeneous and characterized by a regional predominance of particular clonal lineages [8]. Existing data on the global routes of CA-MRSA dissemination and the role of intercontinental travel in contributing towards the emergence of 'pandemic' clones is fragmented and restricted to reports on their sporadic import at a national or sub-national level. Systematic surveillance of CA-MRSA import on a larger scale, however, is hardly in place [9] since CA-MRSA and associated infections are not routinely notifiable in most European countries. To fill this gap, we analysed data collected from May 2011 to November 2016 by StaphTrav (www.staphtrav.eu), a multi-centre network of thirteen European travel clinics aiming at the surveillance of *S. aureus* imported to Europe. Compared to a previous report published on SSTI cases caused by both, methicillin-sensitive and –resistant *S. aureus* and analysing data collected from May 2011 to December 2013 [1], the presented report incorporates an additional 246 case patients with imported SSTI and focuses solely on the clinical and (molecular-) epidemiological characteristics of MRSA and associated disease.

Methods

Study population

StaphTrav is a network of thirteen travel clinics in seven European countries that conducts surveillance on imported *S. aureus* (for details see www.staphtrav.eu). As predetermined in the network protocol, returning travellers and migrants seeking medical advice with acute or resolving SSTI with onset during intercontinental travel or within 30 days after return (case definition) are approached for inclusion into a cross-sectional study. After informed consent by the patient or one legal guardian, nasal and lesion swabs are taken and sent to the central laboratory in Heidelberg for laboratory diagnostics. Clinical and patient information are collected during a face-to-face interview by the attending physician using a standardised questionnaire (publicly accessible on www.staphtrav.eu).

Microbiological and molecular diagnostics

Detection of *S. aureus* was performed using Columbia Agar with 5% sheep blood and mannitol salt agar. Colonies typical for *S. aureus* were confirmed by coagulase test and *nuc* gene PCR, as previously described [1]. Susceptibility to commonly used antibiotics was tested by VITEK®2 (Biomerieux, France) and interpreted according to EUCAST clinical breakpoints. Phenotypic methicillin-resistance of *S. aureus* was confirmed by the presence of *mecA.*

All MRSA were further characterized by SCC*mec* types (I-V) [10], PVL, *spa* and, for MRSA with the *spa* type t008, arginine catabolic mobile element (ACME) *arcA* [11]. *Spa* types were clustered into *spa* clonal complexes (spa-CC) using the Based Upon Repeat Pattern algorithm with parameters set to exclude if repeats were <5 and to cluster if cost ≤4 [12]. CA-MRSA MSLT ST8/*spa* t008/SCCmec IVa/IV consistent with the epidemic USA300 lineage were subject to whole genome sequencing. Genomic DNA was extracted using DNeasy Blood and

Tissue Kit (Qiagen, Germany) after prior lysis with lystostaphin (Genaxxon, Germany). Standard genomic library was prepared and sequenced with Illumina HiSeq (paired-end: 2x150bp) by GATC Biotech AG (Konstanz, Germany). Raw sequences were trimmed for quality using sickle 1.33 (parameters : q > 30, l > 45) [15]. Cleaned sequences were assembled de novo using SPAdes 3.12.0 [16]. Contigs obtained from the assembly were therefore curated for length (> 1000 bp) and coverage (>10 X) to ensure no errors and contamination in the draft genome, giving an average coverage of 100x. Contigs were then annotated using Prokka 1.13 (based on genetic code table 11) [17] and the National Centre for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline.

Core genome (cgMLST) was obtained using Roary version 3.11.2 [18] with a definition of core as 100% of the strains sharing the gene. Phylogenetic tree was calculated from the alignment of the core genome using a generalized time-reversible model with fastTree 2.1.10 [19]. Sequence data of the StaphTrav Project were deposited at NCBI (https://www.ncbi.nlm.nih.gov/) under the Bioproject no. PRJNA486096.

Statistical Analysis

In case of clustered cases of SSTI (e.g. fellow travellers, household members), only the primary case was included into the analysis. Exact (binomial) 95% confidence intervals were calculated for proportions of MRSA among *S. aureus* positive SSTI by geographic region. A contingency table was constructed showing the proportion of MRSA among *S. aureus* by geographic region and their deviation from H0: “the proportion of MRSA is equal of categories of geographic region” tested using the $\chi 2$-test in Stata 13 (Stata Corp).

Ethics

This study was approved by the Ethics Committee, Medical Faculty, Eberhard Karls Universität Tübingen, Germany and by the local institutional boards, if required.

Results

From May 2011 to November 2016, 564 independent cases of SSTI fulfilled the eligibility criteria and had been enrolled: 558 were travellers (345 leisure, 105 humanitarian and aid work, 63 visiting friends and relatives, 24 business, 19 education, 2 other reasons) and 6 were migrants. *S. aureus* accounted for two-thirds (n=374/564, 67%) of all SSTI with onset during or up to 30 days post-travel. Of all patients with SSTI, 9% were caused by MRSA (n=51/564), and of all patients with *S. aureus* SSTI, 14% (n=51/374) were caused by MRSA. The majority of SSTI caused by *S. aureus* (n=237/374, 63%) presented as abscesses.

Risk factors for travel-associated MRSA SSTI

The proportion of MRSA among *S. aureus* positive SSTI varied by geographic region ($P<0.001$, χ^2-test, 5 degrees of freedom) and was highest in Latin America (n=16/57, 28%, 95% CI 17.0-41.5), followed by South- and Southeast Asia (n=27/177, 15%, 10.3-21.4), but was comparably low in Sub-Saharan Africa (n=4/121, 3%, 0.9-8.3). Small sample size led to wide confidence intervals around the estimates for North Africa (n=1/4, 25%, 0.6-80.6), West Asia (n=2/3, 67%, 9.4-99.2) and Australia/Oceania (n=1/12, 8%, 0.2-38.5) (Figure 1).

Molecular characteristics of MRSA from imported SSTI

Figure 2 summarizes the molecular population characteristics of CA-MRSA imported to Europe, 2011-2016. Over one third of all MRSA isolated from SSTI (n=19/51, 37%) belonged to one of the major pandemic clones circulating worldwide (Figure 2A, Table 1). Among these, strains of the USA300 MRSA clone dominated, accounting for almost one-fifth (n=9/51, 18%) of all imported MRSA in our study: four isolates belonged to the ST8/t008 PVL+ SCC*mec* IVa arginine catabolic mobile element (ACME) positive clone and the other five belonged to the Latin-American variant of USA300, ST8/t008 PVL+ SCC*mec* IVc

ACME-negative clone, four of which carried the copper and mercury resistance (COMER) mobile genetic element. The one remaining ST8/t008 PVL+ SCC*mec* IVc ACME-negative COMER-negative isolate (STV073L) was imported from South Sudan (Table 1, Figure 3). The second most commonly isolated MRSA (n=6/51, 12%) were ST30/t019 with SCC*mec* type IVc and PVL+, consistent with the epidemic 'Southwest Pacific' CA-MRSA clone followed by ST772, t657/t345 SCC*mec* V, PVL+ CA-MRSA (n=4/51, 8%) also referred to as the Bengal Bay clone.

Classifying all imported CA-MRSA according to spa-CC yielded the following frequencies: 024/304 (n=14/51, 27%), spa-CC 148/791 (n=5/51, 10%), spa-CC 895 (n=5/51, 10%) and two groups of related *spa*-types with no founder (t345/t657; n=4/51, 8%; t186/t239; n=2/51, 4%). One fourth (n=20/51, 39%) of imported CA-MRSA isolates were unrelated (singletons) and one isolate was not typeable (Figure 2B, Table 2).

Dissemination of CA-MRSA through intercontinental travel

Almost all SSTI caused by USA300 and USA300-LV CA-MRSA were acquired in Latin America. South Pacific CA-MRSA SSTI were acquired in South-East Asia (the Philippines, n=3 and Singapore, n=1), Turkey (n=1) and Ghana (n=1). Four Bengal Bay CA-MRSA were isolated from patients with travel to India (n=2), Egypt (n=1) and Indonesia (n=1) (Table 1). Table 2 summarizes information on other/non-epidemic CA-MRSA acquired abroad.

Virulence, transmission, and co-resistance of imported MRSA and associated morbidity

The majority of CA-MRSA acquired abroad were PVL+ (n=43/51, 84%). About half of CA-MRSA SSTI patients suffered from recurrent infections (n=22/50, 44%) with more than half of those with recurrent *S. aureus* SSTI reporting two or more relapses (15/22, 68%).

S. aureus nasal colonisation was common among patients with MRSA SSTI (n=28/51, 55%), most of the times with the same strain in the nose and lesion (n=24/28, 86%) as determined by *spa* typing. About a quarter of CA-MRSA SSTI patients reported clinically similar SSTI in close contacts (n=13/47, 28%).

Substantial proportions of patients with SSTI caused by CA-MRSA suffered from multiple lesions (n=31/51, 61%) and required surgical drainage (n=22/51, 43%) or even in-patient treatment (n=7/50, 14%).

Approximately half (n=31/51, 56%) of all imported MRSA isolates were co-resistant to at least one oral second-line agent (i.e. trimethoprim-sulfamethoxazole, ciprofloxacin, tetracycline, clindamycin and erythromycin), one third (n=18/51, 35%) to two or more, and one fifth to three or more (n=9/51, 18%) of these alternative oral antibiotics (Table 1 and 2).

Discussion

We show that overall about 14% of imported *S. aureus* SSTI treated at travel clinics is caused by MRSA. Major epidemic clones, such as USA300, Bengal Bay and South Pacific CA-MRSA accounted for about 37% of travel-associated MRSA SSTI. Infections caused by these pathogens were typically acquired at endemic destinations, i.e. Latin America [31], India [32], and the Philippines [33]. Taken together, these observations confirm that travel-associated SSTI drives the spread of epidemic CA-MRSA over long distances [5, 9, 34].

We found that most of the imported MRSA belonging to the USA300 lineage could be attributed to returnees from Latin America (7 of 9) and that many of these were infected with the USA300 Latin-American Variant (USA300-LV; 4 of 7). This genetically related clone has expanded in hospitals and communities across South America in recent years [19, 31, 35]. USA300-LV has become the most prevalent MRSA isolated from blood cultures in Colombia and Ecuador [18] and was found to colonize pigs in Cuba [35] – countries where four of the five cases with USA300-LV detected by our network were acquired. To date, its prevalence among CA-MRSA isolates in the European population is considered rare [36, 37]; however, reports of sporadic import, transmission, and micro-outbreaks suggest its expansive potential [13, 38]. Against this background, our findings identify travellers returning from Latin-America to Europe with SSTI as important targets for the containment of epidemic USA300-LV CA-MRSA.

The presented data indicates that recurrent SSTI (24/51, 47%), infections in non-travelling household contacts (13/47, 28%), and nasal colonisation (28/51, 55%) – in 86% (24/28) with the infecting strain – are common in patients with travel-associated CA-MRSA. In conjunction with previous research that demonstrated identical *spa*-types in non-travelling cases of SSTI secondary to imported cases of *S. aureus* infection [2], these findings support a substantial transmission potential of imported MRSA and suggest that nasal colonization represents an important reservoir from where the pathogen causes relapses and secondary cases. The high proportions of subjects requiring surgical drainage (22/51, 43%) and hospitalisation (7/50, 14%) document that health care contact is common in travellers returning with MRSA SSTI. Hence, to prevent nosocomial transmission, enhanced infection prevention and control measures have to be in place. This requirement becomes even more obvious when contextualizing our findings with the following details from published reports on sporadic in-hospital transmission of CA-MRSA: i) the same epidemic clones as those identified as imports in this study have been involved in nosocomial outbreaks in Europe [7, 38-41], ii) in some of these outbreaks the primary case had recent exposure abroad [7, 39] and iii) suffered from travel-associated SSTI [7, 38].

For the implementation of preventive measures, the substantial proportion of nasal colonization found in the present study suggests twofold: i.) that colonisation screening and pre-emptive isolation may prove useful as part of these measures and ii.) that these measures have to apply also to patients where the lesion has already resolved, as nasal colonisation may outlast infection by weeks or even months. In this context it is noteworthy, that household contacts of patients with travel-associated SSTI have also been colonized by imported CA-MRSA [7, 38, 39] – further expanding the target group for potential colonisation screening to those living with CA-MRSA SSTI patients. Finally, health care institutions should educate their own personnel about the increased risk of introducing CA-MRSA into hospitals after exposure abroad [7, 39] and offer colonisation screening to employees reporting travel-associated SSTI.

S. aureus SSTI presents most commonly as abscess, making surgical drainage the mainstay of therapy. Since adjunctive antibiotic treatment of abscesses is beneficial in treating skin abscesses [42, 43], antimicrobial resistance has to be considered when choosing empiric therapy [44]. In this context, the presented data may prove valuable, as it shows a substantial prevalence of methicillin-resistance among imported *S. aureus* from Latin America only (28%, Figure 1), while this proportion among *S. aureus* from South- and Southeast Asia is around the threshold of resistance that would be considered relevant by most clinical microbiologists (15%), and clearly below in Sub-Saharan Africa (3%).

We established this ongoing surveillance to detect newly emerging methicillin-resistant *S. aureus* clones with epidemic potential. Against this background, we detected four independent introductions of CA-MRSA belonging to MLST ST72/spa-CC148/297/PVL+ from Costa Rica (n=3) and New Zealand (n=1) to Germany. This finding is confirmed by data from the German National Surveillance Laboratory that also reported ST72 in 1.6% of putatively imported CA-MRSA isolates [45]. ST72 is reported to have entered the nosocomial setting and cause blood stream infections in the East Asia/Pacific Region [26] and Latin America [18]. Furthermore, we detected import of CA-MRSA ST398/t034/SCC*mec*V/PVL+ from Vietnam – a clone which is closely related to its PVL-negative, live-stock associated counterpart [27]. Our network detected the introduction of other regionally dominant clones such as the “Queensland” clone from Australia [25] and ST59/t437, highly endemic in East Asia [46], as well as several rare and even previously unreported CA-MRSA (Table 2).

Our study has limitations. First, part of the presented data has been previously published [1] which could be mistaken as duplicate publication. However, by doing so, StaphTrav acknowledges “surveillance” as “ongoing collection, collation, and analysis of data and the timely dissemination of information to those who need to know so that action can be taken [47]." Second, due to the nature of our network of travel-clinics, this report has a focus on the import of *S. aureus* from tropical and subtropical regions to Europe. Thus, additional surveillance activities will be necessary to capture the import of epidemic MRSA from travel to the temperate zone. Third, it is likely that our case population recruited at travel clinics has, on average, suffered from more severe and complicated SSTI when compared to cases seeking care at primary care. This may have led to an over-estimation of the proportion of severe disease among all cases of imported *S. aureus* SSTI. Fourth, small sample size for returnees from Australia, North Africa, and West Asia led to imprecise estimates of the risk of MRSA among all *S. aureus* for these regions thus not allowing meaningful inferences on the effectiveness of oral beta-lactams for the treatment of patients returning from there.

In conclusion, our data supports that long-distance travel is an important driver for the spread of epidemic CA-MRSA over long distances. The presented findings on SSTI as a recurrent and highly transmissible condition in conjunction with reports on nosocomial outbreaks of epidemic CA-MRSA through travel-associated SSTI support the implementation of infection control and prevention measures when travellers with skin infections have contact to the health care system. Our data also suggest that caution should be exerted when caring for their household contacts, since these are commonly affected by secondary SSTI.

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Additional members of the StaphTrav Network that have contributed to this work (ordered by place of institutional affiliation) are: Abraham Goorhuis, Center of Tropical Medicine and Travel Medicine, **Amsterdam**, The Netherlands; Antonia Calvo-Cano, Barcelona Centre for International Health Research (ISGlobal), **Barcelona**, Spain; Christoph Hatz, Andreas Neumayr and Johannes Blum, University of Basel and Swiss Tropical and Public Health Institute, **Basel**, Switzerland; Barbara Friedrich-Jänicke and Frank Mockenhaupt, Charité, **Berlin**, Germany; Michael Ramharter, Department of Tropical Medicine, University Medical Centre Eppendorf and Bernhard Nocht Institute for Tropical Medicine, **Hamburg**, Germany; Sabine Gabrysch, Heidelberg Institute of Public Health, **Heidelberg**, Germany; Mirjam Schunk, Abteilung für Infektions- und Tropenmedizin der Ludwig-Maximilians-Universität, **München**, Germany; Alice Perignon, Service de Maladies Infectieuses et Tropicales, Groupe Hospitalier Pitié-Salpêtrière, **Paris**, France; Günter Slesak, Paul-Lechler Krankenhaus, **Tübingen**, Germany; August Stich, Missionsärztliche Klinik, **Würzburg**, Germany. We thank the TropNet (www.tropnet.net) and EuroTravNet (www.istm.org/eurotravnet) networks in tropical medicine and travel health for their support in identifying member sites for the StaphTrav network.

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Conflicts

All authors, no conflicts

ACCEPTED MANUSCRIPT

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Table 1. Major epidemic CA-MRSA clones and associated SSTI in intercontinental travellers from Europe, 2011-2016

ID	Age	Sex	Travel characteristics			Strain characteristics								Clinical characteristics					
			region	country	purpose	PVL	*spa*	spa-CC	MLST	SCC	COMER	ACME	co-resistance	cont.	rec.	mult.	hosp.	surg.	nasal
USA300																			
108	2	F	Latin America	Suriname	leisure	+	t008	024/304	ST8	IVa	neg.	pos.	Ci, SXT, Er	no	none	yes	no	yes	yes
232	47	M	Latin America	Cuba	VFR	+	t008	024/304	ST8	IVa	neg.	pos.	Ci, Er	no	multiple	no	no	no	yes
262	18	M	Latin America	Bahamas	VFR	+	t008	024/304	ST8	IVa	neg.	pos.	Er	no	single	yes	yes	yes	no
915	31	M	Southeast Asia	Thailand	leisure	+	t008	024/304	ST8	IVa	neg.	pos.	Er	yes	none	yes	no	no	no
USA300-LV																			
73	49	M	Africa	S.-Sudan	aid	+	t008	024/304	ST8	IVc	neg.	neg.	Te	no	none	no	no	no	yes
101	59	M	Latin America	Colombia	leisure	+	t008	024/304	ST8	IVc	pos.	neg.	Ci	missing	none	yes	no	no	yes
209	6	F	Latin America	Colombia	VFR	+	t008	024/304	ST8	IVc	pos.	neg.	Er	yes	multiple	yes	no	no	yes
563	49	M	Latin America	Cuba	VFR	+	t008	024/304	ST8	IVc	pos.	neg.	none	no	multiple	yes	no	yes	yes
658	50	M	Latin America	Cuba	leisure	+	t008	024/304	ST8	IVc	pos.	neg.	none	no	none	no	no	no	no
South Pacific Clone																			
18	31	F	Southeast Asia	Philippines	VFR	+	t019	single	ST30	IVc	n.a.	n.a.	none	yes	none	yes	no	no	yes
282	22	M	Southeast Asia	Philippines	aid	+	t019	single	ST30	IVc	n.a.	n.a.	none	no	single	yes	no	yes	no
483	49	M	Southeast Asia	Philippines	leisure	+	t019	single	ST30	IVc	n.a.	n.a.	none	missing	none	no	yes	yes	no
545	44	M	Middle East	Turkey	VFR	+	t019	single	ST30	IVc	n.a.	n.a.	none	yes	none	yes	no	no	yes
573	53	F	Africa	Ghana	business	+	t019	single	ST30	IVc	n.a.	n.a.	none	no	none	no	yes	yes	yes
597	34	M	Southeast Asia	Singapore	leisure	+	t019	single	ST30	IVc	n.a.	n.a.	none	no	none	yes	no	no	yes
Bengal Bay Clone																			
190	39	F	South Asia	India	leisure	+	t657	NF1	ST772	V	n.a.	-	Ci, SXT, Ge, Er	no	none	no	yes	yes	yes
430	21	F	South Asia	India	leisure	+	t657	NF1	ST772	V	n.a.	-	Ci, SXT, Ge, Er	no	none	yes	no	yes	no
514	21	M	Africa	Egypt	leisure	+	t657	NF1	ST772	V	n.a.	-	Ci, SXT, Ge, Er	yes	none	yes	no	yes	no
579	26	M	Southeast Asia	Indonesia	leisure	+	t345	NF1	ST772	V	n.a.	-	Ci, SXT, Ge, Er	no	multiple	no	no	yes	yes

PVL=Panton Valentine Leukocidin; *spa*=gene encoding for *S. aureus* protein A; MLST=multi locus sequence type; SCC=staphylococcal cassette chromosome *mec* type; COMER=copper and mercury resistance mobile element; ACME=arginine catabolic mobile element; co-resistance=concomitant phenotypic resistance; cont.=household contacts also affected by skin and soft tissue infection; rec.=recurrent skin and soft tissue infection; mult.=multiple lesions per episode; hosp.=hospitalisation after return to home country; surg.=surgical drainage; nasal=nasal carriage of *S. aureus*; neg.=negative; pos.=positive; VFR=visiting friends and relatives; aid=humanitarian and aid work; Ci=ciprofloxacin; Er= erythromycin; Te =tetracyclin; Ge=gentamicin; SXT=trimethoprim-sulfamethoxazole; single=singleton; LV=Latin Variant; n.a.=not assessed; NF=no founder

Table 2. Non-epidemic MRSA and associated SSTI in intercontinental travellers from Europe, 2011-2016

ID	Age	Sex	Travel characteristics			Strain characteristics						Clinical characteristics						MLST assignment [Ref.][b], endemic at travel region [Ref.][c], comment
			region	country	purpose	PVL	*spa* type	%[a]	spa-CC	SCC	co-resist.	cont.	rec.	mult.	hosp.	surg	nasal	
544	56	M	Latin America	Brazil	leisure	+	t197	0.04	024/304	IVc	none	no	none	yes	no	no	no	ST94 [16], [16]
284	25	F	Latin America	Colombia	aid work	+	t13392	<0.01	singleton	IV[d]	none	no	single	no	no	yes	yes	-
932	23	F	Latin America	Costa Rica	aid work	+	t10437	<0.01	148/791	IVa	none	no	single	no	no	no	yes	CC8 [17], -
170	36	M	Latin America	Costa Rica	VFR	+	t148	0.31	148/791	IVa	none	no	none	yes	no	yes	no	ST72 [16], [18]
619	46	M	Latin America	Costa Rica	leisure	+	t3169	0.01	148/791	IVa	Er, Te	no	multiple	yes	no	no	yes	ST72 [30], [18]
635	26	F	Latin America	Costa Rica	aid work	+	t791	0.02	148/791	IVa	none	yes	mis.	yes	no	yes	yes	ST72 [16], [18]
600	64	M	Latin America	Cuba	education	+	t008	6.02	024/304	IV[d]	none	no	multiple	yes	no	yes	yes	ST8/247/250/254 [16], [19]
180	27	M	Latin America	Panama	leisure	+	t2393	0.02	NF2	IVc	none	yes	multiple	yes	no	no	no	ST88 [20], [18]
222	28	F	Latin America	Jamaica	leisure	+	t024	0.67	024/304	IVc	SXT	yes	none	yes	no	no	yes	ST8 [16], [19]
229	29	M	Africa	Madagascar	leisure	+	t186	0.21	NF2	IVa	SXT	no	none	yes	no	no	no	ST88 [16], [20]
168	24	F	Africa	South Africa	aid work	+	t3812	<0.01	singleton	IVc	none	no	multiple	yes	yes	yes	no	-
496	23	F	Middle East	Israel	leisure	+	t6267	<0.01	895	nt	Er	yes	multiple	yes	no	no	yes	CC5 [21], [22]
268	21	F	South Asia	India	aid work	+	t304	0.44	024/304	IVa	Ci, SXT	no	single	no	no	yes	yes	ST8 [16], [23]
495	57	M	South Asia	India	leisure	-	t005	0.67	singleton	IVc	Ci, Cc, Er	no	none	no	no	no	no	ST22/23/60 [16], [24]
506	57	M	South Asia	Sri Lanka	leisure	+	t002	6.8	895	IVc	Cc, Er	no	none	no	no	no	no	CC5/231 [16], [29]
614	44	M	South Asia	Sri Lanka	leisure	-	t895	0.01	895	IVa	Er, Ri	mis	none	yes	no	no	yes	-
439	24	M	South Asia	Sri Lanka	leisure	-	NT	n/a	n/a	IVc	Te	yes	none	yes	no	no	no	-
431	71	M	South Asia	Sri Lanka	leisure	+	t045	0.7	singleton	IVc	Ci, Er,Te	no	none	yes	yes	no	no	ST5/225 [16], [16]
505	61	M	South East Asia	Cambodia	leisure	+	t380	0.01	singleton	V	SXT, Te	no	single	no	no	no	no	-
448	24	M	South East Asia	Malaysia	leisure	-	t5388	0.01	singleton	IVc	SXT	yes	multiple	no	no	no	no	-
194	54	M	South East Asia	Philippines	leisure	-	t002	6.8	895	IVc	Cc, Er, Te	no	none	no	no	no	yes	ST5/225 [16], [16]
444	46	F	South East Asia	Philippines	VFR	+	t104	0.01	024/304	IVc	SXT	no	none	yes	no	yes	no	-
601	31	F	South East Asia	Philippines	leisure	-	t1379	0.01	singleton	IVc	Er, Ri	yes	multiple	no	no	yes	yes	-
147	46	F	South East Asia	Philippines	leisure	-	t9999	<0.01	singleton	IVc	SXT	yes	none	yes	no	no	yes	-
193	22	F	South East Asia	Thailand	leisure	+	t002	6.8	895	IVc	none	mis	none	yes	no	no	yes	CC5/231 [16], [29]
529	56	M	South East Asia	Thailand	leisure	-	t1510	0.04	singleton	IVa	none	no	none	no	no	no	no	-
919	32	M	South East Asia	Thailand	leisure	+	t1671	0.01	singleton	IVa	Er	no	single	no	no	no	yes	-
289	23	F	South East Asia	Thailand	aid work	+	t202	0.1	singleton	IVa	none	no	multiple	yes	yes	yes	no	ST93 [16], [25], "Queensland" clone
599	35	F	South East Asia	Thailand	leisure	+	t437	0.73	singleton	IV[d]	Cc, Er	no	none	no	no	yes	no	ST59 [16], [28]
652	19	F	South East Asia	Vietnam	leisure	+	t034	1.98	singleton	V	Ci, Er, Te	no	multiple	yes	no	yes	yes	ST398 [16], [27], LA-MRSA
257	19	M	South East Asia	Vietnam	aid work	+	t202	0.1	singleton	IVa	none	no	multiple	yes	no	no	yes	ST93 [16], [25], "Queensland" clone
595	20	F	Australia/Oceania	New Zealand	leisure	+	t3169	0.01	148/791	IVa	Er, Te	no	multiple	no	mis	yes	no	ST72 [30], [26] [22]

PVL=Panton Valentine Leukocidin; *spa* type=molecular typing based on DNA sequencing of the repeat region of the gene encoding for protein A of *S. aureus*; spa-CC=spa clonal complex as determined by Based Upon Repeat Pattern algorithm [12]; SCC= staphylococcal cassette chromosome *mec* type; co-resist.=concomitant phenotypic resistance; cont.=case patient reports that household contacts are also affected by skin and soft tissue infection; rec.=recurrent skin and soft tissue infection; mult.=multiple lesions per episode; hosp.=hospitalisation after return to home country; surg.=surgical drainage; nasal=nasal carriage of *S. aureus*; MLST=multi locus sequence type; ST=sequence type; single=singleton; mis=missing; Cc=clindamycin; Er= erythromycin; Te =tetracyclin; Ge=gentamicin; SXT=trimethoprim-sulfamethoxazole; Ci=ciprofloxacin; Ri=rifampin; aid work=humanitarian and aid work; VFR=visiting friends and relatives; NF=no founder; NT=not typable; LA-MRSA=livestock associated MRSA

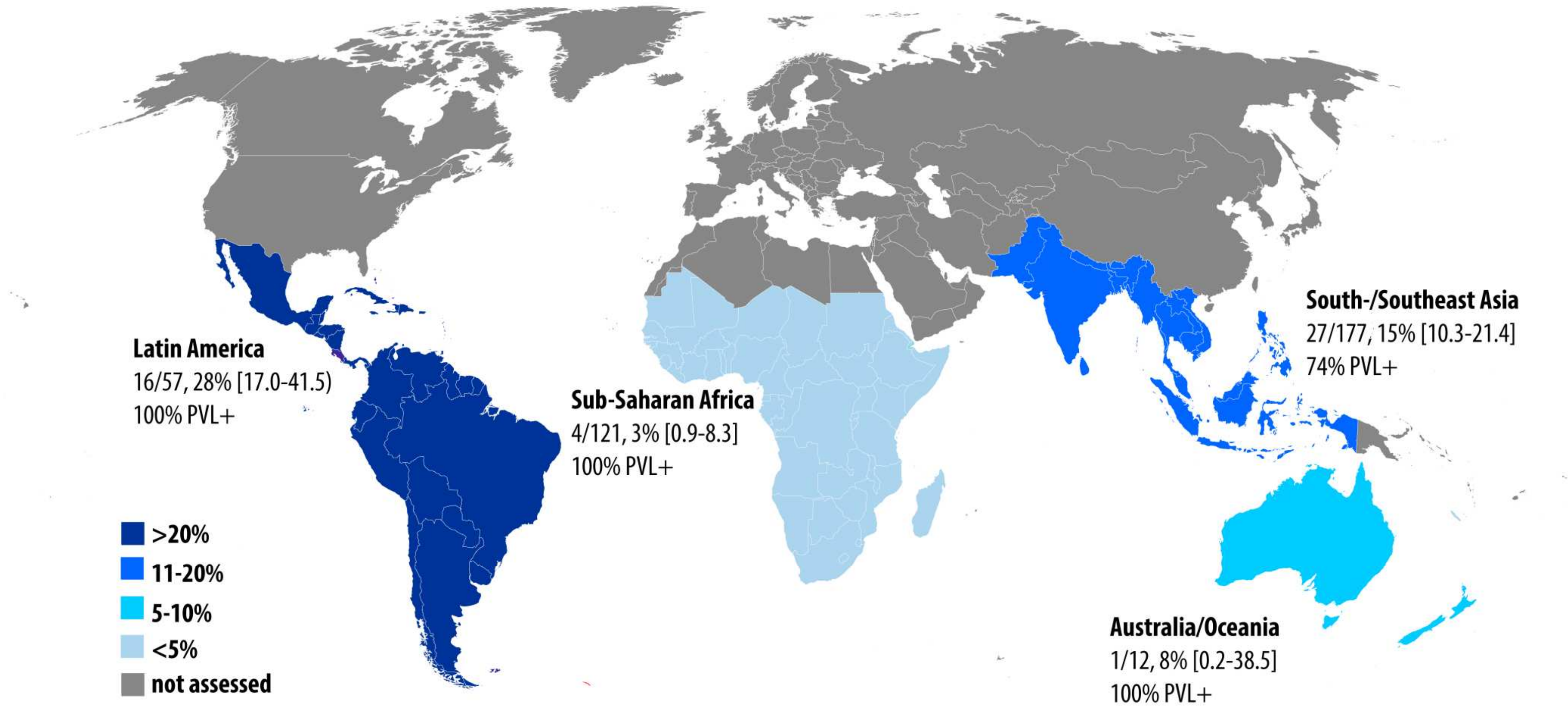
a; % frequency of given *spa*-type among all entries in Ridom spa-server (http://www.spaserver.ridom.de/)

b: reference that reports assignment of *spa* type of this isolate to a particular multi-locus sequence type (ST) or multi-locus clonal complex (CC)

c; reference that reports methicillin-resistant *S. aureus* of given molecular type at region of travel

d; SCC*mec* subtype not covered by PCR assay

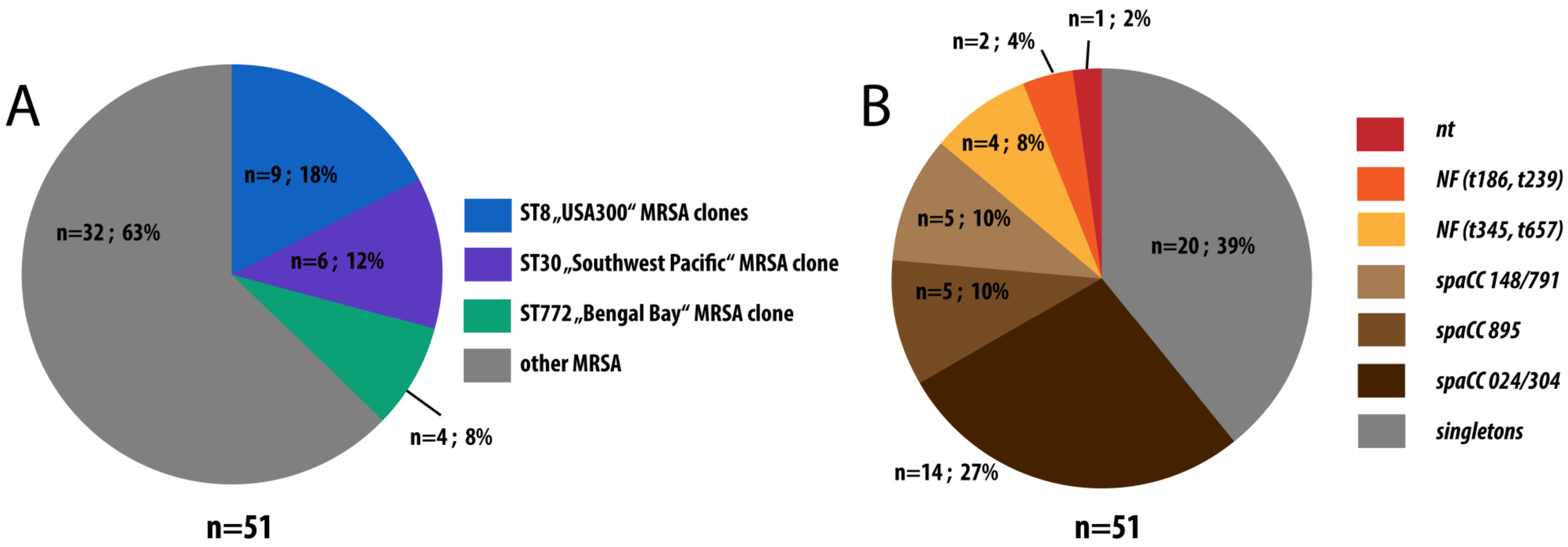
490 Figure 1. Resistance to methicillin in *S. aureus* imported to Europe, by region of travel destination, StaphTrav Network 2011-2016



491

Displayed and codeed in blue are - for destinations with at least 10 *S. aureus* submissions - prevalence estimates of methicillin-resistance [95% confidence interval] among *S. aureus* imported from a geographic region together with estimates of Panton Valentine-Leukocidin (PVL)-positive isolates among methicillin-resistant *S. aureus*. Not displayed are estimates for regions with less than 10 *S. aureus* submitted, i.e. West-Asia (n=2/3, 67% [9.4-99.2], 100% PVL+) and North Africa (n=1/4, 25% [0.6-80.6], 100% PVL+). Chi-squared-test with 5 degrees of freedom for H0: “The proportion of methicillin-resistant *S. aureus* is equally distributed over regions of travel destination.” gives P<0.001.

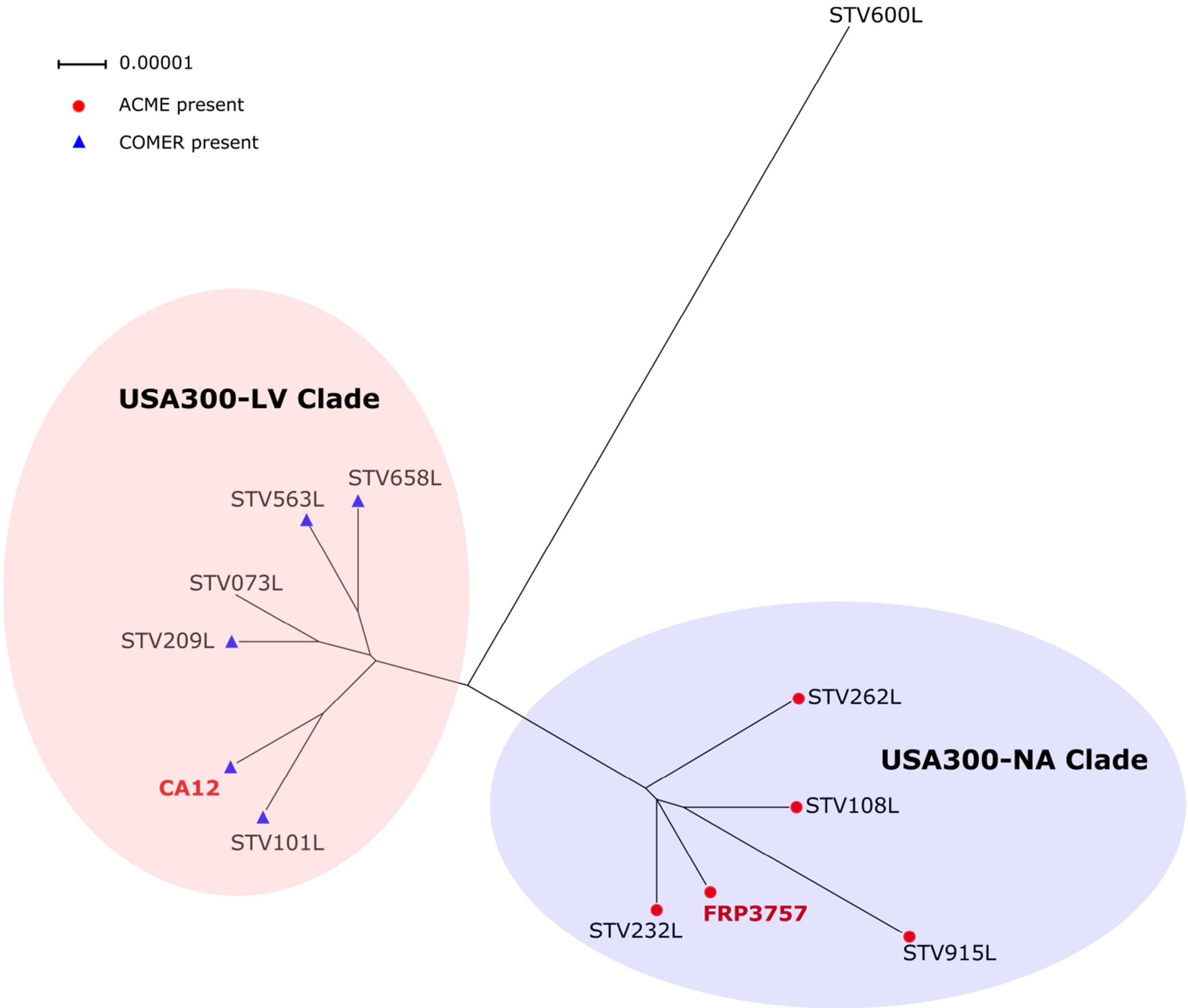
Figure 2. Molecular characteristics of imported MRSA, Europe, StaphTrav Network 2011-2016.



A. Major epidemic clones among imported CA-MRSA

B. Frequency of spa clonal complexes (spa-CC) among imported MRSA

Figure 3. Relatedness of MRSA multi-locus sequence type 8 imported to Europe, 2011-2016 (n=10).



Unrooted maximum likelihood tree based on allelic differences in the core genome (core genome multi-locus sequence typing). Imported isolates of community-associated MRSA MLST ST8/spa t008 belong to two major clades: USA300-North American Clade (USA300-NA), shaded in blue, and USA300-Latin-American Variant (USA300-LV), shaded in red. Reference sequences for North and South American ST8-USA300 MRSA are FRP3757 [14] and CA12 [13], respectively, and marked in red and bold. Presence of the copper and mercury resistance (COMER) mobile element typically found in USA300 of the Latin-American Variant [15] is marked by blue triangles. Presence of the arginine catabolic mobile element (ACME), which is the hallmark of the North American USA300 [15], is marked by red circles. The STV073L isolate, lacking both, COMER and ACME, was acquired in South Sudan, while the remaining isolates in the USA300-LV clade were imported from Cuba (n=2) and Colombia (n=2).