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Wild rodents and shrews are natural hosts of *Staphylococcus aureus*

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ABSTRACT

Laboratory mice are the most commonly used animal model for *Staphylococcus aureus* infection studies. We have previously shown that laboratory mice from global vendors are frequently colonized with *S. aureus*. Laboratory mice originate from wild house mice. Hence, we investigated whether wild rodents, including house mice, as well as shrews are naturally colonized with *S. aureus* and whether *S. aureus* adapts to the wild animal host. 295 animals of ten different species were caught in different locations over four years (2012–2015) in Germany, France and the Czech Republic. 45 animals were positive for *S. aureus* (15.3%). Three animals were co-colonized with two different isolates, resulting in 48 *S. aureus* isolates in total. Positive animals were found in Germany and the Czech Republic in each studied year. The *S. aureus* isolates belonged to ten different *spa* types, which grouped into six lineages (clonal complex (CC) 49, CC88, CC130, CC1956, sequence type (ST) 890, ST3033). CC49 isolates were most abundant (17/48, 35.4%), followed by CC1956 (14/48, 29.2%) and ST890 (9/48, 18.8%). The wild animal isolates lacked certain properties that are common among human isolates, e.g., a phage-encoded immune evasion cluster, superantigen genes on mobile genetic elements and antibiotic resistance genes, which suggests long-term adaptation to the wild animal host. One CC130 isolate contained the *mecC* gene, implying wild rodents might be both reservoir and vector for methicillin-resistant . In conclusion, we demonstrated that wild rodents and shrews are naturally colonized with *S. aureus*, and that those *S. aureus* isolates show signs of host adaptation.

1. Introduction

Antibiotic resistance of human pathogens is on the rise all around the globe. One of the most frequent causes of human infection is the opportunistic bacterium *Staphylococcus aureus* (*S. aureus*). About 30% of the human population is colonized with this pathogen, especially in the area of the anterior nares (Wertheim et al., 2005). *S. aureus* can cause skin and soft tissue infections, e.g. abscesses, as well as life-threatening infections, including pneumonia and sepsis (Tong et al., 2015). Since the prevalence of multi-resistant *S. aureus* strains remains high, and as there is no effective vaccine available, infections have become a more important public health threat (World Health Organization, 2014).

Besides in human beings, *S. aureus* has been found in various animal species, both in livestock, which is in close contact with humans, and

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wild animals (Monecke et al., 2016). The natural colonization of mice and other rodents could be of special interest considering that laboratory mice are the most commonly used experimental model for *S. aureus* infection. Nevertheless, reports on the natural colonization of wild mice and other rodents are scarce and of limited scope (Gomez et al., 2014; Monecke et al., 2016). In contrast, laboratory mice have been investigated more thoroughly and stable colonization with presumably mouse-adapted *S. aureus* strains has recently been shown by our group. The most frequent *S. aureus* lineage in laboratory mice was clonal complex (CC) 88, followed by CC15, CC5, CC188, and CC8 (Holtfreter et al., 2013; Schulz et al., 2017).

Adaptation of *S. aureus* to a host takes place on the genomic level and is determined by the physiological condition of the host, e.g. the immune system, as well as the environmental influences to which the bacteria are exposed, such as antibiotic treatment (Herron-Olson et al., 2007). Typical adaptation mechanisms following zoonotic transmission include mutations of individual genes and the loss and/or gain of mobile genetic elements (MGEs), as has already been shown for *S. aureus* isolates of bovine, ovine, equine, poultry, and laboratory mouse origin (Guinane et al., 2010; Lowder et al., 2009; Murray et al., 2017; Schulz et al., 2017; Viana et al., 2010).

The immune system of an *S. aureus* host exerts a strong selective pressure on the bacterium, which is reflected by the vast array of immune-evading and modulating factors (Thammavongsa et al., 2015). Several staphylococcal virulence factors act host-specifically, e.g. superantigens, the pore-forming toxin Panton-Valentine leukocidin (PVL), and the immune evasion cluster (IEC)-encoded complement-blocking factors chemotaxis inhibitory protein of *S. aureus* (CHIPS), staphylococcal complement inhibitor (SCIN), and staphylokinase (SAK). A prominent sign of *S. aureus* adaptation to an animal host is, therefore, the absence of human-specific virulence factors. This is exemplified by the comparably low prevalence of the IEC, which is encoded on *Sa3int* phages, and the modification of the von Willebrand factor-binding protein (vWbp) (Sung et al., 2008; Viana et al., 2010). Moreover, *S. aureus* isolates from wild animals and laboratory mice frequently lack antibiotic resistance, suggesting a lack of acquisition or alternatively a loss of antibiotic resistance genes (Monecke et al., 2016; Schulz et al., 2017).

The immune systems of laboratory mice and humans show numerous concordances, but also differences, as reviewed recently (Mestas and Hughes, 2004). Even the closely related wild mice and laboratory mice (both are house mouse, *Mus musculus*) differ in some components of their immune systems (Abolins et al., 2017). In consequence, the use of mouse-adapted *S. aureus* strains in their natural host – the mouse – promises to provide a more physiological model for studying *S. aureus*-host interaction and testing novel therapeutics (Holtfreter et al., 2013). Thus, a closer look at the *S. aureus* population in wild rodents could, first, reveal adaptation mechanisms of *S. aureus* to a certain host. Second, the isolated strains could provide a better tool for *S. aureus* infection and vaccination studies in laboratory mice.

The aims of this study were (1) to determine the prevalence and both spatial and temporal distribution of *S. aureus* in rodents and shrews, (2) to characterize the population structure of those *S. aureus* isolates, and (3) to screen the obtained S. *aureus* isolates for signs of host adaptation.

2. Materials and methods

2.1. Study design and ethics statement

The subjects of study were wild rodents and shrews: striped field mouse (*Apodemus agrarius*), yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), water vole (*Arvicola* spp.), field vole (*Microtus agrestis*), common vole (*Microtus arvalis*), house mouse (*Mus musculus*), bank vole (*Myodes glareolus*), common shrew (*Sorex araneus*) and crowned shrew (*Sorex coronatus*) (see Table S1 in the online version at DOI:10.1016/j.ijmm.2017.09.014). During monitoring studies between 2012 and 2015, 295 animals were collected in the wild by snap trapping according to a standard protocol at different locations in Mecklenburg-Western Pomerania (Gristow, Jeeser, Niederhof, Reinkenhagen, Stralsund), Thuringia (Gotha) and Baden-Wuerttemberg (Freiburg, Heimerdingen, Rutesheim, Stühlingen, Weissach) as well as in Amplepuis (Département Rhône, France) and Brno (South Moravian Region, Czech Republic) (Drewes et al., 2017). Animals found dead in live traps were also included in the study. All animals were immediately frozen and stored at −20 °C until dissection. Their noses were aseptically removed from the body and frozen again at −20 °C.

Samples were collected according to relevant legislation and by permission of the responsible State authorities (Regierungspräsidium Stuttgart 35-9185.82/0261; Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern 7221.3-030/09; Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz 22-2684-04-15-107/09).

2.2. S. aureus isolation

The mouse noses were thawed and homogenized in 500 µL enrichment medium (2.5 g/L Tryptone; 18.75 g/L NaCl; 2.5 g/L D-mannitol; 0.625 g/L yeast extract; 4.5 mg/L phenol red in *Aqua bidest*) using zirconia beads (homogenizer Precellys 24, VWR, Darmstadt, Germany). The homogenate was subsequently transferred to a 15 mL tube and cultured aerobically in enrichment medium (total volume 3.5 mL) for 48 h at 37 °C under agitation. Afterwards, serial dilutions (10^{-3}–10^{-6}) were plated on mannitol salt agar plates (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and incubated for 48 h at 37 °C. All distinct colony morphotypes were subcultured on sheep blood agar (Becton, Dickinson and Co.), and the colonies obtained were screened for *S. aureus* with an *S. aureus*-specific latex agglutination test (Staph Xtra Latex kit, ProLexTM, Richmond Hill, ON, Canada) and an *S. aureus*-specific colony multiplex PCR. The amplification of the 16SrRNA gene served as quality control (756 base pair (bp); 16SrRNA_forward primer 5′-AACTCTGTTATTAGGG AAGAACA-3′, 16SrRNA_reverse primer 5′-CCACCTTCCTCCGG TTTGTCACC-3′), and the *S. aureus*-specific gyrase gene (281 bp; Gyr_forward primer 5′-AGTACATCGTCGTATACTATATGG-3′, Gyr_reverse primer 5′-ATCACGTAACAGTTCAAGTGTG-3′) was used to detect *S. aureus* DNA. A single colony was resuspended in 10 µL of DNase- and RNase-free water and heat-inactivated for 10 min at 95 °C. PCRs were performed with the GoTaq® Flexi DNA polymerase system (Promega, Mannheim, Germany). Each reaction mix (25 µL) contained 1x GoTaq® reaction buffer, 100 µM deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP; Roche Diagnostics, Mannheim, Germany), 5 mM $MgCl_2$, 320 nM of each primer, 1.0 U GoTaq® Flexi DNA polymerase and 4.3 µL of the heat-inactivated *S. aureus* suspension. An initial denaturation of DNA at 95 °C for 10 min was followed by 30 cycles of amplification (95 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s), ending with a final extension phase at 72 °C for 7 min. All PCR products were resolved by electrophoresis in 1.5% agarose gels (1x TBE buffer), stained with RedSafe™ (INtRON Biotechnology, Sungnam, Korea) and visualized under UV light.

PCR-positive *S. aureus* isolates were stored as glycerol stocks. For each animal, between 1 and 5 isolates were stored and subsequently genotyped. Isolates originating from the same animal showing the same genotype were counted as one for later analysis. DNA isolation was performed on all isolates using the DNeasy Blood and Tissue Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions, but with an addition of 0.2 mg/mL lysostaphin (Sigma-Aldrich, St. Louis, Missouri, USA) to the lysis buffer.

2.3. Spa genotyping and multi-locus sequence typing (MLST)

Spa genotyping and MLST were performed as described elsewhere (Enright et al., 2000; Harmsen et al., 2003). *Spa* typing was performed on all *S. aureus* isolates. *Spa* types were clustered into clonal lineages

using the BURP algorithm of Ridom Software and the SpaServer (http://www.spaserver.ridom.de/). MLST was performed on selected isolates to confirm the *spa* clustering results. Profiles were submitted to the SpaServer and the MLST database (https://pubmlst.org/saureus/).

2.4. Virulence gene detection using multiplex PCR

A total of 25 *S. aureus* virulence genes, including gyrase (*gyr*), methicillin resistance (*mecA*), Panton-Valentine leukocidin (*lukS-PV*, *lukF-PV*), staphylococcal superantigen (*sea-selu*, *tst*), exfoliative toxin (*eta*, *etd*) and *agr* group 1–4 genes, were detected as described elsewhere (Goerke et al., 2009; Holtfreter et al., 2007).

S. aureus bacteriophage types (*Sa1int-Sa7int*) and the *Sa3int* phage-encoded IEC (*Sa3int*, *sak*, *chp*, *scn*) were detected with multiplex PCR according to published protocols (Schulz et al., 2017). The methicillin-resistance coding *mecC* gene was detected by PCR as described elsewhere (Cuny et al., 2011).

2.5. Virulence gene detection using DNA microarray

Selected isolates were tested using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH, Jena, Germany), which is an array hybridization kit for DNA-based detection of resistance genes and pathogenicity gene markers as well as assignment of unknown *S. aureus* isolates to known reference strains. The Genotyping Kit 2.0 covers 333 target sequences, corresponding to 170 distinct *S. aureus* genes and their allelic variants, including genes for species markers, resistances, exotoxins, adhesins, surface proteins, capsular proteins and *agr* group typing markers. The principle of the assay, related procedures, and a list of targets have been described previously and are available on the company's homepage (https://alere-technologies.com/products/lab-solutions/s-aureus.html) (Monecke et al., 2016).

3. Results and discussion

3.1. Prevalence of S. aureus in rodents and shrews

S. aureus was isolated from 45 of 295 tested animals (15.3%). No *S. aureus* was detected in the following species: crowned shrew, wood mouse, striped field mouse, or water vole; although this might simply be due to the low number of samples obtained from those species (Table 1).

Only for a few species enough animals were caught to allow a reliable approximation of colonization rates, which ranged from 9 to 21% (common vole, yellow-necked mouse, bank vole) (Table 1). For the other species, low sample numbers precluded reliable determination of the prevalence of *S. aureus*.

S. aureus-harboring animals were found in Mecklenburg-Western Pomerania, Baden-Wuerttemberg, Thuringia (all Germany) and Brno (Czech Republic), but not in Amplepuis (France; seven rodents and one shrew tested; not significant) (Fig. 1). The absence of *S. aureus* from the French samples is likely due to the low sample number. Moreover, *S. aureus*-colonized animals were detected in every studied year (2012–2015), which suggests a long-term presence of *S. aureus* in rodents and shrews in the studied region (Table 2).

Table 1
Overview of the tested species and the number of *S. aureus*-positive animals.

Species	*S. aureus*-positive animals
common vole (*Microtus arvalis*)	12/134 (9.0%)
bank vole (*Myodes glareolus*)	13/61 (21.3%)
yellow-necked mouse (*Apodemus flavicollis*)	11/61 (18.0%)
field vole (*Microtus agrestis*)	3/12[a]
common shrew (*Sorex araneus*)	5/12[a]
crowned shrew (*Sorex coronatus*)	0/8[a]
wood mouse (*Apodemus sylvaticus*)	0/4[a]
house mouse (*Mus musculus*)	1/1[a]
striped field mouse (*Apodemus agrarius*)	0/1[a]
water vole (*Arvicola* spp.)	0/1[a]

[a] Percentages were not calculated because of the small sample numbers.

Three animals were co-colonized by two different *S. aureus* isolates. Therefore, a total of 48 different *S. aureus* isolates were obtained. The isolates fell into groups of 10 different *spa* types (t208, t843, t1736, t1773, t2311, t3058, t3830, t4189, t9909, t15027), which in turn were grouped into six lineages (CC49, CC88, CC130, CC1956, sequence type (ST) 890, ST3033). The *spa* type t15027 and the sequence types ST3033 and ST3252 were first described in this study. CC49 strains were isolated most frequently (17/48, 35.4%), followed by CC1956 (14/48, 29.2%), ST890 (9/48, 18.8%), ST3033 (5/48, 10.4%), CC130 (2/48, 4.2%) and CC88 (1/48, 2.1%) (Table 2). All these lineages are uncommon in the human *S. aureus* population, which indicates a distinct population structure in the investigated animals (Cuny et al., 2011; Holtfreter et al., 2016).

Compared to the human *S. aureus* population obtained from healthy volunteers in Germany (Holtfreter et al., 2016; Mehraj et al., 2014), the presented *S. aureus* population in wild rodents and shrews might be less diverse. Mehraj et al. detected 55 different *spa* types among 85 *S. aureus* isolates, while we obtained only ten *spa* types from 48 different isolates. This might indicate longer evolution of *S. aureus* in the human host than in wild rodents and shrews.

3.2. Genetic characterization of S. aureus isolates from rodents and shrews

3.2.1. S. aureus clonal complex 49

CC49 strains (ST49) were isolated from bank voles and yellow-necked mice in Mecklenburg-Western Pomerania and Baden-Wuerttemberg. All isolates were negative for the methicillin resistance genes *mecA* and *mecC*, superantigen genes, and the IEC-encoding *Sa3int* phages, while being positive for *Sa5int* phages (Table 2). Representative isolates (n = 6) were subjected to DNA array analysis (see Table S2 in the online version at DOI: 10.1016/j.ijmm.2017.09.014). All of them were negative for the tested resistance genes, except for *sdrM*, and positive for the leucocidin genes *lukF/lukS* and *lukF-PV(P83)/lukM*.

CC49 strains are rare in other contexts. Methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) CC49 isolates have occasionally been found in humans (Deplano et al., 2014), squirrels (Simpson et al., 2013), pigs (Overesch et al., 2011), rats (Paterson et al., 2012), horses (Haenni et al., 2015), wallabies (Chen et al., 2016), voles and wildcats (Monecke et al., 2016). All tested CC49 isolates from this study were ampicillin-sensitive MSSA, which points at a low selection pressure for this characteristic in wild animals. Several genetic traits indicate adaptation of the CC49 isolates to their wild animal hosts: The absence of both antibiotic resistance genes and the human-specific IEC, along with the presence of *lukM*, which is absent in human CC49 isolates (Monecke et al., 2016), as well as the presence of *Sa5int* phages, which are rare among human isolates (Goerke et al., 2009).

3.2.2. S. aureus clonal complex 1956

CC1956 isolates (ST1956, ST3252) were obtained from common voles and field voles in Mecklenburg-Western Pomerania, Baden-Wuerttemberg and Brno. All isolates lacked *mecA/mecC*, and superantigen genes (Table 2). All isolates tested by DNA array analysis (n = 8) completely lacked antibiotic resistance gene markers (see Table S2 in the online version at DOI:10.1016/j.ijmm.2017.09.014). ST3252 isolates lacked the IEC, but carried both *Sa3int* phages, which commonly encode the IEC in human *S. aureus* isolates, and *Sa5int* phages. In contrast, ST1956 isolates were negative for *Sa3int* phages, but positive for both *Sa5int* and *Sa7int* phages, or for *Sa7int* phages only. It cannot be determined whether the ST3252 *S. aureus* isolates were infected with IEC-positive *Sa3int* phages that lost the IEC over the course of time or

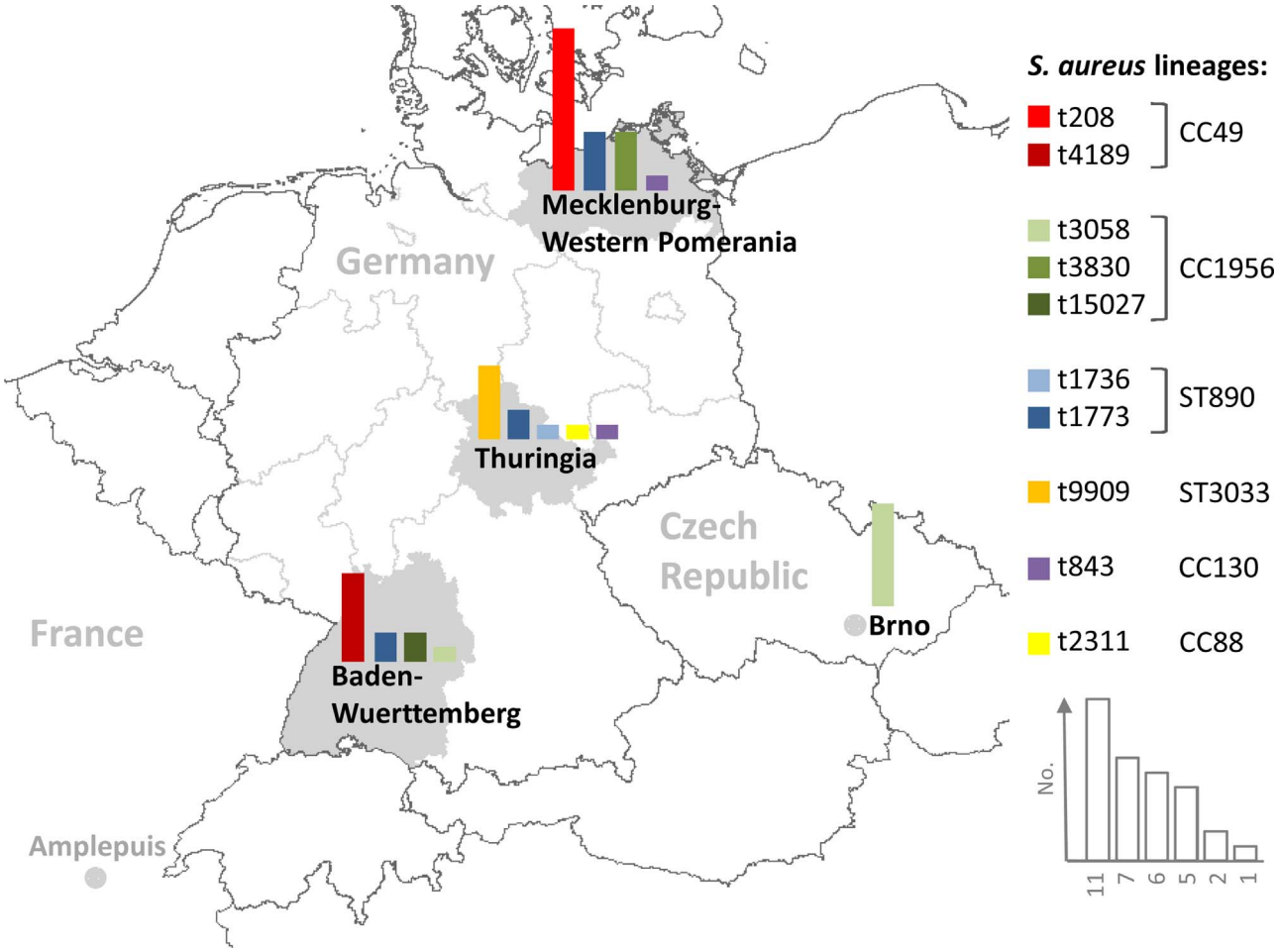

Fig. 1. *S. aureus* lineages CC49, CC1956 and ST890 are widespread among rodents and shrews. The graph illustrates the presence of *S. aureus* lineages among wild rodents and shrews caught at different locations in Germany (Baden-Wuerttemberg, Mecklenburg-Western Pomerania, Thuringia), France (Amplepuis), and the Czech Republic (Brno). No *S. aureus* was detected in Amplepuis. The height of the bars indicates the number of *S. aureus* isolates, their colors show the *spa* type and CC.

with IEC-negative *Sa3int* phages. Two isolates obtained from one field vole are listed as different strains, because one isolate was negative for the *Sa5int* phage while the other isolate was positive (No. 46c/47c, Table 2). However, since mobilization of phages is a common phenomenon within a host (Moore and Lindsay, 2001; Salgado-Pabón et al., 2014), these isolates are probably variants of the same *S. aureus* strain. The scarcity of *Sa3int* phages and especially the IEC in the CC1956 vole lineage may indicate a strong selection against those genetic elements. This is not surprising, since the IEC is highly human-specific and thus generally less frequent in animal isolates (Verkaik et al., 2011).

Similar to CC49 isolates, CC1956 isolates are rare in other animal species and humans; they have only occasionally been detected in cattle (Hasman et al., 2010), common voles and wood mice (Gomez et al., 2014), as well as in vole feces, a beaver (*Castor fiber*), a red squirrel (*Sciurus vulgaris*) and a human (Monecke et al., 2016).

3.2.3. *S. aureus* sequence type 890

ST890 isolates were obtained from yellow-necked mice in Mecklenburg-Western Pomerania, Baden-Wuerttemberg, and Thuringia, as well as from a field vole and a common vole in Thuringia. All isolates lacked *mecA/mecC*, superantigen genes, the IEC, and resistance gene markers, except for the *blaZ* gene (Table 2 and see Table S2 in the online version at DOI:10.1016/j.ijmm.2017.09.014). *BlaZ* was found only in ST890, where it was present in all tested isolates (see Table S2 in the online version at DOI:10.1016/j.ijmm.2017.09.014). Five isolates carried a *Sa7int* phage, the other four a *Sa1int* phage. Surprisingly, three out of four array-tested isolates were negative for the coagulase gene *coA*, which is generally highly conserved among human *S. aureus* strains (Smole et al., 1998). For example, 94.0% (8838/9403) of sequenced *S. aureus* isolates encoded the *coA* gene (https://www.patricbrc.org/; 04.09.2017). Moreover, all murine ST890 isolates lacked an *hlb* hybridization signal (see Table S2 in online version at DOI:10.1016/j.ijmm.2017.09.014).

The related *spa* types t1736 and t1773 have been described in different animals and humans, where they were always assigned to CC130 (Azara et al., 2017; Cuny et al., 2011; Gharsa et al., 2015; Haenni et al., 2014). The corresponding isolates in this study, however, were assigned to ST890 based on MLST and DNA array data. The comparison of array data revealed a high concordance between human CC130 strains and the two CC130 (t843) strains originating from this study (see below), while the ST890 isolates in this collection were distinctive in several ways. They lacked *coA,* some leucocidin genes, and *hlb* as well as *splA/B/E*; they also belonged to a different *agr* type and harbored different adhesion factor allelic variants (see Table S3 in the online version at DOI:10.1016/j.ijmm.2017.09.014). Clearly, the novel ST890 isolates in our study have features that are similar to and others that differ from typical CC130 strains, which may suggest one or more recombination events in those strains. Whether this exchange of large genetic fragments confers host adaptation to the respective host remains to be elucidated.

So far, ST890 isolates have only been found in one human in France, in vole feces (Monecke et al., 2016) and a rabbit carcass in Switzerland (Merz et al., 2016a). The latter two isolates carried *blaZ*, as did the

Table 2
Origin and genetic characterization of the obtained *S. aureus* isolates.

No.[a]	*spa* type	MLST ST[b]	MLST CC	*agr*	*mecA/C*	phage integrases (type)	IEC[c]	MGE-encoded SAgs	Non-MGE-encoded SAgs	Species	Place of capture (region, country)	Place of capture (area)	Date of capture
1	t208	ST49*	CC49	II	–	5	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	13.07.2014
2	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	24.09.2014
3	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	24.09.2014
4	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	24.09.2014
5	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	22.09.2014
6	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	26.09.2014
7	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	22.09.2014
8a	t208	ST49	CC49	II	–	5	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	22.09.2014
9	t208	ST49	CC49	II	–	5	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	23.09.2014
10	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	06.10.2015
11	t208	ST49	CC49	II	–	5	–	–	–	bank vole	MV, Germany	Jeeser	08.10.2015
12	t4189	ST49*	CC49	II	–	5	–	–	–	yellow-necked mouse	BW, Germany	Heimerdingen	28.07.2014
13	t4189	ST49	CC49	II	–	5	–	–	–	bank vole	BW, Germany	Heimerdingen	28.07.2014
14	t4189	ST49	CC49	II	–	5	–	–	–	bank vole	BW, Germany	Heimerdingen	28.07.2014
15	t4189	ST49	CC49	II	–	5	–	–	–	bank vole	BW, Germany	Heimerdingen	27.07.2014
16	t4189	ST49	CC49	II	–	5	–	–	–	bank vole	BW, Germany	Heimerdingen	28.07.2014
17	t4189	ST49	CC49	II	–	5	–	–	–	bank vole	BW, Germany	Heimerdingen	28.07.2014
18	t1736	ST890*	Sg	IV	–	7	–	–	–	field vole	TH, Germany	Gotha	24.09.2013
19	t1773	ST890*	Sg	IV	–	7	–	–	–	common vole	TH, Germany	Gotha	24.09.2013
20a	t1773	ST890	Sg	IV	–	1	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	22.09.2014
21	t1773	ST890	Sg	IV	–	1	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	22.09.2014
22	t1773	ST890	Sg	IV	–	7	–	–	–	yellow-necked mouse	TH, Germany	Gotha	04.08.2014
23	t1773	ST890	Sg	IV	–	7	–	–	–	yellow-necked mouse	BW, Germany	Heimerdingen	26.07.2014
24	t1773	ST890	Sg	IV	–	7	–	–	–	yellow-necked mouse	BW, Germany	Heimerdingen	26.07.2014
25	t1773	ST890	Sg	IV	–	1	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	08.10.2015
26	t1773	ST890	Sg	IV	–	1	–	–	–	yellow-necked mouse	MV, Germany	Jeeser	08.10.2015
27	t2311	ST88*	CC88	III	–	1,2,3	+	–	–	field vole	TH, Germany	Gotha	25.09.2012
28	t843	ST130*	CC130	III	*mecC*	5	–	–	–	yellow-necked mouse	TH, Germany	Gotha	26.09.2013
29	t843	ST130	CC130	III	–	1	–	–	–	house mouse	MV, Germany	Reinkenhagen	10.12.2013
30	t9909	ST3033*	Sg	II	–	3,5,7	–	–	–	common shrew	TH, Germany	Gotha	06.08.2014
31	t9909	ST3033	Sg	II	–	3,5,7	–	–	–	common shrew	TH, Germany	Gotha	05.08.2014
32	t9909	ST3033	Sg	II	–	3,5,7	–	–	–	common shrew	TH, Germany	Gotha	03.10.2014
33	t9909	ST3033	Sg	II	–	3,5,7	–	–	–	common shrew	TH, Germany	Gotha	05.08.2014
34	t9909	ST3033	Sg	II	–	3,5,7	–	–	–	common shrew	TH, Germany	Gotha	03.10.2014
35	t15027	ST3252*	CC1956	IV	–	3,5	–	–	–	common vole	BW, Germany	Rutesheim	22.10.2014
36b	t15027	ST3252*	CC1956	IV	–	3,5	–	–	–	common vole	BW, Germany	Rutesheim	21.10.2014
37b	t3058	ST3252*	CC1956	IV	–	3,5	–	–	–	common vole	BW, Germany	Rutesheim	21.10.2014
38	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	29.11.2014
39	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	29.11.2014
40	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	28.11.2014
41	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	29.11.2014
42	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	29.11.2014
43	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	29.11.2014
44	t3058	ST3252	CC1956	IV	–	3,5	–	–	–	common vole	SMR, Czech Republic	Brno	29.11.2014
45	t3830	ST1956*	CC1956	IV	–	5,7	–	–	–	common vole	MV, Germany	Jeeser	22.09.2014
46c	t3830	ST1956	CC1956	IV	–	7	–	–	–	field vole	MV, Germany	Jeeser	23.09.2014
47c	t3830	ST1956	CC1956	IV	–	5,7	–	–	–	field vole	MV, Germany	Jeeser	23.09.2014
48	t3830	ST1956	CC1956	IV	–	5,7	–	–	–	common vole	MV, Germany	Jeeser	22.09.2014

Abbreviations: MLST, multi-locus sequence typing; ST, sequence type; CC, clonal complex; agr, accessory gene regulator; mec, methicillin resistance; IEC, immune evasion cluster; MGE, mobile genetic elements; SAgs, superantigens; Sg, singleton; MV, Mecklenburg-Western Pomerania; BW, Baden-Wuerttemberg; TH, Thuringia; SMR, South Moravian Region.

[a] Isolates in bold face were tested with the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH, Jena, Germany). Isolates bearing the same letter behind the number were found in the same animal, e.g. 8a and 20a.

[b] Isolates marked with an asterisk (*) were tested by MLST. ST and CC for unmarked isolates were derived from the *spa* type.

[c] Isolates marked with a "+" tested positive for *chp*, *sak* and *scn* by PCR, isolates marked with "−" tested negative for these three genes.

ST890 isolates in this study (this information is not available for the human strain).

3.2.4. S. aureus sequence type 3033

ST3033 strains were only isolated from common shrews in Thuringia. Again, all isolates lacked *mecA/mecC*, superantigen genes, the IEC, and resistance gene markers. The isolates were positive for *Sa3int* phages devoid of the IEC, just like the CC1956 strains. Moreover, the ST3033 isolates harbored *Sa5int* and *Sa7int* phages (Table 2 and see Table S2 in the online version at DOI:10.1016/j.ijmm.2017.09.014). Since ST3033 was a novel singleton ST, no other hosts have been previously described.

3.2.5. S. aureus clonal complex 130

CC130 isolates (ST130) were cultured from a house mouse in Mecklenburg-Western Pomerania and a yellow-necked mouse in Thuringia. Both isolates lacked superantigen genes and the IEC (Table 2 and see Table S2 in the online version at DOI:10.1016/j.ijmm.2017.09.014). The Thuringia isolate (No. 28, Table 2) was the only *mecC*-positive isolate in this study, but lacked *lukF-PV (P83)/lukM*, while the reverse was true in the house mouse-derived isolate. CC130 is the major *mecC*-harboring *S. aureus* lineage, and CC130-MRSA are frequently found in livestock (Paterson et al., 2014).

CC130 is quite common in small ruminants (Merz et al., 2016b), but has also been found in humans and many other wild and domestic animals (Monecke et al., 2016; Walther et al., 2012). Importantly, rodents harboring *mecC*-positive *S. aureus* could represent a dangerous reservoir and vector for those strains, since their spread can hardly be monitored or controlled, putting livestock and even humans at risk (García-Garrote et al., 2014; Harrison et al., 2013; Paterson et al., 2014; Petersen et al., 2013). Such animals are usually found in cities or farming environments, where they can be exposed to *S. aureus* selected under antibiotic pressure. The discovery of a yellow-necked mouse carrying *mecC*-positive *S. aureus* in the wild was, therefore, unexpected.

3.2.6. S. aureus clonal complex 88

A single CC88 isolate (ST88) was obtained from a field vole in Thuringia. It lacked *mecA/mecC*, superantigen genes, and any resistance gene markers. This isolate was the only one in this study carrying *scn, chp, sak* of the IEC. Moreover, it encoded *Sa1int, Sa2int* and *Sa3int* phages (Table 2 and see Table S2 in the online version at DOI:10.1016/j.ijmm.2017.09.014).

CC88 has already been described in humans (Abdulgader et al., 2015), pigs (Fall et al., 2012), dogs (Gómez-Sanz et al., 2013), rooks (Monecke et al., 2016) and laboratory mice (Holtfreter et al., 2013). In a previous study and in a study published in this issue of IJMM, our group observed that CC88 is the predominant *S. aureus* lineage in laboratory mice (Schulz et al., 2017). Schulz et al. compared murine *S. aureus* isolates from laboratory mice with human CC88 strains, and observed several features of host adaptation. In contrast to human CC88 isolates, the corresponding *S. aureus* strains from laboratory mice lacked *Sa3int* phages and superantigen-encoding mobile genetic elements, and were frequently ampicillin-sensitive (Schulz et al., 2017). Moreover, murine CC88 isolates coagulated mouse plasma faster than did human CC88 isolates. The prototypical murine CC88 strain JSNZ was superior to commonly used human-adapted *S. aureus* strain Newman in a mouse colonization model (Holtfreter et al., 2013). While CC88 was predominant in laboratory mice, it was rare in wild rodents and shrews. Moreover, *S. aureus* JSNZ lacks the IEC, while the novel isolate still harbors it, which could be an indication that *S. aureus* JSNZ is better adapted to the animal host than the isolate presented here (see Table S4 in the online version at DOI:10.1016/j.ijmm.2017.09.014).

4. Conclusions

S. aureus colonization of wild rodents and shrews was detected in different areas of Germany and the Czech Republic over a four-year period (2012–2015), which may suggest a long-term persistence of *S. aureus* in the investigated animal populations. Thus, wild rodents (including mice) and shrews are natural hosts of *S. aureus*. In line with our findings, we have recently reported that laboratory mice from various vendors are also naturally colonized with mouse-adapted *S. aureus* lineages. Being natural hosts turns mouse species into better models for *S. aureus* infection and vaccination studies than previously assumed.

Notably, the detected *S. aureus* population was less diverse than the population described in healthy human carriers of the same region (Holtfreter et al., 2016; Mehraj et al., 2014). This is in line with previous studies, which reported a limited diversity of *S. aureus* lineages in, e.g., ruminants and poultry (Guinane et al., 2010; Lowder et al., 2009). Whole-genome studies suggest that humans are the major reservoir for *S. aureus*, and that the present animal-adapted *S. aureus* lineages were derived from a jump from humans to the studied animal host (Guinane et al., 2010; Lowder et al., 2009). As a less likely alternative, the small number of animal-associated lineages might mean that *S. aureus* – although already long present in animals – faced more bottleneck events in the respective animals compared to the human host and therefore could not diversify accordingly. Ongoing whole-genome sequencing studies of the *S. aureus* isolates from rodents and shrews along with lineage-matched human strains will provide some indications as to whether or not the detected *S. aureus* lineages originated from a human host.

Several genetic traits indicate adaptation of the investigated *S. aureus* population to its hosts: the nearly complete absence of antibiotic resistance genes, the lack of the human-specific IEC and lineage-specific superantigen genes, the high prevalence of *Sa5int* phages and low prevalence of *Sa3int* phages. Ongoing whole-genome sequencing will reveal whether these strains have developed further means of host adaptation, such as deletion of non-advantageous genes, allelic variants of fitness and virulence factors, or the acquisition of novel mobile genetic elements.

The scarcity of IEC-encoding *Sa3int* phages in all detected *S. aureus* lineages from wild rodents and shrews may indicate a strong selection against these genetic elements. It is well known that the IEC is highly human-specific and, therefore, generally less frequent in animal isolates (Verkaik et al., 2011). Its loss during the course of adaptation to an animal host has already been described (Price et al., 2012). In detail, Price et al. sequenced 89 CC398 isolates from animals and humans and provided evidence that the animal strains originated from humans and subsequently lost the *Sa3int* phage with its human-specific virulence genes. Remarkably, Katayama et al. showed that *hlb*, which is destroyed by the integration of *Sa3int* phages, promotes skin colonization of mice by *S. aureus* by at least a factor of 50 (Katayama et al., 2013). Moreover, IEC-encoding *Sa3int* phages were rare in murine *S. aureus* isolates colonizing laboratory mice as compared to lineage-matched human strains (Holtfreter et al., 2013). Thus, expelling *Sa3int* and thereby restoring the *hlb* gene seems to be advantageous for colonizing murine species. The same could be true for the species mentioned here.

The *S. aureus* isolates from wild rodents and shrews characterized in this study might be promising candidates for improving *S. aureus* infection and vaccination models. Currently, researchers are using human-adapted strains to infect mice. Using mouse-adapted *S. aureus* strains in their natural host – the mouse – could provide a more physiological model for studying *S. aureus* host interaction and testing novel therapeutics. Indeed, we have previously shown that the murine CC88 strain JSNZ shows better fitness and virulence in mouse colonization and infection models, respectively (Holtfreter et al., 2013). A comparison of representative *S. aureus* isolates from the predominant lineages found in wild rodents and shrews (this study) and laboratory mice (Mrochen et al., IJMM, this issue) in mouse infection experiments will reveal whether they are suitable tools for developing better infection models.

One yellow-necked mouse from Thuringia was colonized with a *mecC*-positive CC130 strain. This raises the question of whether wild

rodents may be reservoirs for MRSA, having important implications for the health of both livestock and humans. In fact, rats living in close vicinity to humans, e.g., in cities or near livestock farms, were found to be colonized with human or livestock-associated MRSA, respectively (Himsworth et al., 2014; Pletinckx et al., 2013). Wild rodents are known to act as reservoirs for various zoonotic pathogens, such as hantaviruses, arenaviruses, cowpox virus, *Leptospira* spp., *Rickettsia* spp. and *Francisella tularensis* (Charrel and de Lamballerie, 2010; Mayer-Scholl et al., 2014; Schex et al., 2011; Schlegel et al., 2014; Wobeser et al., 2009; Wolfs et al., 2002). Our findings suggest that wild rodents and shrews can acquire *S. aureus* strains present in their immediate environment. However, the degree to which these bacteria are maintained in these populations and the potential significance of rodents and shrews as a reservoir of *S. aureus* and in particular MRSA for humans, animals, and the environment remain to be addressed.

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Conflicts of interest

None.

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