International Journal of Medical Microbiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

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

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ARTICLE INFO

Keywords:
Staphylococcus aureus
Colonization
Wild mice
Host adaptation
Immune evasion cluster
mecC

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 *S. aureus*. 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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http://dx.doi.org/10.1016/j.ijmm.2017.09.014
Received 30 July 2017; Received in revised form 12 September 2017; Accepted 19 September 2017

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′-AACTCTGTTATTAGGGAAGAACA-3′, 16SrRNA_reverse primer 5′-CCACCTTCCTCCGGTTTGTCACC-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