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Global spread of mouse-adapted *Staphylococcus aureus* lineages CC1, CC15, and CC88 among mouse breeding facilities

Daniel M. Mrochen[a], Dorothee Grumann[a], Daniel Schulz[a], Janine Gumz[a], Patricia Trübe[a], Kathleen Pritchett-Corning[b,1], Sarah Johnson[c], Werner Nicklas[d], Petra Kirsch[e,2], Karine Martelet[f], Jens van den Brandt[g], Sabine Berg[g], Barbara M. Bröker[a], Siouxsie Wiles[c,h], Silva Holtfreter[a,*]

[a] Department of Immunology, University Medicine Greifswald, Germany
[b] Charles River, Research and Professional Services, Wilmington, MA, United States
[c] Bioluminescent Superbugs Lab, Department of Molecular Medicine and Pathology, University of Auckland, New Zealand
[d] Deutsches Krebsforschungszentrum, Microbiological Diagnostics, Heidelberg, Germany
[e] Tierforschungszentrum, University of Ulm, Ulm, Germany
[f] Charles River, 360 diagnostic (RADS France), L'Arbresle cedex, France
[g] Central Core & Research Facility of Laboratory Animals, University Medicine Greifswald, Germany
[h] Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand

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ABSTRACT

We previously reported that laboratory mice from all global vendors are frequently colonized with *Staphylococcus aureus* (*S. aureus*). Genotyping of a snap sample of murine *S. aureus* isolates from Charles River, US, showed that mice were predominantly colonized with methicillin-sensitive CC88 strains. Here, we expanded our view and investigated whether laboratory mice from other global animal facilities are colonized with similar strains or novel *S. aureus* lineages, and whether the murine *S. aureus* isolates show features of host adaptation. In total, we genotyped 230 *S. aureus* isolates from various vendor facilities of laboratory mice around the globe (Charles River facilities in the USA, Canada, France, and Germany; another US facility) and university- or company-associated breeding facilities in Germany, China and New Zealand. *Spa* typing was performed to analyse the clonal relationship of the isolates. Moreover, multiplex PCRs were performed for human-specific virulence factors, the immune-evasion cluster (IEC) and superantigen genes (SAg). We found a total of 58 different *spa* types that clustered into 15 clonal complexes (CCs). Three of these *S. aureus* lineages had spread globally among laboratory mice and accounted for three quarters of the isolates: CC1 (13.5%), CC15 (14.3%), and CC88 (47.0%). Compared to human colonizing isolates of the same lineages, the murine isolates frequently lacked IEC genes and SAg genes on mobile genetic elements, implying long-term adaptation to the murine host. In conclusion, laboratory mice from various vendors are colonized with host-adapted *S. aureus*-strains of a few lineages, predominantly the CC88 lineage. *S. aureus* researchers must be cautioned that *S. aureus* colonization might be a relevant confounder in infection and vaccination studies and are therefore advised to screen their mice before experimentation.

1. Introduction

The Gram-positive bacterium *Staphylococcus aureus* is a dangerous opportunistic pathogen and a leading cause of bacterial infection in hospitals and in the community world-wide. Apart from being a human pathogen, *S. aureus* also colonizes and infects a large range of hosts, including companion animals, livestock, and wild animals (Fitzgerald and Holden, 2016; McCarthy et al., 2012). The treatment of *S. aureus* infections is hampered by the spread of methicillin-resistant *S. aureus* (MRSA) strains, which have dwelled and spread in hospitals for decades, and are nowadays also found in the community and even livestock (Cuny et al., 2015; DeLeo et al., 2010; World Health Organization, 2014). Unfortunately, all attempts to develop a protective *S. aureus* vaccine have failed so far (Fowler and Proctor, 2014). Thus, novel approaches for the prevention and treatment of *S. aureus* infections are urgently required. The development of new interventional strategies

* Corresponding author.
E-mail address: silva.holtfreter@uni-greifswald.de (S. Holtfreter).
[1] Current address: Office of Animal Resources, Harvard University Faculty of Arts and Sciences, Cambridge, MA, United States.
[2] Current address: Robert Koch Institute, Berlin, Germany.

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relies on robust and clinically relevant infection models. Most researchers use laboratory mice as a surrogate host, because they have a well characterized immune system and are relatively easy and inexpensive to breed. Moreover, cause-effect studies are facilitated by numerous gene knock-out mouse strains. However, we have recently reported that laboratory mice are natural hosts of *S. aureus* and that colonization of specific-pathogen-free mice is far more common than expected (Schulz et al., 2017). We observed that *S. aureus* spreads in mouse colonies by efficient transmission from parents to offspring and can persistently colonize young mice. Just like humans, colonized laboratory mice mount a systemic immune response against *S. aureus*. Moreover, both mice and men frequently suffer from abscess formation. These similarities in the interaction between *S. aureus* and humans or mice suggest that laboratory mice are better models for colonization and infection studies than previously assumed.

Adaptation to new hosts is a complex evolutionary process, involving the loss and/or acquisition of mobile genetic elements (MGEs), such as phages, as well as the accumulation of mutations, resulting in host-specific allelic variants or loss of function (Fitzgerald and Holden, 2016). We have previously genotyped 99 murine *S. aureus* isolates from Charles River Laboratories, USA, and observed that CC88-MSSA is the dominant murine *S. aureus* lineage (Schulz et al., 2017). The murine strains showed features of host adaptation, e.g. the absence of superantigen (SAg)-encoding mobile genetic elements, as well as the lack of the *Sa3int* phages. SAgs are highly potent T cell mitogens which show a 10–100-fold reduced activity on murine as compared to human T cells (Holtfreter and Broker, 2005). *Sa3int* phages encode the human-specific immune evasion gene cluster (IEC) carrying staphylo-kinase (*sak*), staphylococcal complement inhibitor (*scn*) and chemotaxis inhibitory protein of *S. aureus* (CHIPS; *chp*) as well as staphylococcal enterotoxins A or P (*sea*, *sep*) (Sung et al., 2008; van Wamel et al., 2006). These phages are common in human isolates, but usually absent in animal-adapted strains, including the murine *S. aureus* isolates from Charles River, US (Holtfreter et al., 2013; Markham and Markham, 1966; Sung et al., 2008).Worryingly, natural *S. aureus* colonization of laboratory mice primed the adaptive immune system and could hence affect experimental infection and vaccination studies.

The aims of this study were to investigate (1) whether laboratory mice from other commercial but also university-associated breeding facilities are colonized with CC88 strains or novel *S. aureus* lineages, and (2) to look for features of host adaptation by comparing the murine isolates with corresponding human strains.

2. Materials and methods

2.1. Murine S. aureus isolates

S. aureus isolates were provided by several vendors or university breeding facilities. Specimen were obtained during routine health monitoring, and occasionally from euthanized mice suffering from *S. aureus* infections (e.g. preputial gland adenitis). To avoid a sampling bias, we included a maximum of 4 isolates of the same *spa* type, barrier, mouse strain and time point in the study cohort.

CR, USA: The CR strains (n = 121) were provided by Charles River's Research Animal Diagnostic Services (Wilmington, USA). Strains were submitted by different Charles River facilities in Hollister (n = 13), Kingston (n = 10), Raleigh (n = 10), and Canada (n = 6), as well as from Charles River customers (n = 70) (Table 1). Another twelve strains were provided by an anonymous US vendor. *Spa* type, SAg gene patterns, IEC genes and ampicillin resistance of the majority of these strains (n = 84) have been previously reported (Schulz et al., 2017). The PGA strains were obtained from C57BL/6CRL mice with preputial gland adenitis (PGA) from Charles River breeding facilities at Kingston (n = 6) and Hollister (n = 9).

CR, France: 14 *S. aureus* isolates were obtained during routine health monitoring at the Charles River facilities in L'Arbresle cedex, France.

Auckland: 13 *S. aureus* strains were isolated from fecal samples of BALB/c and C57BL/6 mice at the animal facility at the University of Auckland. A single isolate was obtained from a mouse with a severe purulent vaginal infection. JSNZ, the prototypical murine CC88-MSSA strain, was isolated from a C57BL/6 mouse with PGA in 2008 (Holtfreter et al., 2013).

China: DNA of twelve murine *S. aureus* strains was provided by an anonymous Chinese pharmaceutical company. Three samples were obtained from mice that originated from Beijing Vital River Biotechnologies (Charles River, China). Some mice were symptom-free, while others had PGA or facial infections (Table S1).

DKFZ Heidelberg: 37 *S. aureus* isolates were obtained during routine health monitoring, and occasionally from euthanized mice suffering from *S. aureus* infections (e.g. PGA, dermatitis, abscesses) at the animal facilities of the Deutsche Krebsforschungszentrum (DKFZ) in Heidelberg, Germany. Samples were obtained between 1983 and 2015 from various mouse strains, directly derived from several different vendors or obtained from in-house-breeding facilities.

Ulm: Eight *S. aureus* isolates were obtained from various mouse strains during routine health monitoring, and occasionally from euthanized mice at the animal facilities (Tierforschungszentrum) of the University of Ulm, Germany.

Greifswald: This cohort comprises 14 *S. aureus* isolates from stool samples of mostly C57BL/6 mice at the Central Service and Research Facility for laboratory animals at the University Medicine in Greifswald, Germany.

2.2. Human S. aureus isolates

CC-matched human *S. aureus* strains were obtained from several *S. aureus* colonization studies (T, SH, SHIP). The T and SH strains were obtained from healthy blood donors in Northern Germany in 2002 and 2005–2006, respectively. *Spa* types as well as *agr* type, SAg gene patterns and *Saint* phage groups of these strains were previously reported (Holtfreter et al., 2004; Holtfreter et al., 2007). The SHIP studies (SHIP-LEGENDE, SHIP-2, SHIP-Trend-0) are population-based studies in Western Pomerania. They are described in depth elsewhere (Holtfreter et al., 2016; Volzke et al., 2011) (Table S2). Human CC88 isolates were obtained from various sources as previously reported (Schulz et al., 2017).

2.3. Ethics statement

CR, US: Most murine *S. aureus* isolates were obtained during routine health monitoring at Charles River facilities in Hollister CA, Kingston NY, and Wilmington MA, USA (Protocol number P06172002–Holding & Euthanasia of Animals for Diagnostic Testing and Health Monitoring). Few animals (PGA samples) were selected from animals destined for euthanasia, either due to surplus animal production or for their presentation with PGA. All animal work was performed in accordance with United States Public Health Service Policy on Humane Care and Use of Laboratory Animals and the US Animal Welfare Act.

S. aureus isolates from Charles River, France, the University Medicine of Greifswald, the University of Ulm and the DKFZ Heidelberg were obtained during routine health monitoring laboratory rodent colonies. *S. aureus* isolates from the anonymous Chinese company were obtained during routine health monitoring, and from euthanized mice suffering from *S. aureus* infections (e.g. PGA, facial infections).

2.4. S. aureus identification and genotyping

S. aureus was identified by colony morphology on mannitol salt agar (MSA) plates (Becton Dickinson, Franklin Lakes, USA), *S. aureus*–specific latex agglutination test (Staph Xtra Latex kit, ProLexTM, Richmond Hill, ON, Canada) as well as gyrase and nuclease