International Journal of Medical Microbiology 308 (2018) 598–606

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

# 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

ARTICLE INFO

*Keywords:*
*Staphylococcus aureus*
Colonization
Laboratory mice
Global
Host adaptation
Genotype
CC88
Immune evasion cluster

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.

https://doi.org/10.1016/j.ijmm.2017.11.006
Received 21 July 2017; Received in revised form 27 October 2017; Accepted 16 November 2017

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

**Table 1**
Overview on participating animal breeding facilities and detected *S. aureus* lineages.

| Cohort | Facility[1] | Year of isolation (No. of isolates) | detected *S. aureus* lineages (No. of isolates; mouse strains) | N |
|---|---|---|---|---|
| Charles River, Hollister, USA | Vendor | 2004 (5), 2013 (8) | CC1 (8; BL/6), CC188 (5; BL/6) | 13 |
| Charles River, Kingston, USA | Vendor | 2004 (5), 2013 (5) | CC88 (10; BL/6) | 10 |
| Charles River, Raleigh, USA | Vendor | 2004 (10) | CC88 (10; BL/6) | 10 |
| Charles River, St. Constant, CAN | Vendor | 2004 (6) | CC88 (4; BL/6), CC188 (2; BL/6) | 6 |
| Charles River, L'Arbresle cedex, FRA | Vendor | 2014 (13), unkn (1) | CC1 (8; diverse), CC15 (2; diverse), CC88 (3; diverse), singleton (1; diverse) | 14 |
| Charles River, USA | Uni, Pharma (customers) | 2004 (5), 2011 (64), 2012 (1) | CC5 (11; outbred, CD1, Nude), CC8 (4; BALB/C, CD1), CC15 (7; diverse), CC25 (2; BALB/C, outbred), CC30 (3; outbred, Nude), CC72 (1; CD1), CC88 (40; outbred, BL/6, Nude and others), CC101 (1; BALB/c), CC188 (1; outbred) | 70 |
| anon. vendor, USA | Vendor | 2004 (1), 2012 (11) | CC15 (11; mainly BL/6), CC88 (1; BL/6) | 12 |
| DKFZ, Heidelberg, GER | Uni | 1983 (3), 2007 (3), 2008 (9), 2009 (1), 2012 (3), 2013 (1), 2014 (8) | CC1 (5; NMRI, tg), CC15 (3; BL/6, tg), CC20 (3; NMRI, BL/6), CC22 (6; BL/6, CD1, tg), CC30 (1; CD1), CC88 (9; BL/6, tg), CC97 (1; BL/6) | 28 |
| | Vendor* | 2008 (3), 2014 (4), 2015 (2) | CC15 (2; FVB/N), CC30 (1; BL/6), CC88 (3; BL/6), CC398 (2; BL/6), ST4948 (1, C3HHe/N) | 9 |
| University of Auckland, Auckland, NZL | Uni | 2008 (1), 2011 (12) | CC88 (13; BALB/C, BL/6) | 13 |
| | Vendor* | 2011 (11) | CC1 (4; CD1), CC88 (7; CD1 and BL/6) | 11 |
| University Medicine Greifswald, Greifswald, GER | Uni | 2011 (4), 2013 (10) | CC1 (3; BL/6), CC7 (3; diverse), CC15 (4; Albino BL/6), CC88 (4; BL/6) | 14 |
| University of Ulm, Ulm, GER | Uni | 2007 (4), 2008 (4) | CC15 (4; BL/6, tg, NMRI), CC88 (4; diverse) | 8 |
| Anon. pharmaceutical company, Bejing, CHN | Pharma | 2014 (12) | CC1 (3; BL/6), CC8 (9; BL/6) | 12 |

Abbreviations: BL/6, C57BL/6; tg, transgenic; Uni, university-associated animal breeding facility; Pharma, pharmaceutical company-associated animal facility.
Vendor* are mice from commercial vendors that were analyzed at the University facility right after quarantine.
[1] Animal facilities were classified as commercial vendors ("Vendor") as opposed to customer facilities at universities (including the DKFZ; "Uni") and pharmaceutical industry ("Pharma").

PCR as previously reported (Schulz et al., 2017). *Spa* genotyping and multilocus sequence typing (MLST) were performed as described elsewhere (Enright et al., 2000; Harmsen et al., 2003). *Spa* types were clustered into clonal lineages using the BURP algorithm of the Ridom Software and the spaserver database (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/).

### 2.5. Virulence gene detection

Multiplex PCRs were applied to detect a total of 25 *S. aureus* species markers, resistance and virulence genes, including gyrase (*gyr*), penicillin binding protein 2A (*mecA*), Panton-Valentine leukocidin (*pvl*), staphylococcal superantigens (*sea-selu*, *tst*), exfoliative toxins (*eta*, *etd*) and *agr* group 1–4 (Goerke et al., 2009; Holtfreter et al., 2007). The *Sa3int* phage-encoded immune evasion cluster (IEC; *Sa3int*, *sak*, *chp*, *scn*) was detected by multiplex PCR according to published protocols (Schulz et al., 2017).

### 2.6. Antibiotic resistances

The minimal inhibition concentration (MIC) values for penicillin were determined according to EUCAST guidelines. Briefly, *S. aureus* colonies from blood agar plates were suspended in 0.9% (w/v) sodium chlorite solution to a McFarland of 0.5 and subsequently diluted 1:50 in Mueller Hinton broth 2 (Sigma-Aldrich, Germany). 50 µL of the bacterial suspension were incubated with serial dilutions of penicillin (Sigma-Aldrich, Germany) (0.016–125 µg/mL). Bacterial growth was visually inspected after 24 und 48 h. Isolates with a MIC value above 0.125 µg/mL were considered as penicillin- resistant.

Methicillin resistance was determined for all murine and human isolates by PCR amplification of the *mecA* and *mecC* genes as previously reported (Holtfreter et al., 2016). *mecA*-positive strains were subsequently subjected to a disc diffusion antimicrobial susceptibility assay using 30 µg cefoxitin (BBL™ Sensi-Disc™, BD Germany).

### 2.7. Statistics

Categorical data were assessed by using Pearson's chi-square test. P values of $\leq 0.05$ were considered statistically significant.

## 3. Results

### 3.1. The study cohort comprises S. aureus isolates from 11 animal facilities from around the globe

To characterize the *S. aureus* population in laboratory mice on a global scale, we obtained 230 *S. aureus* isolates from various vendors (Charles River, US; Charles River, Canada; Charles River, France, an anonymous US vendor), four research institutes (University of Auckland, New Zealand; University Medicine of Greifswald, Germany; DKFZ, Germany; University of Ulm, Germany) and a pharmaceutical company (Beijing, China) (Table 1). Most isolates (n = 121) were provided by Charles River's Research Animal Diagnostic Services (Wilmington, USA). These strains originated from customers (universities and pharmaceutical companies) (n = 74), as well as different Charles River breeding facilities in the US (i.e. Hollister, Kingston, Raleigh) and Canada. We also included a comprehensive sample collection from the DKFZ, comprising *S. aureus* isolates from the early 1980s until 2015 (n = 37). Some of the DKFZ isolates (n = 11) were obtained from the mice from commercial vendors immediately upon arrival and were hence regarded as vendor-derived samples. For details on the *S. aureus* isolates from the other vendors, university-associated animal facilities and the included pharmaceutical company, please refer to Table 1. To avoid a sampling bias, we included a maximum of four isolates from the same genotype (as determined by an identical *spa* type and virulence gene pattern), barrier, mouse strain and time point in the study cohort.

### 3.2. S. aureus lineages CC1, CC15, and CC88 have spread in laboratory mice around the globe

We have previously reported that CC88 strains are the predominant

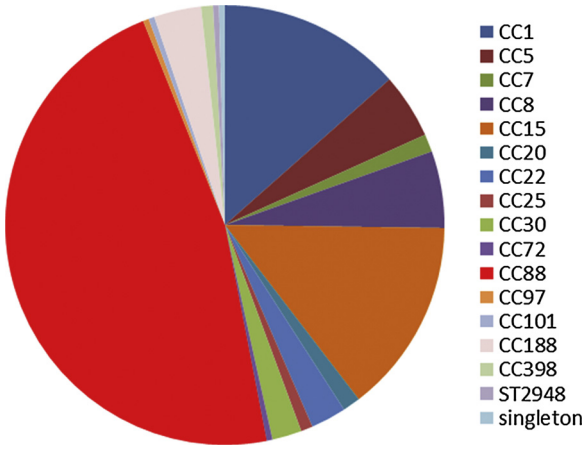


**Fig. 1.** CC88 is the predominant *S. aureus* lineage among laboratory mice. *Spa* genotyping was employed to resolve the population structure of murine *S. aureus* isolates. *Spa* types were clustered into MLST-derived CCs.

*S. aureus* lineage in laboratory mice derived from Charles River facilities in the US. To determine whether laboratory mice from different geographical locations and facilities are colonized with CC88 or rather facility-specific lineages, we *spa*-typed all murine *S. aureus* isolates. We detected a total of 58 different *spa* types that clustered into 15 clonal complexes (CCs) and a single sequence type (ST2948) (Fig. 1). One *spa* type singleton (t2176) could not be assigned to a lineage. Almost half of the murine isolates (47.0%) belonged to CC88, followed by CC15 (14.3%), CC1 (13.5%), CC8 (5.7%), CC5 (4.8%), and CC188 (3.5%) (Fig. 1, Tables 1 and S1). The earliest isolates were obtained from murine abscesses at the DKFZ in 1983 and belonged to CC20 (*spa* type t164) (Table 1 and S1), which was not detected again thereafter.

Fig. 2 illustrates the global spread of *S. aureus* lineages within animal breeding facilities of commercial vendors and research facilities. *S. aureus* isolates derived from Charles River customers were excluded from this analysis (n = 70). Overall, three *S. aureus* lineages had spread globally and were frequently isolated from laboratory mice: CC1, CC15, and CC88.

CC88 was by far the predominant *S. aureus* lineage in laboratory mice and has spread around the globe. CC88 isolates were found in mice from almost all tested vendors and university-associated breeding facilities (except in China) on three continents (Fig. 2, Table 1 and S1). This lineage is prevailing in C57BL/6 mice from two US Charles River facilities (Kingston, Raleigh). CC88 isolates were also detected at the Charles River facilities in Canada and France, as well as another (anonymous) US American vendor. Moreover, they were frequently detected in the university-associated animal breeding facilities, e.g. Greifswald, Heidelberg, Ulm, and Auckland. Since some mice bought from vendors were sampled straight after arrival at the research institute (DKFZ) or quarantine (Auckland), one can speculate that CC88 strains are also present in Jackson Laboratories, US, and Charles River facilities in Germany. This lineage is likely a long-standing companion of laboratory mice, as the population is genetically diverse (22 different *spa* types) and the oldest CC88 isolates were obtained from C57BL/6 mice as early as in 2004 (Charles River, US), and 2008 (DKFZ, Germany).

The *S. aureus* lineage CC1 has also spread around the globe, being present in vendor or university facilities from five countries on four continents (Fig. 2, Table 1 and S1). In detail, CC1 isolates were detected in the Hollister branch of Charles River, US, but not in the other North-American Charles River facilities. Moreover, CC1 strains were found in animals from Charles River France, from a Chinese pharmaceutical company (n = 3), as well as from all tested university-associated animal facilities.

CC15 strains were also wide-spread: We detected these strains in mice from two vendors (US vendor; Charles River, France), and two university-associated animal facilities (Greifswald; DKFZ) (Fig. 2). The lineages CC7, CC8, CC22, and CC188 were less frequently found in laboratory mice from breeding facilities (Fig. 2). CC188 isolates were only detected in the Hollister and St Constant branch of Charles River,

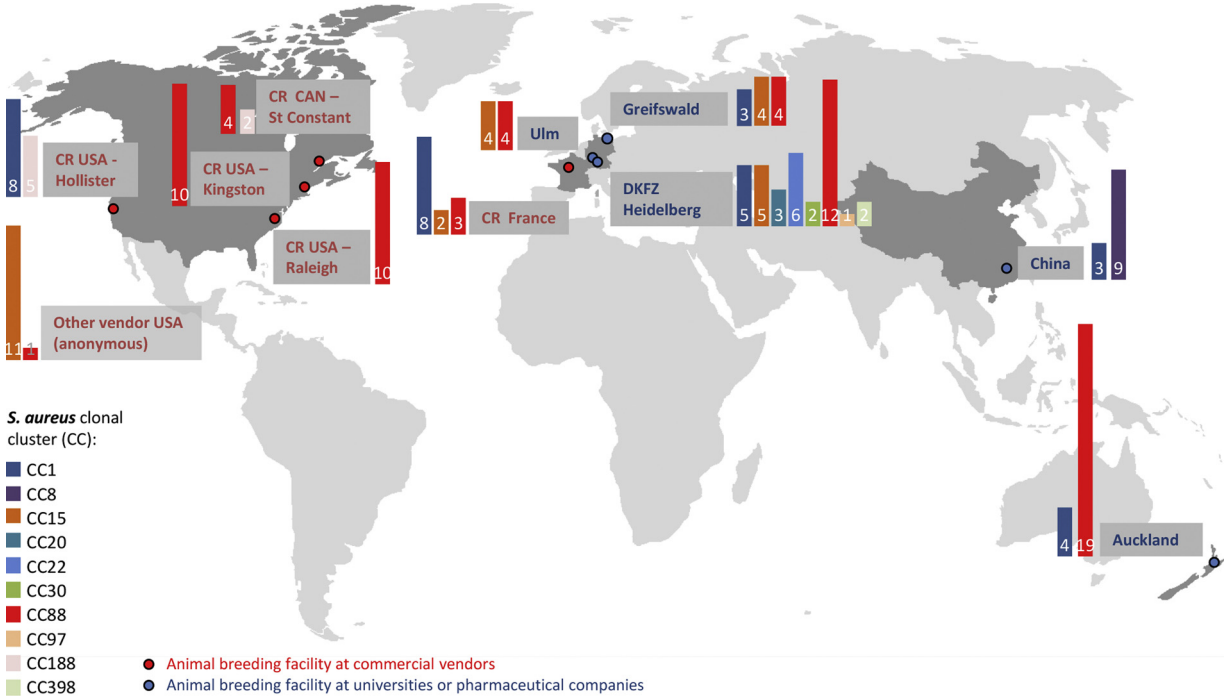


**Fig. 2.** Global spread of mouse-adapted *S. aureus* lineages CC1, CC15, and CC88 in mouse breeding facilities. The graph illustrates the occurrence of *S. aureus* lineages (CCs) at each vendor (shown in red; three branches of Charles River, USA; Charles River, Canada; Charles River, France, an anonymous US vendor), four research institutes (blue; University of Auckland, New Zealand; University Medicine of Greifswald, Germany; DKFZ, Germany; University of Ulm, Germany) and a pharmaceutical company (blue; China). The absolute number of isolates per (CC) is depicted in the bars.

**Table 2**
Prevalence of IEC-encoding Sa3int phages, MGE-encoded superantigen genes, and penicillin resistance in murine and matched human isolates.

| Genetic feature | Clonal cluster | % positive murine *S. aureus* isolates (absolute No.) | | % positive human *S. aureus* isolates (absolute No.) | | p value |
|---|---|---|---|---|---|---|
| MGE-encoded SAg genes | CC1 | 32.3% | (10/31) | 72.2% | (13/18) | $p < 0.01$ |
| | CC5 | 54.5% | (6/11) | 75.0% | (15/20) | n.s. |
| | CC8 | 38.4% | (5/13) | 90.0% | (18/20) | $p < 0.01$ |
| | CC15 | 0.0% | (0/33) | 0.0% | (0/20) | n.s. |
| | CC22 | 0.0% | (0/6) | 15.0% | (3/20) | n.s. |
| | CC88 | 1.9% | (2/108) | 45.8% | (11/24) | $p < 0.001$ |
| | CC188 | 75% | (6/8) | 0.0% | (0/5) | $p < 0.01$ |
| | all strains | 16.5% | (38/230) | 44.9% | (57/127) | $p < 0.001$ |
| IEC-encoding Sa3int phages[1] | CC1 | 48.4% | (15/31) | 55.5% | (10/18) | n.s. |
| | CC5 | 54.5% | (6/11) | 90.0% | (18/20) | $p < 0.05$ |
| | CC8 | 53.3% | (8/15) | 90.0% | (18/20) | $p < 0.05$ |
| | CC15[2] | 0.0% | (0/33) | 0.0% | (0/20) | n.s. |
| | CC22 | 0.0% | (0/6) | 90.0% | (18/20) | $p < 0.001$ |
| | CC88 | 13.0% | (14/108) | 100.0% | (24/24) | $p < 0.001$ |
| | CC188 | 100.0% | (8/8) | 20.0% | (4/5) | n.s. |
| | all strains | 28.3% | (65/230) | 72.4% | (92/127) | $p < 0.001$ |
| Penicillin resistance | CC1 | 28.6% | (8/28[3]) | 72.2% | (13/18) | $p < 0.01$ |
| | CC5 | 9.1% | (1/11) | 50.0% | (10/20) | $p < 0.05$ |
| | CC8 | 75.0% | (3/4[3]) | 75.0% | (15/20) | n.s. |
| | CC15 | 60.6% | (20/33[3]) | 80.0% | (17/20) | n.s. |
| | CC22 | 100.0% | (6/6) | 65.0% | (13/20) | n.s. |
| | CC88 | 0.0% | (0/107[3]) | 70.6% | (12/17) | $p < 0.001$ |
| | CC188 | 85.7% | (7/8) | 80.0% | (4/5) | n.s. |
| | all strains | 26.9% | (54/217[3]) | 70.0% | (84/120) | $p < 0.001$ |

Abbreviations: IEC, immune evasion cluster; MGE, mobile genetic element

[1] The immune evasion cluster (IEC) encodes staphylokinase, staphylococcal complement inhibitor, chemotaxis inhibitory protein of S. aureus, as well as staphylococcal enterotoxins A or P.

[2] All CC15 isolates carry immobilized remnants of the Sa3int phage with most of the phage genome including the integrase missing. The IEC locus, however, is partially intact.

[3] Penicillin resistance could not be determined for all isolates, because in some cases only DNA was available.

suggesting that this lineage was introduced at some point and has successfully spread within these facilities thereafter.

### 3.3. Murine S. aureus isolates show features of host adaptation

We have previously reported that murine *S. aureus* isolates from Charles River facilities in the US are adapted to their murine host, because in contrast to matched human strains they lacked MGE-encoded SAg genes, the *Sa3int* phages encoding the human specific IEC genes, and ampicillin resistance (Schulz et al., 2017). To test whether murine isolates from this study share these features, we compared their virulence gene profile and penicillin resistance with matched human colonizing isolates (Table 2).

#### 3.3.1. MGE-encoded SAg genes

MGE-encoded SAg genes were less frequent in murine CC1, CC8, CC88 and CC188 as compared to the matched human strains (Table 2). For example, almost all murine CC88 strains lacked the MGE-encoded SAg genes, whereas they were common in human CC88 isolates (1.9% vs. 45.8%; $p < 0.001$). If SAg genes were present in murine strains, the pattern was also often different from human strains. For example, murine CC1 isolates frequently carried *sec*, *sel* and *seq*, which are likely co-localized on a pathogenicity island. On the contrary, human CC1 strains (*spa* type t127) lacked the *sec/sel/seq*-encoding element, but often carried *seb* and/or *sek/seq,* which are located on a related pathogenicity island (Tables S1 and S2). Surprisingly, murine CC188 were often SAg gene-positive (6/8), while none of the lineage-matched human isolates encoded SAg genes. All SAg-positive murine CC188 strains carried the *Sa3int* phage-encoded SAg *sep,* belonged to the same *spa* type, and were derived from Charles River facilities in Hollister and Canada (Table S1). The murine *sep* sequence was identical to the sequence found in human *S. aureus* isolates (data not shown).

#### 3.3.2. IEC-encoding Sa3int phages

IEC-encoding phages are common in human isolates, but frequently lacking in animal-adapted strains, including the murine *S. aureus* isolates from Charles River, US (Holtfreter et al., 2013; Sung et al., 2008). In this study, the IEC-encoding *Sa3int* phages were less prevalent in murine CC5, CC8, CC22, and CC88 isolates than their human counterparts (Table 2). The most striking example is CC88: Only 13.0% of murine CC88 strains carried IEC-encoding *Sa3int* phages compared to 100% of the human CC88 isolates ($p < 0.001$). The lineage CC15 is a special case: These strains harbor remnants of *Sa3int* phages including the IEC genes *scn* and *chp* in the same location in the bacterial genome, while most of the phage genome including *sak* and *Sa3int* are missing (Schulz et al., 2017). Hence, *scn* and *chp* were present in all murine and human CC15 isolates.

#### 3.3.3. Penicillin resistance

Penicillin resistance was less frequent in murine CC1, CC5, and CC88 isolates than in their matched human counterparts (Table 2). For example, all murine CC88 isolates were sensitive to penicillin, while 70.6% of the human isolates were resistant to this antibiotic. Interestingly, two murine strains (both CC398) carried the *mecA* gene, but were phenotypically sensitive to cefoxitin (Table S1). All other murine strains were *mecA* and *mecC*-negative.

In summary, most *S. aureus* lineages have one or more genetic features that could be associated with adaptation to the murine host.

## 4. Discussion

### 4.1. The lineages CC1, CC15, and CC88 are common and widespread among laboratory mice

We detected a total of 15 lineages in our *S. aureus* strain collection from laboratory mice with CC88 being the predominant lineage, followed by CC15, CC1, CC8, CC5, and CC188. These findings are in very good agreement with a previous study from our group that focused on *S. aureus* isolates from Charles River, US (n = 99), and reported that CC88 accounts for 54.5% of murine isolates, followed by CC15, CC5,

CC188, and CC8 (Schulz et al., 2017).This is no surprise, because most of these Charles River isolates (n = 84) were included in the presented strain collection. However, there were also some differences. For example, the lineage CC1 was not detected in the previous study, but now found in mice from the Charles River facilities in Hollister and France as well as in two university-associated facilities.

Among the 15 observed lineages, only three lineages had spread globally: CC88, CC15, and CC1. All these lineages were very likely long-standing companions of laboratory mice, because they were genetically highly diverse, as indicated by a *spa* type diversification, and (except for CC15) showed features of host adaptation, i.e. loss of IEC-encoding *Sa3int* phages, MGE-encoded SAg genes and penicillin resistance. The lineage CC88, for example, has persisted in the Charles River facilities for at least 13 years. Interestingly, a single CC88 isolate (t2311) was recently detected in a field vole captured in a remote area in Central Germany (Mrochen et al.; IJMM this issue). Thus, it seems possible, that a CC88 sublineage has been persisting in small rodents for a long time and has been part of the endogenous flora of laboratory mice since their derivation from common house mice. Alternatively, this lineage might have been introduced into wild small rodents and laboratory mice independently by humans. However, this seems more unlikely because the CC88 lineage is very rare among the North-American and European human *S. aureus* populations (Gorwitz et al., 2008; Holtfreter et al., 2016). A direct transmission of CC88 strains from wild mice to laboratory mice is nigh impossible at vendor and university facilities, because pest control is strictly managed. Ongoing whole genome sequencing of CC88 isolates from laboratory mice, wild mice, and humans will clarify the evolutionary events.

The lineage CC15 was also a common colonizer of laboratory mice from different breeding facilities. This lineage is a well-known human lineage that has spread around the globe. Among *S. aureus* carriers frequencies vary between 10 and 19% (Holtfreter et al., 2016; Mehraj et al., 2014; Ruimy et al., 2009). CC15 isolates have only occasionally been reported from animals, including companion animals (Cuny et al., 2010), primates (Schaumburg et al., 2015), moose, rooks (Monecke et al., 2016), and common voles (Gomez et al., 2014). We did not detect any CC15 isolates in Muroidea and common shrews in Central Europe (Mrochen et al., IJMM, this issue). Hence, it seems likely that the CC15 sublineage in laboratory mice was introduced by contact persons. CC15 strains generally lack SAg genes and carry remnants of the *Sa3int* phage, so these genetic features were not helpful in identifying host adaptation features in this lineage. Whole genome sequencing will likely shed some light on adaptation mechanisms of murine CC15 isolates.

CC1 also represents a widespread and common lineage in laboratory mice. We detected CC1 isolates in vendor- and university-associated breeding facilities from five countries on four continents. Similar to CC15, CC1 is a common lineage among humans including both MSSA and community-acquired MRSA strains (Holtfreter et al., 2016; Monecke et al., 2011). CC1-MSSA and −MRSA are also frequently found in cattle (Alba et al., 2015), and occasionally in wild animals (Loncaric et al., 2013; Monecke et al., 2016).

### 4.2. There is almost no overlap between S. aureus populations in laboratory mice and wild rodents

Apart from studying the molecular epidemiology of *S. aureus* in laboratory mice, we also investigated the prevalence and molecular epidemiology of *S. aureus* in wild rodents and shrews in Central Europe (Mrochen et al., IJMM, this issue). Notably, there was almost no overlap in the population structure between laboratory mice (*Mus musculus*) and the screened wild Muroidea, which included *Mus musculus* (house mouse), *Apodemus flavicollis* (yellow-necked mouse), *Myodes glareolus* (bank vole), *Microtus agrestis* (field vole), *Microtus arvalis* (common vole), and the unrelated *Sorex araneus* (common shrew). Among wild rodents we most frequently observed CC49, followed by CC1956, ST890, ST3033, CC130, and a single CC88 isolate. As discussed above, CC88 might have been part of the endogenous flora of laboratory mice since their separation from common house mice.

Routes of transmission among laboratory mice differ strongly from those observed among wild mice, resulting in different *S. aureus* populations in both cohorts. Laboratory mice live in an almost sterile environment with their cage mates and caretakers being the only source for acquiring *S. aureus*. In contrast, wild mice actively explore a number of different environments and will therefore likely encounter various *S. aureus* strains throughout their lifetime. Depending on their habitat, these strains could be of human, livestock, or wild animal origin. Indeed, it has been reported that wild rats and mice living around pig barns are colonized with pig MRSA strains, while human CA-MRSA, such as USA300, was frequently isolated from Canadian urban rats, as well as mice living around an Afghanistan army camp (Himsworth et al., 2014; Pletinckx et al., 2013; Schlegel et al., 2012).

### 4.3. Apart from CC88, laboratory mice are colonized by S. aureus lineages that are common in humans

Apart from CC88, most other lineages that we detected in laboratory mice, e.g. CC1, CC15, CC22, and CC30 represent common human lineages that are only occasionally found in companion animals or wild animals (Cuny et al., 2010; Strommenger et al., 2006). The only exceptions are CC5 and CC8. CC5 is common in humans, but has recently spread into domestic poultry and wild fowl (Lowder et al., 2009; Monecke et al., 2016). CC8 has a broad host reservoir, including humans, horses, and wildlife (Holtfreter et al., 2016; Nowakiewicz et al., 2016; Walther et al., 2009). Therefore, we propose that the lineages CC1, CC5, CC8, CC15, CC22, and CC30 have been introduced into the mouse colonies by the staff at the animal facilities, and have thereafter adapted to their murine host and spread within the colony. Notably, the most common human lineages CC30 and CC45 were not at all or rarely detected in our murine strain collection, suggesting that these lineages have some fitness disadvantages in the murine host.

Nevertheless, human-to-mouse transmission is likely a rare event. We have previously screened staff and mice at a local university-associated animal facility for *S. aureus* colonization and observed no overlap in the genotypes (Schulz et al., 2017). Indeed, vendors and university-associated breeding facilities efficiently limit human-to-animal transmission by using isolators or other forms of containment housing (with *S. aureus* regarded as an unwanted pathogen), protected cage changing stations, performing regular microbiological screenings and adhering to a strict hygiene practice (Baker, 2003).

Our data suggest that mouse–mouse transmission represents the most effective route. We present evidence that each vendor facility harbors its own specific *S. aureus* genotype (Fig. 2). Moreover, we previously reported that parental mice pass their *S. aureus* strain on to the offspring with a 100% success rate. This very efficient vertical transmission allows the persistence of defined *S. aureus* clones within a barrier room for years. Moreover, the spread of *S. aureus* between cages can be facilitated by insufficient disinfection of cages, bottles etc. because *S. aureus* is able to survive on various non-living surfaces for several weeks.

We also observed some isolates from lineages that are typically referred to as livestock-associated, i.e. CC97 (n = 1) and CC398 (n = 2) (Fitzgerald and Holden, 2016; McCarthy et al., 2012; Monecke et al., 2016). All of them were isolated from mice at customer facilities, suggesting that they have been transferred into the facility by people with pets or close contact with livestock.

Overall, our data suggest that most murine *S. aureus* lineages in laboratory mice are probably the result of a small number of host jumps of *S. aureus* strains of human origin, followed by genetic adaptation to mice. Thus, human *S. aureus* strains may be evolutionary precursors of modern laboratory mouse- adapted strains. The evolutionary history of the predominant lineage C88 might be different though, since this

lineage is rare in humans but was occasionally detected in wild mice.

### 4.4. Most murine S. aureus lineages show features of host adaptation

A comparison of murine and matched human *S. aureus* isolates showed that murine strains are probably adapted to their murine host. These strains frequently lacked SAg-encoding MGEs (lineages CC1, 8, 88 and 188), the *Sa3int* phages encoding the human-specific IEC genes (CC5, 8, 22, and 88) and penicillin resistance (CC1, 5, and 88). SAgs have a 10–100-fold reduced activity on murine as compared to human T cells (Holtfreter and Broker, 2005). The elimination of SAg genes in murine *S. aureus* strains, wherever their genetic location permits this, reflects the expendability of these toxins in mice. Similarly, *Sa3int* phages encode immune evasion factors, which show no or negligible activity in the murine system (de Haas et al., 2004; Gladysheva et al., 2003; Holtfreter and Broker, 2005; Rooijakkers et al., 2005). These phages integrate into the *hlb* gene, which encodes the sphingomyelinase hemolysin beta (Hlb). This toxin mediates hemolysis of ruminant erythrocytes and is an important virulence factor in mouse infection models (Katayama et al., 2013). In conclusion, the *hlb*-encoded toxin provides a clear advantage in the murine host, whereas the IEC-encoded factors do not, resulting in a selection against *hlb*-integrating IEC-encoding phages in murine *S. aureus* isolates.

Finally, penicillin resistance, which is conferred by a ß-lactamase, was scarce among murine isolates. When facing bacterial infections in mouse colonies, vendors prefer to cull the animals over using antibiotics, thereby eliminating any selective pressure for acquiring or maintaining this resistance gene. The difference between murine and human isolates is even more striking, considering that most of the matched human strains were obtained from healthy human carriers and were thus probably exposed to an only moderate antibiotic pressure as compared to *S. aureus* isolates from hospitals. We have two possible explanations for this finding: Either the host jump to laboratory mice occurred before the acquisition and spread of penicillin resistance in the *S. aureus* population, or ß-lactamase-encoding plasmids or transposons were deleted following the host jump.

Notably, two murine CC398 isolates were phenotypically cefoxitin sensitive, even though the *mecA* gene could be amplified by PCR. Whole genome sequencing will reveal the structure of the *mecA*-encoding staphylococcal chromosomal cassette and will clarify whether a mutation led to a loss-of-function.

### 4.5. S. aureus colonization can impact vaccination and infection studies

The frequent colonization of laboratory mice with *S. aureus* is of concern, because it could significantly affect *S. aureus* infection and vaccination studies. Working with either *S. aureus*-free or consistently primed mice will improve the validity and reproducibility of murine infection models. There are two ways to ensure that researchers work with *S. aureus*-free mice: First, specific-and-opportunistic-pathogen-free (SOPF) mice are guaranteed *S. aureus*-free. However, SOPF mice are expensive and only available for a few commonly used mouse strains. Second, researchers can buy *S. aureus*-free SPF mice. Depending on the vendor, between 1 and 20% of SPF mice are colonized with *S. aureus*. Importantly, the colonization status is not reported for immunocompetent mice in Europe (Mahler Convenor et al., 2014; Schulz et al., 2017). Therefore, European researchers will have to approach their vendor, or screen their mice for colonization or seroconversion as reported in Holtfreter et al. to ensure that they work with *S. aureus*-free mice (Holtfreter et al., 2013). In contrast, US researchers can easily identify *S. aureus*-free barriers at their vendor of choice, because all US vendors publish *S. aureus* screening results in their health certificates.

Since all humans are immunologically primed against *S. aureus*, infection studies using primed mice might represent the most physiological situation. To our best knowledge, the impact of nasal *S. aureus* colonization on subsequent infection has not been addressed in animal models so far. However, several studies reported that prior exposure due to either sub-lethal intraperitoneal inoculations or mild skin infections can partially protect from a subsequent lethal challenge with the same strain (Murphy et al., 2014; Brown et al., 2015; Selle et al., 2016; Montgomery et al., 2014; van den Berg et al., 2015). We have previously reported that colonized SPF mice mount a systemic antibody response against a panel of *S. aureus* proteins, including numerous *S. aureus* vaccine candidates from past or current clinical trials (Schulz et al., 2017). Unrecognized colonization and hence priming may, therefore, be a significant confounder of murine infection and vaccination studies, especially if the proportion of immunized mice varies between experimental groups (Broker et al., 2014; Brown et al., 2015; Murphy et al., 2014; Stentzel et al., 2015).

### 4.6. Murine infection models could be improved by using mouse-adapted S. aureus strains

The murine *S. aureus* isolates characterized in this study could become valuable tools for future *S. aureus* infection and vaccination studies. Currently, researchers are using human-adapted strains to infect mice, resorting to unphysiologically high infection doses to compensate for poor adaptation. We propose that the use of mouse-adapted *S. aureus* strains in their natural host – the mouse – will provide a more physiological model for studying *S. aureus* host interaction and testing novel therapeutics. As a proof of principle, we have previously shown that the murine CC88 strain JSNZ shows a better fitness and virulence in mouse colonization and infection models, respectively (Holtfreter et al., 2013). We are currently testing representative *S. aureus* isolates from the predominant lineages found in laboratory mice (this study) and wild mice (Mrochen et al., IJMM, this issue) for their suitability in murine infection models. However, one has to keep in mind that mice and mouse-adapted strains are not suited to study the effect of human-specific virulence factors, such as PVL and SAgs (Loffler et al., 2010).

## 5. Summary

In summary, the *S. aureus* lineages CC1, CC15, and CC88 have spread within commercial and university-associated animal breeding facilities around the globe. While the first two lineages are likely of human origin and have adapted to their new murine host following host jump, the origin of CC88 isolates remains to be clarified. *S. aureus* colonization of laboratory mice is of prime importance for *S. aureus* basic research and vaccine development, because colonized mice are immunologically primed and hence will deal more effectively with an *S. aureus* challenge.

## Conflict of interests

K. Pritchett-Corning was previously employed at Charles River, USA. The company had no influence on the study design, experiments, data interpretation, and publication.

## Funding information

This work was supported by a Sir Charles Hercus Fellowship from the Health Research Council of New Zealand (SW, 09/099, http://www.hrc.govt.nz/), Deutsche Forschungsgemeinschaft (SH, PT, BB, GRK1870; DS, BB, CRC-TRR34, http://www.dfg.de/) and the Bundesministerium für Bildung und Forschung (BB, DM, InVAC, https://www.bmbf.de/). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

## Acknowledgement

We would like to thank Erika Friebe, and Fawaz Al Sholui for

technical support, Liu Jin for providing *S. aureus* strains, and Stefan Weiß for support in data visualization.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijmm.2017.11.006.

## References


Alba, P., Feltrin, F., Cordaro, G., Porrero, M.C., Kraushaar, B., Argudin, M.A., Nykasenoja, S., Monaco, M., Stegger, M., Aarestrup, F.M., Butaye, P., Franco, A., Battisti, A., 2015. Livestock-associated methicillin resistant and methicillin susceptible *Staphylococcus aureus* sequence type (CC)1 in european farmed animals: high genetic relatedness of isolates from italian cattle herds and humans. PLoS One 10, e0137143.

Baker, D.G., 2003. Natural Pathogens of Laboratory Mice. Their Effects on Research. American Society for Microbiology (ASM) Press, Washington.

Broker, B.M., Holtfreter, S., Bekeredjian-Ding, I., 2014. Immune control of *Staphylococcus aureus* – regulation and counter-regulation of the adaptive immune response. Int. J. Med. Microbiol. 304, 204–214.

Brown, A.F., Murphy, A.G., Lalor, S.J., Leech, J.M., O'Keeffe, K.M., Mac Aogain, M., O'Halloran, D.P., Lacey, K.A., Tavakol, M., Hearnden, C.H., Fitzgerald-Hughes, D., Humphreys, H., Fennell, J.P., van Wamel, W.J., Foster, T.J., Geoghegan, J.A., Lavelle, E.C., Rogers, T.R., McLoughlin, R.M., 2015. Memory Th1Cells are protective in invasive *Staphylococcus aureus* infection. PLoS Pathog. 11, e1005226.

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nubel, U., Ohlsen, K., Strommenger, B., Walther, B., Wieler, L., Witte, W., 2010. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. Int. J. Med. Microbiol. 300, 109–117.

Cuny, C., Wieler, L.H., Witte, W., 2015. Livestock-associated MRSA: the impact on humans. Antibiotics 4, 521–543.

de Haas, C.J.C., Veldkamp, K.E., Peschel, A., Weerkamp, F., Van Wamel, W.J.B., Heezius, E.C.J.M., Poppelier, M.J.J.G., Van Kessel, K.P.M., van Strijp, J.A.G., 2004. Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. J. Exp. Med. 199, 687–695.

DeLeo, F.R., Otto, M., Kreiswirth, B.N., Chambers, H.F., 2010. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 375, 1557–1568.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. 38, 1008–1015.

Fitzgerald, J.R., Holden, M.T., 2016. Genomics of natural populations of *Staphylococcus aureus*. Annu. Rev. Microbiol. 70, 459–478.

Fowler Jr., V.G., Proctor, R.A., 2014. Where does a *Staphylococcus aureus* vaccine stand? Clin. Microbiol. Infect. 20 (Suppl. 5), 66–75.

Gladysheva, I.P., Turner, R.B., Sazonova, I.Y., Liu, L., Reed, G.L., 2003. Coevolutionary patterns in plasminogen activation. Proc. Natl. Acad. Sci. U. S. A. 100, 9168–9172.

Goerke, C., Pantucek, R., Holtfreter, S., Schulte, B., Zink, M., Grumann, D., Broker, B.M., Doskar, J., Wolz, C., 2009. Diversity of prophages in dominant *Staphylococcus aureus* clonal lineages. J. Bacteriol. 191, 3462–3468.

Gomez, P., Gonzalez-Barrio, D., Benito, D., Garcia, J.T., Vinuela, J., Zarazaga, M., Ruiz-Fons, F., Torres, C., 2014. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the mecC gene in wild small mammals in Spain. J. Antimicrob. Chemother. 69, 2061–2064.

Gorwitz, R.J., Kruszon-Moran, D., McAllister, S.K., McQuillan, G., McDougal, L.K., Fosheim, G.E., Jensen, B.J., Killgore, G., Tenover, F.C., Kuehnert, M.J., 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001–2004. J. Infect. Dis. 197, 1226–1234.

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Turnwald, D., Vogel, U., 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J. Clin. Microbiol. 41, 5442–5448.

Himsworth, C.G., Miller, R.R., Montoya, V., Hoang, L., Romney, M.G., Al-Rawahi, G.N., Kerr, T., Jardine, C.M., Patrick, D.M., Tang, P., Weese, J.S., 2014. Carriage of methicillin-resistant *Staphylococcus aureus* by wild urban Norway rats (Rattus norvegicus). PLoS One 9, e87983.

Holtfreter, S., Broker, B.M., 2005. Staphylococcal superantigens: do they play a role in sepsis? Arch. Immunol. Ther. Exp. (Warsz) 53, 13–27.

Holtfreter, S., Bauer, K., Thomas, D., Feig, C., Lorenz, V., Roschack, K., Friebe, E., Selleng, K., Lovenich, S., Greve, T., Greinacher, A., Panzig, B., Engelmann, S., Lina, G., Broker, B.M., 2004. egc-Encoded superantigens from *Staphylococcus aureus* are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. Infect. Immun. 72, 4061–4071.

Holtfreter, S., Grumann, D., Schmudde, M., Nguyen, H.T., Eichler, P., Strommenger, B., Kopron, K., Kolata, J., Giedrys-Kalemba, S., Steinmetz, I., Witte, W., Broker, B.M., 2007. Clonal distribution of superantigen genes in clinical *Staphylococcus aureus* isolates. J. Clin. Microbiol. 45, 2669–2680.

Holtfreter, S., Radcliff, F.J., Grumann, D., Read, H., Johnson, S., Monecke, S., Ritchie, S., Clow, F., Goerke, C., Broker, B.M., Fraser, J.D., Wiles, S., 2013. Characterization of a mouse-adapted *Staphylococcus aureus* strain. PLoS One 8, e71142.

Holtfreter, S., Grumann, D., Balau, V., Barwich, A., Kolata, J., Goehler, A., Weiss, S., Holtfreter, B., Bauerfeind, S.S., Doring, P., Friebe, E., Haasler, N., Henselin, K., Kuhn, K., Nowotny, S., Radke, D., Schulz, K., Schulz, S.R., Trube, P., Vu, C.H., Walther, B., Westphal, S., Cuny, C., Witte, W., Volzke, H., Grabe, H.J., Kocher, T., Steinmetz, I., Broker, B.M., 2016. Molecular epidemiology of *Staphylococcus aureus* in the general population in northeast Germany: results of the study of health in pomerania (SHIP-TREND-0). J. Clin. Microbiol. 54, 2774–2785.

Katayama, Y., Baba, T., Sekine, M., Fukuda, M., Hiramatsu, K., 2013. Beta-hemolysin promotes skin colonization by *Staphylococcus aureus*. J. Bacteriol. 195, 1194–1203.

Loffler, B., Hussain, M., Grundmeier, M., Bruck, M., Holzinger, D., Varga, G., Roth, J., Kahl, B.C., Proctor, R.A., Peters, G., 2010. *Staphylococcus aureus* panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog. 6, e1000715.

Loncaric, I., Stalder, G.L., Mehinagic, K., Rosengarten, R., Hoelzl, F., Knauer, F., Walzer, C., 2013. Comparison of ESBL–and AmpC producing Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from migratory and resident population of rooks (Corvus frugilegus) in Austria. PLoS One 8, e84048.

Lowder, B.V., Guinane, C.M., Ben Zakour, N.L., Weinert, L.A., Conway-Morris, A., Cartwright, R.A., Simpson, A.J., Rambaut, A., Nubel, U., Fitzgerald, J.R., 2009. Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U. S. A. 106, 19545–19550.

Mahler Convenor, M., Berard, M., Feinstein, R., Gallagher, A., Illgen-Wilcke, B., Pritchett-Corning, K., Raspa, M., 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab. Anim.

Markham, N.P., Markham, J.G., 1966. Staphylococci in man and animals: distribution and characteristics of strains. J. Comp. Pathol. 76, 49–56.

McCarthy, A.J., Lindsay, J.A., Loeffler, A., 2012. Are all meticillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. Vet. Dermatol. 23, 267–275 (e253-264).

Mehraj, J., Akmatov, M.K., Strompl, J., Gatzemeier, A., Layer, F., Werner, G., Pieper, D.H., Medina, E., Witte, W., Pessler, F., Krause, G., 2014. Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* nasal carriage in a random sample of non-hospitalized adult population in northern Germany. PLoS One 9, e107937.

Monecke, S., Coombs, G., Shore, A.C., Coleman, D.C., Akpaka, P., Borg, M., Chow, H., Ip, M., Jatzwauk, L., Jonas, D., Kadlec, K., Kearns, A., Laurent, F., O'Brien, F.G., Pearson, J., Ruppelt, A., Schwarz, S., Scicluna, E., Slickers, P., Tan, H.L., Weber, S., Ehricht, R., 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One 6, e17936.

Monecke, S., Gavier-Widen, D., Hotzel, H., Peters, M., Guenther, S., Lazaris, A., Loncaric, I., Muller, E., Reissig, A., Ruppelt-Lorz, A., Shore, A.C., Walter, B., Coleman, D.C., Ehricht, R., 2016. Diversity of *Staphylococcus aureus* isolates in european wildlife. PLoS One 11, e0168433.

Montgomery, C.P., Daniels, M., Zhao, F., Alegre, M.L., Chong, A.S., Daum, R.S., 2014. Protective immunity against recurrent *Staphylococcus aureus* skin infection requires antibody and interleukin-17A. Infect Immun. 82 (May (5)), 2125–2134. http://dx.doi.org/10.1128/IAI.01491-14. Epub 2014 Mar 10.

Murphy, A.G., O'Keeffe, K.M., Lalor, S.J., Maher, B.M., Mills, K.H., McLoughlin, R.M., 2014. *Staphylococcus aureus* infection of mice expands a population of memory gammadelta T cells that are protective against subsequent infection. J. Immunol. 192, 3697–3708.

Nowakiewicz, A., Ziolkowska, G., Zieba, P., Gnat, S., Wojtanowicz-Markiewicz, K., Troscianczyk, A., 2016. Coagulase-positive Staphylococcus isolated from wildlife: identification, molecular characterization and evaluation of resistance profiles with focus on a methicillin-resistant strain. Comp. Immunol. Microbiol. Infect. Dis. 44, 21–28.

Pletinckx, L.J., Verhegghe, M., Crombe, F., Dewulf, J., De Bleecker, Y., Rasschaert, G., Butaye, P., Goddeeris, B.M., De Man, I., 2013. Evidence of possible methicillin-resistant *Staphylococcus aureus* ST398 spread between pigs and other animals and people residing on the same farm. Prev. Vet. Med. 109, 293–303.

Rooijakkers, S.H., Ruyken, M., Roos, A., Daha, M.R., Presanis, J.S., Sim, R.B., van Wamel, W.J., van Kessel, K.P., van Strijp, J.A., 2005. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat. Immunol. 6, 920–927.

Ruimy, R., Armand-Lefevre, L., Barbier, F., Ruppe, E., Cocojaru, R., Mesli, Y., Maiga, A., Benkalfat, M., Benchouk, S., Hassaine, H., Dufourcq, J.B., Nareth, C., Sarthou, J.L., Andremont, A., Feil, E.J., 2009. Comparisons between geographically diverse samples of carried *Staphylococcus aureus*. J. Bacteriol. 191, 5577–5583.

Schaumburg, F., Pauly, M., Anoh, E., Mossoun, A., Wiersma, L., Schubert, G., Flammen, A., Alabi, A.S., Muyembe-Tamfum, J.J., Grobusch, M.P., Karhemere, S., Akoua-Koffi, C., Couacy-Hymann, E., Kremsner, P.G., Mellmann, A., Becker, K., Leendertz, F.H., Peters, G., 2015. *Staphylococcus aureus* complex from animals and humans in three remote African regions. Clin. Microbiol. Infect. 21 (34), e341–348.

Schlegel, M., Baumann, K., Breithaupt, A., Binder, A., Schotte, U., Ruhl, S., Krohmann, C., Eßbauer, S., Frangoulidis, D., Kayßer, P., Meyer, H., Riehm, J., Faulde, M., Lewitzki, J., Sauer, S., Ulrich, R.G., Teifke, J.P., 2012. Spielen Nagetiere Als Überträger von Zoonoseerregern im Einsatzgebiet der Bundeswehr in Afganistan eine Rolle? (What about the Role of Rodents as Vectors for Zoonotic Pathogens in Mission Areas of the Bundeswehr?). Wehrmedizinische Monatsschrift 8.

Schulz, D., Grumann, D., Trübe, P., Pritchett-Corning, K., Johnson, S., Reppschläger, K., Gumz, J., Sundaramoorthy, N., Michalik, S., Berg, S., Brandt J. v. d. Fister, R., Monecke, S., Uy, B., Schmidt, F., Bröker, B.M., Wiles, S., Holtfreter, S., 2017. Laboratory mice are frequently colonized with *Staphylococcus aureus* and mount a systemic immune response–note of caution for in vivo infection experiments. Front. Cell. Infect. Microbiol. 7.

Selle, M., Hertlein, T., Oesterreich, B., Klemm, T., Kloppot, P., Müller, E., Ehricht, R., Stentzel, S., Bröker, B.M., Engelmann, S., Ohlsen, K., 2016. Global antibody response to *Staphylococcus aureus* live-cell vaccination. Sci. Rep. 6 (Apr (22)). http://dx.doi.org/10.1038/srep24754.

Stentzel, S., Sundaramoorthy, N., Michalik, S., Nordengrun, M., Schulz, S., Kolata, J., Kloppot, P., Engelmann, S., Steil, L., Hecker, M., Schmidt, F., Volker, U., Roghmann,

M.C., Broker, B.M., 2015. Specific serum IgG at diagnosis of *Staphylococcus aureus* bloodstream invasion is correlated with disease progression. J. Proteomics 128, 1–7.

Strommenger, B., Kehrenberg, C., Kettlitz, C., Cuny, C., Verspohl, J., Witte, W., Schwarz, S., 2006. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. J. Antimicrob. Chemother. 57, 461–465.

Sung, J.M., Lloyd, D.H., Lindsay, J.A., 2008. *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154, 1949–1959.

van den Berg, S., de Vogel, C.P., van Belkum, A., Bakker-Woudenberg, I.A., 2015. Mild *Staphylococcus aureus* skin infection improves the course of subsequent endogenous *S. aureus* Bacteremia in Mice. PLoS One. 10 (Jun (6)), e0129150. http://dx.doi.org/10.1371/journal.pone.0129150. eCollection 2015.

van Wamel, W.J., Rooijakkers, S.H., Ruyken, M., van Kessel, K.P., van Strijp, J.A., 2006. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J. Bacteriol. 188, 1310–1315.

Volzke, H., Alte, D., Schmidt, C.O., Radke, D., Lorbeer, R., Friedrich, N., Aumann, N., Lau, K., Piontek, M., Born, G., Havemann, C., Ittermann, T., Schipf, S., Haring, R., Baumeister, S.E., Wallaschofski, H., Nauck, M., Frick, S., Arnold, A., Junger, M., Mayerle, J., Kraft, M., Lerch, M.M., Dorr, M., Reffelmann, T., Empen, K., Felix, S.B., Obst, A., Koch, B., Glaser, S., Ewert, R., Fietze, I., Penzel, T., Doren, M., Rathmann, W., Haerting, J., Hannemann, M., Ropcke, J., Schminke, U., Jurgens, C., Tost, F., Rettig, R., Kors, J.A., Ungerer, S., Hegenscheid, K., Kuhn, J.P., Kuhn, J., Hosten, N., Puls, R., Henke, J., Gloger, O., Teumer, A., Homuth, G., Volker, U., Schwahn, C., Holtfreter, B., Polzer, I., Kohlmann, T., Grabe, H.J., Rosskopf, D., Kroemer, H.K., Kocher, T., Biffar, R., John, U., Hoffmann, W., 2011. Cohort profile: the study of health in Pomerania. Int. J. Epidemiol. 40, 294–307.

Walther, B., Monecke, S., Ruscher, C., Friedrich, A.W., Ehricht, R., Slickers, P., Soba, A., Wleklinski, C.G., Wieler, L.H., Lubke-Becker, A., 2009. Comparative molecular analysis substantiates zoonotic potential of equine methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 47, 704–710.

World Health Organization,, 2014. In: Press, W. (Ed.), Antimicrobial Resistance: Global Report on Surveillance. World Health Organization, Geneva, Switzerland.