Staphylococcus aureus in animals

Andreas F. Haag[1], J. Ross Fitzgerald[2] and José R. Penadés[1]

[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, G12 8TA, Glasgow, UK

[2]The Roslin Institute, University of Edinburgh, Edinburgh, UK

Introduction

The genus *Staphylococcus* currently comprises 81 species and subspecies (https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date/prokaryotic-nomenclature-up-to-date.html) and most members of the genus are mammalian commensals or opportunistic pathogens that colonize niches such as skin, nares and diverse mucosal membranes. Several species are of significant medical or veterinary importance. *Staphylococcus pseudintermedius* (1) is a leading cause of pyoderma in dogs and is considered to be a significant reservoir of antimicrobial resistance factors for the genus (2, 3). *S. pseudintermedius* is very similar to *S. intermedius* and can be distinguished from other coagulase-positive staphylococci by positive arginine dihydrolase and acid production from β-gentiobiose and D-mannitol (4), or using a multiplex-PCR approach targeting the nuclease gene *nuc* (5). *Staphylococcus saprophyticus* is the second leading cause of uncomplicated urinary tract infections (6). While *Staphylococcus epidermidis* is a normal component of the epidermal microbiota, it is a leading cause of biofilm contamination of medical devices (7). The most promiscuous and most significant human pathogenic staphylococcal species is *Staphylococcus aureus*, which is the causal agent of a variety of disease symptoms that can range from cosmetic to lethal manifestations. *S. aureus* is distinguished from most members of the genus by its abundant production of secreted coagulase, an enzyme which converts serum fibrinogen to fibrin and promotes clotting. However, the *Staphylococcus intermedius* group and some strains of *Staphylococcus lugdunensis* have coagulase activity (5, 8, 9).

Despite the prevalence of literature characterising staphylococcal pathogenesis in humans, *S. aureus* is a major cause of infection and disease in a plethora of animal hosts leading to a significant impact on public health and agriculture (10). Infections in animals are deleterious to animal health, and animals can act as a reservoir for staphylococcal transmission to

humans. While about 20-30% of the human population carry *S. aureus*, the prevalence of *S. aureus* varies from host species to species, and up to 90% of chicken, 42% of pigs, 29% in sheep and between 14 to 35% in cows and heifers are carriers (11, 12). The economic importance of various animal species strongly determines the abundance of available literature on the subject and as such it is not surprising that *S. aureus* colonisation and infection has only been superficially investigated in wild animals. Nevertheless, *S. aureus* has been isolated from a plethora of wild life sources such as red squirrels [exudative dermatitis; (13)], black bear [endocarditis; (14)], zebra [cutaneous granuloma; (15)], raccoon [Botriomycosis (16)], dolphin [pyogenic meningoencephalitis (17)], harbour seal [systemic infections (18)], black rhinoceros [skin lesion, sepsis (19)], boars [nasal carriage (20, 21)], Rhesus macaques [Nasal carriage van den Berg et al., 2011 (22)], great apes [nasal carriage and sepsis (23)], chaffinch [healthy carriage (24)], mallard [sepsis (25)], red deer, griffon vulture and Iberian ibex [carriage (21)].

Animal isolates of *S. aureus* have been reported to exhibit distinct phenotypic properties that vary depending on the host of origin and six biotypes have been described: human, β-haemolytic human, bovine, caprine, avian-abattoir and non-host specific. These biotypes have, by and large, withstood the application of sophisticated characterisation methods; isolates from different hosts, characterised by multilocus enzyme electrophoresis (MLEE), cluster together suggesting host specificity and a limited ability of strains to be transmitted from one host species to another (10). These observations were further corroborated by genotyping methods such as pulse field gel electrophoresis and strains belonging to specific biotypes grouped in the same or closely related pulsotype (26).

DNA sequence-based approaches such as Multi-Locus-Sequence Typing (MLST) (27) have been extensively used to analyse population structures. At present, more than 3300 different sequence types isolated from more than 4700 *S. aureus* samples have been collated within the MLST reference database (http://saureus.mlst.net/). The database contains isolates from a range of species with human strains predominating by a large margin. Nevertheless, MLST showed that some clonal complexes (CCs) are predominant in, and associated with, specific hosts. In particular, it was shown that animal-associated strains belonged to specific clonal lineages whereas human strains did not (28, 29). Today we know that 87% of *S. aureus* isolates from colonization and infections in humans represent 11 widely disseminated clonal

complexes: CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45, CC51, and CC121. Clonal complexes CC8, CC15, CC22, CC30, CC45, CC30, CC45, and the rarer clonal complexes CC80 and CC152 are primarily associated with isolates from humans (30). MLST-based phylogenetic analysis provided the first long-term picture of the evolution of both human and animal strains (31, 32) and indicated that *S. aureus* has coevolved with its human host over a long time and that it had acquired the ability to infect animals on multiple occasions via human-to-animal host jumps. These host jumps eventually lead to specific strain lineages spreading and adapting within new animal hosts (32). Animal to human host jumps have also been documented (33), but are less frequent. Additionally, a number of methicillin-resistant *S. aureus* (MRSA) strains with low host specificity attributed to CC130 and CC398 have emerged over the past decades.

The main clonal complexes associated with ruminants are CC97, CC133, CC522, and CC151, while clonal lineage ST385 is mainly represented by isolates from poultry (30, 34-38). Comparative analyses of the genomes of MSSA isolates attributed to ST5 from humans and poultry, and of MSSA/MRSA of CC398 revealed that the livestock subpopulations of these clonal complexes originated from ancestral populations in humans (39-41). In contrast, human-associated isolates of the ST97 lineage were clearly shown to have originated from ruminants (33). *S. aureus* colonisation or infection in companion animals are usually caused by human-related genotypes (42)], yet some colonisation factors can determine host specificity (38).

S. aureus has colonised diverse animal species following host-switching events and subsequent adaptation through acquisition and/or loss of mobile genetic elements as well as further host-specific mutations allowing it to expand into new host populations (Figure 1, Table 1). Close contact between animals and humans can facilitate host-switching events, and there is a significant body of evidence indicating that with the beginning of animal domestication in the Neolithic period (10,000-2,000 BC) as well as the increased industrialisation of livestock farming, have provided a platform for animal-to-human transmission of pathogens (43). While host jumps are generally accompanied by the acquisition or loss of larger MGEs, not all host jumps are associated with such large-scale events. Recently, Viana *et al.* showed that single amino acids substitutions in the *dlt*B gene were sufficient to confer infectivity of human ST121 isolates for rabbits (44). Overall, *S. aureus*

92 can readily cross species barrier and infect new hosts. This ability is largely associated with
93 the large proportion of MGEs within the *S. aureus* genome and its capacity to exchange these
94 through contact with its environment. The animal host can provide reservoirs for new
95 virulence traits and antibiotic resistances and the increased contact between humans and
96 animals through industrialised agriculture coupled with its globalisation necessitate tight
97 monitoring of pathogenic animal *S. aureus* to understand the development and spread of
98 staphylococcal lineages.

99 In this chapter, we will be giving an overview of *S. aureus* in animals, how this bacterial species
100 was, and is, being transferred to new host species and the key elements thought to be
101 involved in the adaptation to new ecological host niches. We will also highlight animal hosts
102 as a reservoir for the development and transfer of antimicrobial resistance determinants.

103

104 ***S. aureus* in ruminants**

105 *S. aureus*, next to *Escherichia coli* and several Streptococcal species such as *Streptococcus*
106 *uberis*, and *Streptococcus agalactiae* is a major cause of mastitis in dairy cows and incurs a
107 significant economic loss to the dairy industry. Mastitis in dairy cows results in reduced yields,
108 the need for veterinary intervention and the loss of milk that has to be discarded either due
109 to pathogen or antibiotic contamination. If treatment of the udder is unsuccessful, the animal
110 is often culled. *S. aureus* is associated with both clinical and more commonly sub-clinical
111 mastitis, both of which frequently result in persistent and recurrent infections with a low cure
112 rate after antibiotic therapy (45). Mastitis leads to the influx of leucocytes into the udder and
113 various thresholds for leukocyte numbers have been established for categorising good milk
114 quality. Taking cow milk as an example, milk with more than 200,000 leukocytes per millilitre
115 is considered to be infected, and, in the European Union (EU), when more than 400,000 cells
116 per millilitre are found, the milk is deemed unfit for human consumption. Apart from the
117 considerable economic losses incurred through *S. aureus* derived mastitis, mammary gland
118 infections pose a considerable public health problem. *S. aureus* can be shed from infected
119 glands and most staphylococcal isolates from dairy milk possess genes encoding enterotoxins.
120 Thus, contamination of bulk milk can lead to food poisoning from fermented raw milk
121 products (46, 47).

S. aureus can be found in healthy cows (carriers) on the teat skin, nasal cavity and rectum (11). However, the main reservoirs within a dairy herd are infected udders and teat skin. Infected animals can shed bacteria through their milk and transmission occurs primarily from udder to udder during milking via contact with contaminated milking machines, farmer's hands or contaminated bedding (48). Other environmental transmission routes are less frequent; although *S. aureus* can survive in the environment for some time, it requires animal colonisation to ensure its survival.

The majority of bovine infections worldwide is cause by a subset of specific, bovine-adapted *S. aureus* strains (28). The substantial genetic variation between different lineages (49, 50) suggests that there might be lineage-specific differences in the molecular mechanisms involved in *S. aureus* pathogenesis.

Animal microbiota provide a reservoir of antibiotic resistance genes that can be acquired from their ecological niches and selected for by the use of antibiotics in agriculture. The ability of some animal-adapted *S. aureus* strains to colonise and infect humans can give rise to the development of new epidemic clones with hitherto uncharacterised virulence capacity (32). This becomes particularly clear in strains of the CC97 lineage, which is one of the major clones associated with bovine mastitis (28). Moreover, an increased number of bovine-to-human transmissions have been reported in recent years (37, 51, 52). A closer analysis revealed that at least two CC97 subclades for human infection had emerged that originated in bovine-to-human host jumps and had thereafter spread through the human population (33). This provided further evidence that animals can provide a reservoir for the development of new *S. aureus* clones that can rapidly spread from animal to human and then through the population. Richardson *et al.* recently showed, using genomics based approaches, that cows are a major reservoir for re-infection of humans and multiple host-switching events, both human-to-cow and cow-to-human, have occurred over the past 3000 years (43).

Bovine *S. aureus* isolates of the CC8 lineage closely resemble human isolates and Resch *et al.* used this observation to further study the genetic basis of host adaptation (53). They compared a total of 14 CC8 isolates from cows with subclinical mastitis, nine CC8 isolates from colonised or infected human patients and nine isolates belonging to typical bovine lineages (CC389, CC71, CC151, CC504 and CC479). They observed that CC8 isolates segregated into a unique group that was separate from typical bovine CCs and that within this group isolates

segregated into three subgroups. The main segregating parameter was the content of MGEs within the individual strains and they showed that strains of the mixed human-bovine isolate clusters contained β-haemolysin converting prophages. Conversely, the bovine isolates were devoid of this phage and harboured an additional, new non-*mec* staphylococcal cassette chromosome containing an LPXTG-surface protein with similarity to proteins present in environmental bacteria, often found as milk contaminants (53).

Bar-Gal *et al.* compared pheno- and genotypic characteristics of bovine isolates from Israel, Germany, the USA and Italy using a Bayesian phylogenetic comparison of several key genes (*nuc*, *coa*, *luk*F and *clf*A), *spa* and *agr* typing, followed by CC assignment, and assessed the presence of a broad range of virulence factors and antimicrobial resistance genes (54). This analysis enabled them to cluster different isolates according to their host of origin. Sheep and goat isolates generally showed lower variability and fewer CCs compared to bovine isolates. Within the bovine clade, the authors described two subclades in which isolates matched strains found in Israel or abroad. Their data therefore corroborate other studies suggesting staphylococcal coevolution with its respective host, and might indicate the existence of multiple host jumps by bovine *S. aureus* strains that have occurred in diverse geographical locations (54). Overall, the authors found that 27 virulence associated factors showed a different prevalence in bovine compared to goat and sheep isolates. The authors noted a higher rate of strains carrying capsule type 8 in sheep and goat isolates compared to cow isolates, where both capsule types 5 and 8 were approximately equally distributed. Superantigen genes *ssl*07 and *ssl*08 were found in almost all bovine strains (>93%) but were only present in less than 44% of sheep and goat strains. Strikingly, all bovine strains carried the *hys*A2 gene encoding hyaluronate lyase, while only 48% of goat and sheep strains did. Cow strains showed a higher prevalence of leucocidins D and E, while leucocidins F-P83 and LukM appeared to be more prominent in goat and sheep strains (54). As noted above, infection of the mammary glands triggers the influx of large numbers of leucocytes that are deployed to fight off the infection. Leucocidins play an important role in bovine mastitis and can kill immune cells (leukocytes) thus protecting the pathogen (55-59). In agreement with this, the *luk*F*/luk*M genes are associated with the most prevalent CCs found in mastitic cattle (CC151, CC479, CC133, some CC97, most CC522) (60). Leucocidins show different specificity for immune cells (particularly phagocytic cells) of various hosts through recognition of

different host cell receptor alleles and this can be related to the formation of hybrids among the different LukF and LukS paralogs. Leucocidins LukMF' and LukPQ are mainly associated with zoonotic disease and found amongst animal-derived *S. aureus* strains (61). Several additional virulence factors located primarily on MGEs, such as superantigens (62) and ruminant-specific alleles of the von Willebrand-binding protein (vWbp) (63), have also been found to be strongly associated with bovine hosts (43)

S. aureus strains in ruminants appear to be undergoing a significant amount of DNA exchange leading to the emergence of hybrid clones. A recent study by Spoor *et al.* (64) showed that the CC71 lineage of livestock-associated (LA) *S. aureus* strains evolved from an ancestor belonging to the major bovine lineage CC97. The authors showed that multiple large-scale import and recombination events involving other *S. aureus* lineages occupying the same ruminant niche had occurred, and that these affected a 329 kb region surrounding the chromosomal origin of replication. These recombination events resulted in allele replacement and either loss or gain of genes influencing host-pathogen interactions. In particular, the CC71 lineage acquired factors involved in innate immune evasion and bovine extracellular matrix adherence. The ability to take up and integrate large DNA segments from environmental staphylococcal strains highlights the pathogen's capacity for rapid evolution and adaptation.

In small ruminants, *S. aureus* is major cause of mastitis and septicaemia, by infections that may have a thromboembolic origin (65). These infections can also be secondary to parasite infestation which allows *S. aureus* of the normal skin flora to enter the bloodstream (66) and in lambs can lead to fatal toxaemia or to chronic disease with organ dissemination and abscess formation. In goats, staphylococcal infection can be secondary to parapox virus infection, leading to chorioptic mange or contagious pustular dermatitis (67). Morel's disease in sheep and goats is caused by a subspecies of *S. aureus*, *S. aureus* subsp. *anaerobius*, primarily affecting young animals. The disease is manifested through the formation of abscesses in superficial lymph nodes usually located in the mandibular region. This disease is thought to be caused by a single bacterial clone worldwide (ST1465), which has undergone long-term adaptation and is restricted to small ruminants (68).

Methicillin-resistant *S. aureus* (MRSA) in animals was first isolated from the milk of dairy cows with mastitis in Belgium in the early 1970s (69) and has since then been isolated from cows around the globe (70-76). MRSA strains harbour an MGE known as SCCmec, containing the

mec gene, which codes for an additional penicillin binding protein that has low affinity for β-lactam antibiotics and therefore mediates resistance to nearly all compounds of this antibiotic class (besides ceftobiprole and ceftarolin). The mainly pig-related LA-MRSA CC398 has also been isolated from bovine udder infections (77), altogether in line with the elevated host promiscuity of this CC. Several cattle-associated MRSA lineages (ST130, ST425, and ST1943), that had previously been thought to be bovine-restricted, have been recently isolated from human disease or carriage in Europe (78). Moreover, a newly identified *mec* determinant, named *mec*C (also known as *mecA*$_{LGA251}$), which shares 70% homology with *mec*A, was identified among MRSA strains of CC130, CC705, and ST425 recovered from cattle and humans (79). The *mec*C allele is associated with a unique SCC*mec* element designated SCC*mec*XI and is thought to be present in about 1.4% of bovine *S. aureus* isolates in as many as 2.8% of herds.

A recent study investigated the molecular profile of *S. aureus* strains isolated form bovine mastitis in the Shanghai and Zhejiang areas of China (80). The study identified a total of 19 sequence types with the dominant STs being ST97, ST520, ST188, ST398, ST7 and ST9. The majority of isolates were found to be methicillin-sensitive (198/212) with ST97 being the most predominant lineage among MSSA strains and ST9-MRSA-SCC*mec*XII the most common MRSA clone. The study revealed that the molecular virulence profiles of different lineages differed significantly. The predominant lineage causing bovine mastitis in eastern China was the MSSA ST97, but there was some indication that toxigenic MRSA ST9 lineages were also present, and it was suggested that their spread and distribution should be monitored in the future. ST9-MRSA strains, containing the SCC*mec*XII cassette have also been identified in nasal swabs from live pigs in China (80-82) and this cassette has also been identified in isolates from humans in Taiwan (83). These strains were shown to have a specific MGE profile encoding vWbp on a SaPIbov4-like pathogenicity island (83). vWbp's are responsible for the activation of host pro-thrombin and the formation of fibrin strands, thereby promoting the development of infectious lesions. SaPI-borne vWbp are distinct from their genomic homologs and have been shown to be responsible for the coagulation of ruminant plasma (63) and may therefore have an important role in the animal host specificity of *S. aureus*. The vWbp variants encoded by the SaPIbov4-like pathogenicity island shared only between 67 and 93% protein sequence identity to the previously characterized SaPI-vWbp (63). Nevertheless,

246 they were able to coagulate bovine and caprine plasma. However, the ability of these vWbps
247 to coagulate human plasma was not assessed in the study (83).

248 In a study investigating the prevalence of MRSA strains in contaminated milk and dairy
249 products in southern Italy, 8.3% of all isolates (40/484) were methicillin resistant. Of these
250 MRSA strains, the most prevalent sequence types in this study were ST152 (67.5%) followed
251 by ST398 (25%), ST1 (5% and ST5 (2.5%) (84). 92.5% (37/40) and 5% of isolates harboured
252 SCC*mec* type V or Iva, respectively, while 2.5% of isolates (1/40) harboured a no-further
253 defined methicillin resistance determinant.

254 MRSA of CC130, which has recently gained attention, carries *mec*C instead of *mec*A and is
255 primarily associated with ruminants and wildlife that share the same habitats suggesting that
256 there might be mutual exchange of strains (85). The *mec*C gene is also found in the dairy
257 associated lineage ST425 causing mastitis in cows. Both CC130 and ST425 isolates have been
258 isolated from human infections (79, 86, 87).

259 **_S. aureus_ in rabbits**

260 Staphylococcal infection causes substantial economic losses in commercial cuniculture and
261 clinical signs of *S. aureus* infection are present in more than 60% of rabbitries (88, 89).
262 Infection of rabbits with *S. aureus* is associated with suppurative dermatitis, abscesses,
263 pododermatitis and mastitis (90-93), with chronic mastitis being the main reason for culling
264 diseased animals in rabbitries (88, 91). Most chronic staphylococcal infections in rabbits are
265 caused by the ST121 lineage; less common lineages, such as ST96, can also be involved (94,
266 95). Infection of mammary glands with ST121 strains resulted in elevated levels of
267 granulocytes and reduced numbers of B cells, T cells, $CD4^+$ T cells and $CD8^+$ T cells compared
268 to mammary glands infected with ST96 strains (96). The authors of the study suggested that
269 this observation might be explained by strain-specific difference in host interactions leading
270 to altered perception by the host's immune system. However, further studies will be required
271 to verify this hypothesis.

272 Among *S. aureus* strains isolated in rabbitries, two main strain types were initially classified,
273 according to their virulence, into high virulence strains with the capacity to rapidly spread
274 through entire flocks, and low virulence strains that cause more limited infections (97). In
275 accordance with this, high and low virulence strains can induce either severe or mild

symptoms, respectively, in a rabbit skin infection model, indicating the presence of either different virulence factors or differences in virulence factor expression levels (98). Interestingly, most low virulence strains could be grouped into poultry or human biotypes, whereas high virulence strains were members of a mixed biotype that produced β-haemolysin and showed no staphylokinase activity (99). Classical high-virulence strains belong to the biotype "mixed CV-C" and are sensitive to phages 3A/3C/55/71 of phage group II, suggesting a clonal origin of these high-virulence strains (100). Subsequent molecular typing studies found that the majority of high virulence strains belonged to ST121 and to a lesser extent ST425 with *agr* types 4 and 2, respectively (95).

Viana *et al.* analysed a total of 178 strains from chronic mastitis in rabbits that presented with a range of disease manifestations including abscesses, suppurative mastitis with a lobular pattern, cellulitis and mixed lesions. The majority of isolates belonged to the high virulence ST121 (166/178) with sequence types ST398, 96, 45, 1, DVL879 and SLV9 (7, 1, 1, 2, 1, respectively) comprising the rest. However, disease symptoms could not be correlated to any specific genotype or sequence type (94). Rabbit isolates are significantly different from those found in humans and ruminants suggesting the presence of host-specific factors selective for rabbit-specific sequence types (93). The phylogenetic origin of the ST121 lineage was eventually traced back to a human-to-rabbit host jump approximately 40 years ago (44). Comparative analysis of the accessory genomes of ST121 strains showed that the majority of human strains contained MGEs, which encode potent toxins involved in human disease pathogenesis such as Panton-Valentine leucocidin (PVL) and exfoliative toxins (ETs), and all except one contained a β-haemolysin-converting phage (Φ*Sa*3) encoding the human-specific immune evasion cluster (IEC). None of the rabbit strains contained PVL- or ET-encoding MGEs, indicating that these were dispensable for *S. aureus* infection of rabbits (44). Interestingly, the rabbit strains did not contain any MGEs that were unique to rabbit *S. aureus* indicating that the acquisition of rabbit-specific MGEs was not required to cause infection. Instead, the authors found that single non-synonymous mutations at the 5'-end of the *dlt*B gene were sufficient to confer rabbit infectivity in human ST121 strains. DltB is an integral membrane protein encoded by the *dlt*ABCD operon that is likely responsible for the translocation and incorporation of D-alanine into teichoic acids and lipoteichoic acids in *S. aureus* (101). However, Viana *et al.* (44) showed that neither the D-alanine nor the bacterial cell wall

composition was altered in in strains harbouring the rabbit-infective *dlt*B mutants. This would therefore suggest an additional function for DltB during rabbit infections and the authors propose that DltB, a member of the membrane-bound O-acetyltransferases (MBOAT) that transfer organic acids, typically fatty acids, to hydroxyl groups, has a role in signalling that could be responsible (44). Rabbit-associated *S. aureus* strains therefore appear to be representatives of infective strains that require little adaptation to jump between humans and rabbits and further studies will be required to determine how these relatively recent epidemic strains have evolved.

Rabbitries endeavour to prevent *S. aureus* infection by limiting the introduction of new animals and by reducing contact between rabbit flocks. Unfortunately, antibiotic treatments, disinfection of cages and environments, as well as vaccinations have so far proved inefficient in eliminating *S. aureus* infections in rabbitries (97). Consequently, culling of entire flocks followed by thorough disinfection of the cages is the only efficient strategy for dealing with *S. aureus* epidemics in cuniculture.

***S. aureus* in chickens and other poultry**

The growth of commercial poultry farming has provided a fertile field for staphylococcal infections and zoonotic transfer. (102). *S. aureus* is among the leading causes of bacterial infections in poultry (10) causing a wide range of diseases including septic arthritis, subdermal abscesses, gangrenous dermatitis and septicaemia (103). As with other hosts, staphylococcal strains associated with poultry cluster into specific clonal complexes that appear to have either evolved together with their avian host or adapted after zoonotic transmission. For example, CC385 has so far been identified only among avian hosts, whereas strains of CC5 and CC398 have been isolated from chickens, humans and other mammals (39, 104, 105). One of the predominant staphylococcal lineages causing disease in the poultry industry is CC5 (40, 103). Dissemination of CC5 into chicken involved a single transmission from human approximately 40 years ago (40) followed by a significant number of genetic recombination events leading to host adaptation. At least 44 recombination events in 33 genes have accumulated in poultry isolates and a further 47 genes were found to be more frequent in poultry compared to human isolates. Interestingly, many of these genes were common among chicken isolates from other clonal complexes indicating that horizontal transfer of these genes between CCs may have a potential role in host adaptation (102). On a phenotypic

level, these genetic alterations contribute to *S. aureus* adaptation to their poultry host and poultry isolates show enhanced growth at 42°C (the core body temperature of the adult chicken (106)) and greater erythrocyte lysis on chicken blood agar for chicken compared to human isolates (102). Conversely, most human isolates but only around half of the chicken isolates were able to lyse human erythrocytes (102). The improved growth of chicken isolates at 42°C is thought to be related to two poultry-associated genes (SAAV_0062 and SAAV_0064) that share more than 85% nucleotide identity with genes important for growth at elevated temperatures, including *dna*K and *dna*E (102).

Furthermore, the host jump from human to poultry was also accompanied by genetic changes such as the loss of several genes involved in human disease pathogenesis and the acquisition of avian-specific mobile genetic elements (40). For example, the poultry strain ED98 had acquired 2 prophages, 2 plasmids and a SaPI, and these MGEs are widely distributed among avian, but completely absent from human strains (40). A similar observation has been made with bovine-adapted strains (107).

Plasmids can confer virulence traits as well as antibiotic resistances (pT181, pT127, pC194, pC221, pC223 and pUB112) (108) and can contribute to the spread of disease. Such plasmids are present in *S. aureus* isolates causing a variety of difficult to treat chicken diseases (102, 103, 109). A recent study has focused on identifying bottlenecking and drift-related genetic changes, and on separating them from genetic changes conferring advantages in the poultry niche, and on showing adaptation over time to the avian host (102). By sampling a total of 191 isolates from diseased chickens from the UK, USA and Netherlands they confirmed that the major staphylococcal lineage in these infections was CC5 and that human and chicken isolates within CC5 clustered in distinct subgroups (102). They further identified an increased recombination frequency within the CC5 poultry relative to human isolate genomes and a tight clustering of chicken isolates once recombination events were compensated for. Changes in chicken-derived genomes localised within 33 genes and consisted of 196 substitution and 44 recombination sites. 47 genes were more frequently present in CC5 chicken compared to human isolates with 38 of these being shared among CC5 and CC1 and 41 genes shared between CC5 and CC398 poultry isolates, respectively. All 47 poultry-associated genes were present in strains of the CC385 lineage. Recombination regions in poultry isolates were associated with both the core genome and plasmids and many clustered

within three distinct genomic regions comprising genes with putative roles in heat shock response, haemolysis, adhesion, mobile elements and transposons. Furthermore, a total of 58 poultry-associated genes and genetic elements were predicted to be involved in the transfer of mobile genetic elements containing gene with predicated function as transposases, in conjugation as well as pathogenicity islands and two hotspot regions containing phage related elements (102).

Several genes that have so far been found only in poultry isolates are implicated in increased pathogenicity in chickens (41, 110). These include *scp*B, encoding a putative cysteine protease (Staphostatin A) (40, 111) which is found on an avian disease-associated plasmid (pAvX) in CC5 and CC385 strains (112). The CC385 lineage has been isolated from various wild and reared birds suggesting that it has had long-term avian host restriction (40, 43).

Multi-host CC398: A melting pot and reservoir for virulence and resistance development

MRSA strains of the CC398 complex have been studied in detail. This lineage is likely derived from a human MSSA clone that has successfully jumped into pigs where it acquired methicillin resistance and changes to its accessory genome (39). Despite these changes it has retained the ability to infect humans and it has been found in other animals suggesting that CC398 strains are more promiscuous infecting agents than other CCs (43). CC398 is the main lineage of LA-MRSA strains in Europe, whereas other lineages have been isolated frequently in other geographical areas (113-115). CC9 LA-MRSA isolates are predominantly isolated in Asia whereas CC398 and CC5 are relatively common in North America (116). Methicillin resistance is conferred by the acquisition of SCC*mec* elements that contain various *mec* genes. Presently, at least 13 different structural types of SCC*mec* are known (30, 79-83).

The proportion of *S. aureus* infections caused by MRSA has increased significantly from the end of the 1980s until 2000 worldwide (30). MRSA infections of humans could be initially grouped into either healthcare-associate (HA-) or community-associated (CA-) MRSA based on epidemiological criteria (117). HA-MRSA and CA-MRSA strains can be differentiated by their structural and functional genomic traits (118). However, these epidemiological criteria have become increasingly blurred as HA-MRSA have been found within the community and CA-MRSA strains were identified as the causative agents within the hospital setting (119, 120). In addition to these two categories of MRSA, animals can act as a reservoir for the

development and transmission of so-called livestock-associated (LA-) MRSA that have been found to cause infections within the human community. All three MRSA types differ in their genotype and associated genotypic traits from each other allowing, for now, a clear segregation into specific lineages associated with specific origins of the pathogen.

In pigs, *S. aureus* usually does not cause much disease; skin infections in pigs are typically caused by *Staphylococcus hyicus* and have only been occasionally documented to be caused by *S. aureus* (67, 121, 122). Consequently, *S. aureus* had not been monitored extensively in pigs. However, it has recently been realized that pigs represent a major reservoir for MRSA, after all.

CC398-MRSA and CC398-MSSA staphylococcal strains were first identified among pig farmers in France (122, 123). While CC398-MRSA strains rapidly spread among pigs and other livestock, they are considered to spread only infrequently beyond animals and personnel in direct contact with an infected animal (124-126). Most LA-CC398 strains are resistant to β-lactams, macrolides, lincosamides, streptogramines, tetracyclines, and in part to fluoroquinolones as well as to cotrimoxazole. They are susceptible to glycopeptides, daptomycin, tigecyclin, rifampicin, fusidic acid, fosfomycin, and with few exceptions also to linezolid (30). Initial studies suggested a possible human origin for LA-CC398 that was transferred to pigs and subsequently acquired methicillin resistance driven by the pressure of antibiotics in animal feeds (39). However, a more recent analysis indicated that both human MSSA and LA-CC398 emerged in parallel around 1970 (127)

The CC398 lineage is the most commonly detected MRSA lineage among European livestock and thus was given the name of livestock-associated MRSA (LA-MRSA) with *spa* types t011, t034 and t108 being the most prevalent among the LA-MRSA CC398 strains (128, 129). CC398 MRSA strains are non-typable by *Sma*I-pulsed-field gel electrophoresis (PFGE) (130), comprise only a small set of *spa*-types and harbour a novel *Sau1-hsdS1* type 1 restriction-modification system (131). LA-MRSA isolates typically carry SCC*mec* type IVa or V, which are different from those carried by other MRSA genotypes commonly found in community and healthcare settings (132). They often exhibit co-resistance to many non-β-lactam antimicrobials (e.g. macrolide (70%), trimethoprim (65%), gentamicin (14%), ciprofloxacin (8%), and trimethoprim-sulfamethoxazole (4%)), including those commonly used in animal production (133). The majority of CC398 LA-MRSA isolates do not produce toxins such as Panton–

Valentine leucocidin (PVL) or enterotoxins (134). Following the reduction in cost of next-generation sequencing approaches, further characterisation of *S. aureus* CC398 isolates has been possible through the increased availability of whole genome sequencing data for this CC allowing a more detailed insight into CC398's host adaptation (see below sections).

There is a frequent transmission of CC398 LA-MRSA between livestock and farmers (135-139) and, until recently, strains of this lineage were rarely found outside this group (125). However, a rising number of cases of MRSA CC398 has recently been observed in humans within the healthcare environment (140). These findings show a strong epidemiological link with livestock contact (124). The origin of LA-CC398-MRSA is believed to be a human MSSA strain harbouring the Φ*Sa*3 phage. This phage carries a so-called immune evasion cluster that encodes many human-specific immunomodulatory factors including the *sea*, *sep*, *scn*, *chp* and *sak* genes (encoding staphylococcal enterotoxin A and P, staphylococcal complement inhibitor, chemotaxis inhibitory protein and plasminogen activator staphylokinase, respectively) and integrates within the *hlb* gene (141). The *hlb* gene encodes a sphingomyelinase known as beta-toxin or β-haemolysin, which can lyse sheep erythrocytes. The factors encoded in the Φ*Sa*3 phage specifically interfere with the human immune response (142, 143) and about 90% of clinical human-derived isolates contain the Φ*Sa*3 phage within their genome (141). Given that the immunomodulatory factors encoded in Φ*Sa*3 specifically target human immune factors, it is not surprising that the Φ*Sa*3 phage is missing from the genomes of CC398 lineages adapted to livestock (30, 39). In general, porcine LA-MRSA CC398 lack the Φ*Sa*3 phage and are *mec*A positive while human-specific CC398 are *mec*A negative and Φ*Sa*3 positive (144, 145).

Studies indicate that the adaptation of CC398 to its host is connected to the loss and/or acquisition of mobile genetic elements, including Φ*Sa*3,since the major changes that were revealed in these studies occurred within the CC398 accessory genome (134, 146). In particular, a new staphylococcal pathogenicity island (SaPI-S0385) was identified in strain S0385 that appears to be a composite of the 5'-sequence of SaPIbov1 (up to and including the excisionase gene) and SaPI5 (packaging module) and contains a unique region at its 3'-end encoding two putative extracellular proteins with similarity to staphylococcal complement inhibitor (SCIN) and vWbp, respectively. Both proteins also have a conserved homologue in

460 the core genome of S0385 however, no studies have been performed to determine whether
461 these conferred advantages to *S. aureus* within the porcine host (146).

462 In parallel, animal-independent human colonisation and infection by CC398-MSSA strains has
463 occurred and spread worldwide with a particular high incidence rate in China where this clone
464 accounts for almost 20% of skin and soft tissue infections (147).

465 While CC398 has spread successfully among pigs in Europe, CC9 is the most commonly
466 isolated lineage in farmed pigs in South-East Asia (82). Strains belonging to this CC are
467 genetically distinct from strains of the CC398 lineage and their genome is consistent with an
468 independent zoonotic event leading to its emergence. CC9 MSSA strains colonise humans and
469 transmission between humans and pigs has been reported in the United Kingdom (122). The
470 characterised CC9 isolates were deficient in the type IV restriction modification system (RM)
471 which poses a major restriction barrier for the acquisition of foreign DNA. Loss of the type IV
472 RM system has been observed in *S. aureus* strains prone to acquire the *van*A gene from
473 enterococci (148). Furthermore, two novel transposon-like elements containing genes with a
474 high degree of similarity to genes from coagulase negative staphylococci or enterococci have
475 been identified in CC9 strains but so far have not been found in *S. aureus* strains belonging to
476 other lineages (82). Overall, these observations might suggest that the newly emerged LA-
477 CC9 strains could have an enhanced capacity for the uptake of foreign DNA. However,
478 experimental verification remains to be provided. In line with this observation, the analysed
479 CC9 strains contained SCC*mec* type XII cassettes with a class C2 *mec* and *ccr*C gene complex
480 (82). Such CC9 strains have also been isolated from cattle in China (81). The genes encoded
481 by the SCC*mec* type XII elements are similar to genes found in coagulase negative
482 staphylococci which could represent a potential source of this element. The CC9 MRSA strains
483 thus represent a significant threat to humans as well as livestock, owing to their apparent
484 ability to acquire novel genetic elements and to their propensity for interspecies transmission.

485 **MRSA in companion animals – cats, dogs, horses**

486 Generally, MRSA strains of companion animals differ from those in livestock and meat
487 production animals. *S. aureus* strains isolated from companion animals are mainly of human
488 origin and are passed between human owners and their animals (38, 149-151). Dogs and cats
489 are not typically colonised by *S. aureus* but rather form transient associations that can on

490 occasion lead to severe infections (38, 152). MRSA infections in companion animals are
491 predominantly skin and soft tissue infections; previous antibiotic treatments of human
492 owners, the number of antimicrobial courses, the number of hospitalisation days, implant
493 devices, surgical interventions and contact with humans who have been previously
494 hospitalised, account for major risk factors to these animals (153, 154). Overall, these risk
495 factors are similar to those defining HA-MRSA infections in humans (155).

496 *S. aureus* MRSA strains isolated from horses, and humans in close contact with horses differ
497 from those spread throughout the human population. A CA-MRSA clone (CC8) was isolated
498 from horses in Canada and was well-adapted to the animal host (156). In Europe, CC398 MRSA
499 strains have also been isolated from horses and horse-to-human transmission has been
500 shown (157, 158). MRSA was first reported in horses in 1999 during a 13-month outbreak in
501 a veterinary teaching hospital in Michigan. These were horses that had undergone surgical
502 procedures and were subsequently infected with MRSA that appeared to have originated
503 from colonised surgical staff (159). MRSA has since then been detected among horses in
504 Europe, America and Asia (160). Consistent with the risk factors, disease presentation in
505 horses mirrors that observed in humans in the clinic. Skin and soft tissue MRSA infections,
506 bacteraemia, septic arthritis, osteomyelitis, implant-related infections, metritis, omphalitis,
507 catheter-related infections and pneumonia have all been reported in horses (160). MRSA
508 infections in horses have been linked to strains carried by clinical personnel (CC1, CC254 and
509 CC398) and nasal colonisation of veterinarians, veterinary personnel, and students was also
510 observed indicating transmission to or from humans (161).

511 The main CC isolated from horses is CC8 and equine isolates are distinct from human strains
512 of the general population but not from strains isolated from close-contact personnel. A recent
513 study has shown that equine CC8 isolates had acquired a phage encoding a novel equine allele
514 of the staphylococcal inhibitor of complement (*scn*) as well as an equine-specific form of the
515 bi-component leucocidins, LukPQ, that exhibited equine-specific activity (43, 162, 163).
516 Acquisition of antibiotic resistance determinants influences the clustering of equine and pig
517 isolates, suggesting a role for the acquisition of resistance in host adaptation (43). Adaptation
518 to horses also involves the acquisition of SaPI-encoded paralogues of the von Willebrand-
519 binding factor able to coagulate equine plasma (63). Since phages are required for the

activation of SaPIs (164) it will be interesting to see whether the newly identified horse-specific phage is also able to activate and transfer this horse specific SaPI.

Monkeys in Sub-Saharan Africa

Studies on species to species transmission of *S. aureus* have largely focused on LA-transmission. Yet non-human primates are readily colonised by *S. aureus* in captivity and in the wild (165). In a recent study, Senghore and colleagues investigated the transmission of *S. aureus* from humans to green monkeys in The Gambia (166). The study revealed multiple anthroponotic transmissions of *S. aureus* from humans to green monkeys and the emergence of a monkey-associated clade of *S. aureus* approximately 2700 years ago. Development of this monkey-associated clade was accompanied by the loss of the Φ*Sa*3 phage carrying genes known to play important roles in human colonisation. More recent anthroponotic transmissions included well-characterised human lineages and are thought to be the result of human encroachment on monkey habitats. However, the authors did not observe any monkey to human transmission (166). Non-human primates (and bats) in sub-Saharan Africa are colonised by the related but distinct staphylococcal species *S. argentus* and *S. schweitzeri*. While *S. schweitzeri* was isolated from monkeys from all study sites no transmission of these strains to humans was observed. In contrast, human-associated *S. aureus* sequence types (ST1, ST6, ST15) were detected in domestic animals and nonhuman primates indicating a human-to-monkey transmission in the wild (165).

***Staphylococcus aureus* host switching and the role of mobile genetic elements**

A recent study by Richardson *et al.* used a population-genomic approach to better characterise how *S. aureus* adapts to multiple different hosts and causes colonisation and disease (43). The study found that humans act as a major hub for the pathogen for both ancient and recent host-switching events leading to the emergence of endemic livestock strains. Cows were shown to be the most frequent recipient of *S. aureus* host jumps but also appeared to be the main animal reservoir for reinfection of humans and the emergence of animal-derived human epidemic clones (33, 43). The study identified 14 host jumps from humans to cows (median number of host jumps per tree as distributions from all subsamples

and trees in the study) dating back as early as 2000 BC to as recently as 2012 AD. Cows were also shown to act as a source of *S. aureus* for small ruminants such as goats and sheep. A pan-genome-wide association analysis identified host-specific accessory gene pools specific for birds, pigs and horses, respectively. Accessory genomes from human, cow, sheep and goat strains also clustered in a host-specific manner but exhibited greater diversity in gene content. The authors suggested that these differences might have been caused either through a range of cryptic host niches occupied by the pathogen, or because the time elapsed since the host-switching event, had been too short to allow sufficient diversification to result in the clear separation of human and ruminant accessory genome clusters. Alternatively, specific gene sets or combinations of gene sets might confer a more generalist host tropism. However, it was noted that clustering in equine and pig isolates was influenced by the acquisition of host-specific antimicrobial resistance determinants. Host-switching events were shown to be correlated with the acquisition via horizontal gene transfer of host-niche-specific genetic elements that confer selective advantages to the pathogen for survival within the new host. The study identified a total number of 36 distinct MGEs (including predicted plasmids, transposons, *S. aureus* pathogenicity islands and prophages). For instance, the β-haemolysin-converting phage Φ*Sa*3, which encodes modulators of the human innate immune response, was primarily associated with human strains, whereas several pathogenicity islands contain ruminant-specific superantigens or von Willebrand factor-binding proteins (62, 63). Conversely, equine isolates were shown to contain a prophage, integrated in the lipase precursor gene (*geh*), encoding equine-specific alleles of the staphylococcal inhibitor of complement (*scn*) and a bi-component leucocidin LukPQ (Table 1) (162, 163). The study also identified numerus previously uncharacterised MGEs. A novel plasmid SCC*mec* element encoding resistance to heavy metal ions (a common pig-feed supplement) was linked to human-to-porcine host-switching events. Furthermore, *S. aureus* isolates from animals had acquired several gene clusters encoding bacteriocins that would enable them to compete with the resident bacterial flora. Interestingly, the MGEs in *S. aureus* pig isolates showed an increased guanine-cytosine content and reduced codon-adaptation index that indicated a distinct genealogical origin for these MGEs which may be related to pathogenicity islands identified in the pig-associated zoonotic pathogen *Streptococcus suis* (43). Host-switching events are therefore accompanied by the rapid acquisition of MGEs that confer the capacity for survival within a new host niche, mainly by targeting the host's innate immune response.

582 Acquisition of resistance to antimicrobials and heavy metals allow the pathogen to survive
583 under high selective pressures; subsequent positive selection via point mutations or
584 recombination (102) acts on the core genome to modify metabolic pathways and to further
585 adapt *S. aureus* to its new host.

586 *S. aureus* host adaptation was found to coincide, depending on the host, both with gain and
587 loss of gene function. While avian strains contained a higher proportion of functional genes
588 compared to strains from other host species, ruminant strains showed an increase in
589 pseudogenes. Many of these pseudogenes in ruminants were found to be associated with
590 nutrient transport, including carbohydrates, and could indicate metabolic remodelling in
591 response to distinct nutrient availability. *S. aureus* was shown to further adapt to its host
592 niche in response to the availability of distinct nutrients. The authors showed that strains
593 isolated from dairy cattle exhibited an enhanced ability to utilise lactose as carbon source
594 supporting the concept that *S. aureus* undergoes genetic diversification in response to the
595 nutrients that differ in availability in different niches (43).

596 The study also revealed that staphylococcal antibiotic and heavy metal resistance genes are
597 unevenly distributed among isolates from different animal hosts and showed a clear
598 correlation to antibiotic usage practices within medicine and agriculture. For example,
599 human, pig and ruminant isolates harboured a collection of key resistance determinants that
600 were absent in avian isolates, in line with antimicrobial usage practises (43).

601 The acquisition of specific mobile genetic elements and core genome mutations plays a crucial
602 role in *S. aureus* host adaptation (40, 44, 63). For instance, the presence/acquisition of mobile
603 genetic elements not found in human strains could be clearly associated with host jumps from
604 humans to avian and porcine hosts (39, 40, 43). Host-specific functional effectors of *S. aureus*
605 pathogenicity such as leucocidins, superantigens and von Willebrand factor-binding proteins
606 are frequently located on MGEs (61, 162, 163, 167-170).

607 **Conclusions**

608 In the last decades an increasing number of studies have demonstrated that *S. aureus* is able
609 to colonise and infect a plethora of different eukaryotic hosts. While *S. aureus* can cause
610 severe infections in some animals, others show less severe symptoms and are mainly
611 colonised, acting as a staphylococcal reservoir for human reinfection. This is particularly true

for *S. aureus* lineages found in pigs and dairy cows. Due to the use of specific antibiotics and growth enhancing supplements, these strains have necessarily acquired mechanisms of resistance to these agents from various environmental sources. Current farming practices make farm animals ideal breading grounds for the development and/or acquisition of new resistance mechanism that can then spread into the community and pose a significant risk to the human population. There is an ever-increasing amount of data detailing host-switching events for *S. aureus,* with humans acting as the major exchange hub for strain lineages. These data highlight the ability of *S. aureus* to function as a multi-host pathogen and to evolve and adapt to new hosts. The ability of *S. aureus* to readily adapt to new environments and rapidly take up new genetic material via horizontal gene transfer makes the bacterium a most versatile coloniser, able to spread into new niches. Moreover, it can also rapidly adapt to new stresses and antibiotics with the result of an ever-continuing arms race between *S. aureus* and humankind.

References

1. Devriese LA, Vancanneyt M, Baele M, Vaneechoutte M, De Graef E, Snauwaert C, Cleenwerck I, Dawyndt P, Swings J, Decostere A, Haesebrouck F. 2005. *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int J Syst Evol Microbiol 55:1569-73.
2. Guardabassi L, Loeber ME, Jacobson A. 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Veterinary Microbiology 98:23-27.
3. Guardabassi L, Schwarz S, Lloyd DH. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. Journal of Antimicrobial Chemotherapy 54:321-332.
4. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. 2007. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J Clin Microbiol 45:2770-8.
5. Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K. 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. Journal of Clinical Microbiology 48:765-769.
6. Hovelius B, Mardh PA. 1984. *Staphylococcus saprophyticu*s as a common cause of urinary-tract infections. Reviews of Infectious Diseases 6:328-337.
7. Fey PD, Olson ME. 2010. Current concepts in biofilm formation of *Staphylococcus epidermidis*. Future Microbiology 5:917-933.
8. Mateo M, Maestre JR, Aguilar L, Cafini F, Puente P, Sanchez P, Alou L, Gimenez MJ, Prieto J. 2005. Genotypic versus phenotypic characterization, with respect to susceptibility and identification, of 17 clinical isolates of *Staphylococcus lugdunensis*. Journal of Antimicrobial Chemotherapy 56:287-291.
9. Riegel P, Jesel-Morel L, Laventie B, Boisset S, Vandenesch F, Prevost G. 2011. Coagulase-positive *Staphylococcus pseudintermedius* from animals causing human endocarditis. International Journal of Medical Microbiology 301:237-239.
10. Peton V, Le Loir Y. 2014. *Staphylococcus aureus* in veterinary medicine. Infect Genet Evol 21:602-15.
11. Roberson JR, Fox LK, Hancock DD, Gay JM, Besser TE. 1994. Ecology of *Staphylococcus aureus* Isolated from Various Sites on Dairy Farms1. Journal of Dairy Science 77:3354-3364.
12. Nagase N, Sasaki A, Yamashita K, Shimizu A, Wakita Y, Kitai S, Kawano J. 2002. Isolation and Species Distribution of Staphylococci from Animal and Human Skin. Journal of Veterinary Medical Science 64:245-250.
13. Simpson VR, Davison NJ, Kearns AM, Pichon B, Hudson LO, Koylass M, Blackett T, Butler H, Rasigade JP, Whatmore AM. 2013. Association of a *luk*M-positive clone of *Staphylococcus aureus* with fatal exudative dermatitis in red squirrels (*Sciurus vulgaris*). Veterinary Microbiology 162:987-991.
14. McBurney S, Veitch AM, Daoust P-Y. 2000. Bacterial Valvular Endocarditis in a Black Bear from Labrador. Journal of Wildlife Diseases 36:788-791.
15. Pandey GS, Nomura Y, Kobayashi K, Fujise H, Yamada T. 1998. Cutaneous Staphylococcal Granuloma in a Free Living Zebra (*Equus burchelli*) in Zambia. Journal of Veterinary Medical Science 60:137-138.
16. Hamir AN. 2010. Systemic *Staphylococcus aureus* Infection in a Free-ranging Raccoon (*Procyon lotor*). Journal of Wildlife Diseases 46:665-668.
17. Colgrove GS, Migaki G. 1976. Cerebral abscess associated with stranding in a dolphin. J Wildl Dis 12:271-4.
18. Van Pelt RW, Dietrich RA. 1973. Staphylococcal infection and toxoplasmosis in a young harbor seal. Journal of wildlife diseases 9:258-261.
19. Clausen B, Ashford WA. 1980. Bacteriologic survey of black rhinoceros (*Diceros bicornis*). J Wildl Dis 16:475-80.

20. Meemken D, Blaha T, Hotzel H, Strommenger B, Klein G, Ehricht R, Monecke S, Kehrenberg C. 2013. Genotypic and phenotypic characterization of *Staphylococcus aureus* isolates from wild boars. Appl Environ Microbiol 79:1739-42.
21. Porrero MC, Mentaberre G, Sanchez S, Fernandez-Llario P, Casas-Diaz E, Mateos A, Vidal D, Lavin S, Fernandez-Garayzabal JF, Dominguez L. 2014. Carriage of *Staphylococcus aureus* by free-living wild animals in Spain. Appl Environ Microbiol 80:4865-70.
22. van den Berg S, van Wamel WJ, Snijders SV, Ouwerling B, de Vogel CP, Boelens HA, Willems RJ, Huijsdens XW, Verreck FA, Kondova I, Heidt PJ, Verbrugh HA, van Belkum A. 2011. Rhesus macaques (*Macaca mulatta*) are natural hosts of specific *Staphylococcus aureus* lineages. PLoS One 6:e26170.
23. Nagel M, Dischinger J, Turck M, Verrier D, Oedenkoven M, Ngoubangoye B, Le Flohic G, Drexler JF, Bierbaum G, Gonzalez JP. 2013. Human-associated *Staphylococcus aureus* strains within great ape populations in Central Africa (Gabon). Clin Microbiol Infect 19:1072-7.
24. Paterson GK, Larsen AR, Robb A, Edwards GE, Pennycott TW, Foster G, Mot D, Hermans K, Baert K, Peacock SJ, Parkhill J, Zadoks RN, Holmes MA. 2012. The newly described *mecA* homologue, *mecA*$_{LGA251}$, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. J Antimicrob Chemother 67:2809-13.
25. Wobeser G, Kost W. 1992. Starvation, staphylococcosis, and vitamin A deficiency among mallards overwintering in Saskatchewan. J Wildl Dis 28:215-22.
26. Hennekinne JA, Kerouanton A, Brisabois A, De Buyser ML. 2003. Discrimination of *Staphylococcus aureus* biotypes by pulsed-field gel electrophoresis of DNA macro-restriction fragments. J Appl Microbiol 94:321-9.
27. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38:1008-15.
28. Smith EM, Green LE, Medley GF, Bird HE, Fox LK, Schukken YH, Kruze JV, Bradley AJ, Zadoks RN, Dowson CG. 2005. Multilocus sequence typing of intercontinental bovine *Staphylococcus aureus* isolates. J Clin Microbiol 43:4737-43.
29. Smyth DS, Feil EJ, Meaney WJ, Hartigan PJ, Tollersrud T, Fitzgerald JR, Enright MC, Smyth CJ. 2009. Molecular genetic typing reveals further insights into the diversity of animal-associated *Staphylococcus aureus*. J Med Microbiol 58:1343-53.
30. Cuny C, Wieler LH, Witte W. 2015. Livestock-Associated MRSA: The Impact on Humans. Antibiotics (Basel) 4:521-43.
31. Shepheard MA, Fleming VM, Connor TR, Corander J, Feil EJ, Fraser C, Hanage WP. 2013. Historical zoonoses and other changes in host tropism of *Staphylococcus aureus,* identified by phylogenetic analysis of a population dataset. PLoS One 8:e62369.
32. Weinert LA, Welch JJ, Suchard MA, Lemey P, Rambaut A, Fitzgerald JR. 2012. Molecular dating of human-to-bovid host jumps by *Staphylococcus aureus* reveals an association with the spread of domestication. Biol Lett 8:829-32.
33. Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, Aarestrup FM, Kearns AM, Larsen AR, Skov RL, Fitzgerald JR. 2013. Livestock origin for a human pandemic clone of community-associated methicillin-resistant *Staphylococcus aureus*. MBio 4.
34. Kock R, Friedrich A, On Behalf Of The Original Author Group C. 2014. Systematic literature analysis and review of targeted preventive measures to limit healthcare-associated infections by meticillin-resistant *Staphylococcus aureus*. Euro Surveill 19.
35. Cuny C, Friedrich A, Kozytska S, Layer F, Nübel U, Ohlsen K, Strommenger B, Walther B, Wieler L, Witte W. 2010. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. International Journal of Medical Microbiology 300:109-117.
36. Fitzgerald JR. 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends in Microbiology 20:192-198.

37. Espinosa-Gongora C, Moodley A, Lipinska U, Broens EM, Hermans K, Butaye P, Devriese LA, Haesebrouck F, Guardabassi L. 2014. Phenotypes and genotypes of old and contemporary porcine strains indicate a temporal change in the *S. aureus* population structure in pigs. PLoS One 9:e101988.
38. Pantosti A. 2012. Methicillin-Resistant *Staphylococcus aureus* Associated with Animals and Its Relevance to Human Health. Front Microbiol 3:127.
39. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM. 2012. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio 3.
40. Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nubel U, Fitzgerald JR. 2009. Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci U S A 106:19545-50.
41. Lowder BV, Fitzgerald JR. 2010. Human origin for avian pathogenic *Staphylococcus aureus*. Virulence 1:283-4.
42. Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou Elnaga Y, Stamm I, Kopp PA, Kohn B, Witte W, Jansen A, Conraths FJ, Semmler T, Eckmanns T, Lubke-Becker A. 2012. Sharing more than friendship--nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS One 7:e35197.
43. Richardson EJ, Bacigalupe R, Harrison EM, Weinert LA, Lycett S, Vrieling M, Robb K, Hoskisson PA, Holden MTG, Feil EJ, Paterson GK, Tong SYC, Shittu A, van Wamel W, Aanensen DM, Parkhill J, Peacock SJ, Corander J, Holmes M, Fitzgerald JR. 2018. Gene exchange drives the ecological success of a multi-host bacterial pathogen. Nature Ecology & Evolution doi:10.1038/s41559-018-0617-0.
44. Viana D, Comos M, McAdam PR, Ward MJ, Selva L, Guinane CM, Gonzalez-Munoz BM, Tristan A, Foster SJ, Fitzgerald JR, Penades JR. 2015. A single natural nucleotide mutation alters bacterial pathogen host tropism. Nat Genet 47:361-366.
45. Schukken YH, Gunther J, Fitzpatrick J, Fontaine MC, Goetze L, Holst O, Leigh J, Petzl W, Schuberth HJ, Sipka A, Smith DG, Quesnell R, Watts J, Yancey R, Zerbe H, Gurjar A, Zadoks RN, Seyfert HM, members of the Pfizer mastitis research c. 2011. Host-response patterns of intramammary infections in dairy cows. Vet Immunol Immunopathol 144:270-89.
46. Le Maréchal C, Thiéry R, Vautor E, Le Loir Y. 2011. Mastitis impact on technological properties of milk and quality of milk products—a review. Dairy Science & Technology 91:247-282.
47. Le Loir Y, Baron F, Gautier M. 2003. *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research 2:63-76.
48. Sakwinska O, Giddey M, Moreillon M, Morisset D, Waldvogel A, Moreillon P. 2011. *Staphylococcus aureus* host range and human-bovine host shift. Appl Environ Microbiol 77:5908-15.
49. Budd KE, Mitchell J, Keane OM. 2016. Lineage associated expression of virulence traits in bovine-adapted *Staphylococcus aureus*. Vet Microbiol 189:24-31.
50. McCarthy AJ, Lindsay JA. 2010. Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173.
51. Menegotto F, Gonzalez-Cabrero S, Lorenzo B, Cubero A, Cuervo W, Gutierrez MP, Simarro M, Orduna A, Bratos MA. 2012. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Spanish hospital over a 4-year period: clonal replacement, decreased antimicrobial resistance, and identification of community-acquired and livestock-associated clones. Diagn Microbiol Infect Dis 74:332-7.

52. Udo EE, Aly NY, Sarkhoo E, Al-Sawan R, Al-Asar AS. 2011. Detection and characterization of an ST97-SCC*mec*-V community-associated meticillin-resistant *Staphylococcus aureus* clone in a neonatal intensive care unit and special care baby unit. J Med Microbiol 60:600-4.
53. Resch G, Francois P, Morisset D, Stojanov M, Bonetti EJ, Schrenzel J, Sakwinska O, Moreillon P. 2013. Human-to-bovine jump of *Staphylococcus aureus* CC8 is associated with the loss of a beta-hemolysin converting prophage and the acquisition of a new staphylococcal cassette chromosome. PLoS One 8:e58187.
54. Bar-Gal GK, Blum SE, Hadas L, Ehricht R, Monecke S, Leitner G. 2015. Host-specificity of *Staphylococcus aureus* causing intramammary infections in dairy animals assessed by genotyping and virulence genes. Veterinary Microbiology 176:143-154.
55. Fournier C, Kuhnert P, Frey J, Miserez R, Kirchhofer M, Kaufmann T, Steiner A, Graber HU. 2008. Bovine *Staphylococcus aureus*: association of virulence genes, genotypes and clinical outcome. Res Vet Sci 85:439-48.
56. Piccinini R, Borromeo V, Zecconi A. 2010. Relationship between *S. aureus* gene pattern and dairy herd mastitis prevalence. Vet Microbiol 145:100-5.
57. Piccinini R, Tassi R, Dapra V, Pilla R, Fenner J, Carter B, Anjum MF. 2012. Study of *Staphylococcus aureus* collected at slaughter from dairy cows with chronic mastitis. J Dairy Res 79:249-55.
58. Younis A, Krifucks O, Fleminger G, Heller ED, Gollop N, Saran A, Leitner G. 2005. *Staphylococcus aureus* leucocidin, a virulence factor in bovine mastitis. J Dairy Res 72:188-94.
59. Alonzo F, 3rd, Torres VJ. 2014. The bicomponent pore-forming leucocidins of *Staphylococcus aureus*. Microbiol Mol Biol Rev 78:199-230.
60. Schlotter K, Ehricht R, Hotzel H, Monecke S, Pfeffer M, Donat K. 2012. Leukocidin genes lukF-P83 and lukM are associated with *Staphylococcus aureus* clonal complexes 151, 479 and 133 isolated from bovine udder infections in Thuringia, Germany. Vet Res 43:42.
61. Spaan AN, van Strijp JAG, Torres VJ. 2017. Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. Nature Reviews Microbiology 15:435.
62. Deringer JR, Ely RJ, Monday SR, Stauffacher CV, Bohach GA. 1997. Vβ-dependent stimulation of bovine and human T cells by host-specific staphylococcal enterotoxins. Infect Immun 65:4048-54.
63. Viana D, Blanco J, Tormo-Mas MA, Selva L, Guinane CM, Baselga R, Corpa J, Lasa I, Novick RP, Fitzgerald JR, Penades JR. 2010. Adaptation of *Staphylococcus aureus* to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. Mol Microbiol 77:1583-94.
64. Spoor LE, Richardson E, Richards AC, Wilson GJ, Mendonca C, Gupta RK, McAdam PR, Nutbeam-Tuffs S, Black NS, O'Gara JP, Lee CY, Corander J, Fitzgerald JR. 2015. Recombination-mediated remodelling of host-pathogen interactions during *Staphylococcus aureus* niche adaptation. Microb Genom 1:e000036.
65. Pyrah ITG, Scott PR, Penny CD. 1994. Possible involvement of *Staphylococcus aureus* as a primary pathogen in lamb septicaemia. Veterinary Record 134:679-680.
66. Bates P. 2003. Bacterial associations with the sheep scab mite (*Psoroptes ovis*). Veterinary Record 152:206-208.
67. Foster AP. 2012. Staphylococcal skin disease in livestock. Veterinary Dermatology 23:342-e63.
68. de la Fuente R, Ballesteros C, Bautista V, Medina A, Orden JA, Domínguez-Bernal G, Vindel A. 2011. *Staphylococcus aureus* subsp. *anaerobius* isolates from different countries are clonal in nature. Veterinary Microbiology 150:198-202.
69. Devriese LA, Van Damme LR, Fameree L. 1972. Methicillin (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. Zentralbl Veterinarmed B 19:598-605.

70. Lee JH. 2003. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 69:6489-94.
71. Kaszanyitzky EJ, Egyed Z, Janosi S, Keseru J, Gal Z, Szabo I, Veres Z, Somogyi P. 2004. Staphylococci isolated from animals and food with phenotypically reduced susceptibility to β-lactamase-resistant β-lactam antibiotics. Acta Vet Hung 52:7-17.
72. Kwon NH, Park KT, Moon JS, Jung WK, Kim SH, Kim JM, Hong SK, Koo HC, Joo YS, Park YH. 2005. Staphylococcal cassette chromosome *mec* (SCC*mec*) characterization and molecular analysis for methicillin-resistant *Staphylococcus aureus* and novel SCC*mec* subtype IVg isolated from bovine milk in Korea. J Antimicrob Chemother 56:624-32.
73. Juhasz-Kaszanyitzky E, Janosi S, Somogyi P, Dan A, van der Graaf-van Bloois L, van Duijkeren E, Wagenaar JA. 2007. MRSA transmission between cows and humans. Emerg Infect Dis 13:630-2.
74. Umoh VJ, Adesiyun AA, Gomwalk NE. 1990. Antibiogram of staphylococcal strains isolated from milk and milk-products. Zentralbl Veterinarmed B 37:701-6.
75. Costa EO, Benites NR, Guerra JL, Melville PA. 2000. Antimicrobial susceptibility of *Staphylococcus* spp. isolated from mammary parenchymas of slaughtered dairy cows. J Vet Med B Infect Dis Vet Public Health 47:99-103.
76. Erskine RJ, Walker RD, Bolin CA, Bartlett PC, White DG. 2002. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. J Dairy Sci 85:1111-8.
77. Fessler A, Scott C, Kadlec K, Ehricht R, Monecke S, Schwarz S. 2010. Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. J Antimicrob Chemother 65:619-25.
78. Holmes MA, Zadoks RN. 2011. Methicillin resistant *S. aureus* in human and bovine mastitis. J Mammary Gland Biol Neoplasia 16:373-82.
79. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA. 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mec*A homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11:595-603.
80. Li T, Lu H, Wang X, Gao Q, Dai Y, Shang J, Li M. 2017. Molecular Characteristics of *Staphylococcus aureus* Causing Bovine Mastitis between 2014 and 2015. Front Cell Infect Microbiol 7:127.
81. Wu Z, Li F, Liu D, Xue H, Zhao X. 2015. Novel Type XII Staphylococcal Cassette Chromosome mec Harboring a New Cassette Chromosome Recombinase, CcrC2. Antimicrob Agents Chemother 59:7597-601.
82. Yan X, Li Z, Chlebowicz MA, Tao X, Ni M, Hu Y, Li Z, Grundmann H, Murray S, Pascoe B, Sheppard SK, Bo X, Dijl JM, Du P, Zhang M, You Y, Yu X, Meng F, Wang S, Zhang J. 2016. Genetic features of livestock-associated *Staphylococcus aureus* ST9 isolates from Chinese pigs that carry the lsa(E) gene for quinupristin/dalfopristin resistance. Int J Med Microbiol 306:722-729.
83. Zhou W, Li X, Osmundson T, Shi L, Ren J, Yan H. 2018. WGS analysis of ST9-MRSA-XII isolates from live pigs in China provides insights into transmission among porcine, human and bovine hosts. J Antimicrob Chemother doi:10.1093/jac/dky245.
84. Basanisi MG, La Bella G, Nobili G, Franconieri I, La Salandra G. 2017. Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. Food Microbiology 62:141-146.
85. Loncaric I, Kubber-Heiss A, Posautz A, Stalder GL, Hoffmann D, Rosengarten R, Walzer C. 2014. *mec*C- and *mec*A-positive meticillin-resistant *Staphylococcus aureus* (MRSA) isolated from livestock sharing habitat with wildlife previously tested positive for *mec*C-positive MRSA. Vet Dermatol 25:147-8.

86. Paterson GK, Harrison EM, Holmes MA. 2014. The emergence of *mec*C methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 22:42-7.
87. Cuny C, Layer F, Strommenger B, Witte W. 2011. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mec*A homologue in humans in Germany. PLoS One 6:e24360.
88. Rosell JM, de la Fuente LF. 2009. Culling and mortality in breeding rabbits. Prev Vet Med 88:120-7.
89. Rosell JM, de la Fuente LF. 2018. Mastitis on Rabbit Farms: Prevalence and Risk Factors. Animals (Basel) 8.
90. Corpa JM, Hermans K, Haesebrouck E. 2010. Main pathologies associated with *Staphylococcus aureus* infections in rabbits: a review. 2010 17:11.
91. Segura P, Martinez J, Peris B, Selva L, Viana D, Penades JR, Corpa JM. 2007. Staphylococcal infections in rabbit does on two industrial farms. Vet Rec 160:869-72.
92. Okerman L, Devriese LA, Maertens L, Okerman F, Godard C. 1984. Cutaneous staphylococcosis in rabbits. Vet Rec 114:313-5.
93. Viana D, Selva L, Segura P, Penades JR, Corpa JM. 2007. Genotypic characterization of *Staphylococcus aureus* strains isolated from rabbit lesions. Vet Microbiol 121:288-98.
94. Viana D, Selva L, Callanan JJ, Guerrero I, Ferrian S, Corpa JM. 2011. Strains of *Staphylococcus aureus* and pathology associated with chronic suppurative mastitis in rabbits. Vet J 190:403-7.
95. Vancraeynest D, Haesebrouck F, Deplano A, Denis O, Godard C, Wildemauwe C, Hermans K. 2006. International dissemination of a high virulence rabbit *Staphylococcus aureus* clone. J Vet Med B Infect Dis Vet Public Health 53:418-22.
96. Guerrero I, Ferrian S, Penades M, Garcia-Quiros A, Pascual JJ, Selva L, Viana D, Corpa JM. 2015. Host responses associated with chronic staphylococcal mastitis in rabbits. Vet J 204:338-44.
97. Hermans K, Devriese LA, Haesebrouck F. 2003. Rabbit staphylococcosis: difficult solutions for serious problems. Veterinary Microbiology 91:57-64.
98. Meulemans L, Hermans K, Duchateau L, Haesebrouck F. 2007. High and low virulence *Staphylococcus aureus* strains in a rabbit skin infection model. Veterinary Microbiology 125:333-340.
99. Devriese LA, Hendrickx W, Godard C, Okerman L, Haesebrouck F. 1996. A New Pathogenic *Staphylococcus aureus* Type in Commercial Rabbits. Journal of Veterinary Medicine, Series B 43:313-315.
100. Hermans K, De Herdt P, Devriese LA, Hendrickx W, Godard C, Haesebrouck F. 1999. Colonization of rabbits with *Staphylococcus aureus* in flocks with and without chronic staphylococcosis. Vet Microbiol 67:37-46.
101. Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Gotz F. 1999. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem 274:8405-10.
102. Murray S, Pascoe B, Meric G, Mageiros L, Yahara K, Hitchings MD, Friedmann Y, Wilkinson TS, Gormley FJ, Mack D, Bray JE, Lamble S, Bowden R, Jolley KA, Maiden MCJ, Wendlandt S, Schwarz S, Corander J, Fitzgerald JR, Sheppard SK. 2017. Recombination-Mediated Host Adaptation by Avian *Staphylococcus aureus*. Genome Biol Evol 9:830-842.
103. Bystron J, Podkowik M, Piasecki T, Wieliczko A, Molenda J, Bania J. 2010. Genotypes and enterotoxin gene content of *S. aureus* isolates from poultry. Vet Microbiol 144:498-501.
104. Monecke S, Ruppelt A, Wendlandt S, Schwarz S, Slickers P, Ehricht R, Jackel SC. 2013. Genotyping of *Staphylococcus aureus* isolates from diseased poultry. Vet Microbiol 162:806-12.
105. Abdelbary MM, Wittenberg A, Cuny C, Layer F, Kurt K, Wieler LH, Walther B, Skov R, Larsen J, Hasman H, Fitzgerald JR, Smith TC, Wagenaar JA, Pantosti A, Hallin M, Struelens MJ, Edwards G, Bose R, Nubel U, Witte W. 2014. Phylogenetic analysis of *Staphylococcus aureus* CC398

reveals a sub-lineage epidemiologically associated with infections in horses. PLoS One 9:e88083.
106. Richards SA. 1971. The significance of changes in the temperature of the skin and body core of the chicken in the regulation of heat loss. J Physiol 216:1-10.
107. Herron-Olson L, Fitzgerald JR, Musser JM, Kapur V. 2007. Molecular correlates of host specialization in *Staphylococcus aureus*. PLoS One 2:e1120.
108. Ehrlich SD. 1977. Replication and expression of plasmids from *Staphylococcus aureus* in *Bacillus subtilis*. Proc Natl Acad Sci U S A 74:1680-2.
109. Pyzik E, Marek A. 2013. Plasmid profile analysis and evaluation of antibiotic susceptibility of *Staphylococcus aureus* strains isolated from table chicken eggs. Pol J Vet Sci 16:307-12.
110. Abdalrahman LS, Stanley A, Wells H, Fakhr MK. 2015. Isolation, Virulence, and Antimicrobial Resistance of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) Strains from Oklahoma Retail Poultry Meats. Int J Environ Res Public Health 12:6148-61.
111. Takeuchi S, Kinoshita T, Kaidoh T, Hashizume N. 1999. Purification and characterization of protease produced by *Staphylococcus aureus* isolated from a diseased chicken. Vet Microbiol 67:195-202.
112. Takeuchi S, Matsunaga K, Inubushi S, Higuchi H, Imaizumi K, Kaidoh T. 2002. Structural gene and strain specificity of a novel cysteine protease produced by *Staphylococcus aureus* isolated from a diseased chicken. Vet Microbiol 89:201-10.
113. Cui S, Li J, Hu C, Jin S, Li F, Guo Y, Ran L, Ma Y. 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China. J Antimicrob Chemother 64:680-3.
114. Guardabassi L, O'Donoghue M, Moodley A, Ho J, Boost M. 2009. Novel lineage of methicillin-resistant *Staphylococcus aureus*, Hong Kong. Emerg Infect Dis 15:1998-2000.
115. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC. 2013. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. PLoS One 8:e53738.
116. Sun J, Yang M, Sreevatsan S, Davies PR. 2015. Prevalence and Characterization of *Staphylococcus aureus* in Growing Pigs in the USA. PLoS One 10:e0143670.
117. Salgado CD, Farr BM, Calfee DP. 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. Clin Infect Dis 36:131-9.
118. Otto M. 2013. Community-associated MRSA: what makes them special? Int J Med Microbiol 303:324-30.
119. Tavares A, Miragaia M, Rolo J, Coelho C, de Lencastre H, group C-MMw. 2013. High prevalence of hospital-associated methicillin-resistant *Staphylococcus aureus* in the community in Portugal: evidence for the blurring of community-hospital boundaries. Eur J Clin Microbiol Infect Dis 32:1269-83.
120. Liu C, Graber CJ, Karr M, Diep BA, Basuino L, Schwartz BS, Enright MC, O'Hanlon SJ, Thomas JC, Perdreau-Remington F, Gordon S, Gunthorpe H, Jacobs R, Jensen P, Leoung G, Rumack JS, Chambers HF. 2008. A population-based study of the incidence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* disease in San Francisco, 2004-2005. Clin Infect Dis 46:1637-46.
121. van Duijkeren E, Jansen MD, Flemming SC, de Neeling H, Wagenaar JA, Schoormans AH, van Nes A, Fluit AC. 2007. Methicillin-resistant *Staphylococcus aureus* in pigs with exudative epidermitis. Emerg Infect Dis 13:1408-10.
122. Armand-Lefevre L, Ruimy R, Andremont A. 2005. Clonal comparison of *Staphylococcus aureus i*solates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11:711-4.
123. van Belkum A, Melles DC, Peeters JK, van Leeuwen WB, van Duijkeren E, Huijsdens XW, Spalburg E, de Neeling AJ, Verbrugh HA, Dutch Working Party on S, Research of M-S. 2008.

975 Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and
976 humans. Emerg Infect Dis 14:479-83.
977 124. Deiters C, Gunnewig V, Friedrich AW, Mellmann A, Kock R. 2015. Are cases of Methicillin-
978 resistant *Staphylococcus aureus* clonal complex (CC) 398 among humans still livestock-
979 associated? Int J Med Microbiol 305:110-3.
980 125. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. 2009. Nasal colonization of
981 humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without
982 exposure to pigs. PLoS One 4:e6800.
983 126. Larsen J, Petersen A, Sorum M, Stegger M, van Alphen L, Valentiner-Branth P, Knudsen LK,
984 Larsen LS, Feingold B, Price LB, Andersen PS, Larsen AR, Skov RL. 2015. Meticillin-resistant
985 *Staphylococcus aureus* CC398 is an increasing cause of disease in people with no livestock
986 contact in Denmark, 1999 to 2011. Euro Surveill 20.
987 127. Ward MJ, Gibbons CL, McAdam PR, van Bunnik BA, Girvan EK, Edwards GF, Fitzgerald JR,
988 Woolhouse ME. 2014. Time-Scaled Evolutionary Analysis of the Transmission and Antibiotic
989 Resistance Dynamics of *Staphylococcus aureus* Clonal Complex 398. Appl Environ Microbiol
990 80:7275-82.
991 128. Kock R, Harlizius J, Bressan N, Laerberg R, Wieler LH, Witte W, Deurenberg RH, Voss A, Becker
992 K, Friedrich AW. 2009. Prevalence and molecular characteristics of methicillin-resistant
993 *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related
994 MRSA into hospitals. Eur J Clin Microbiol Infect Dis 28:1375-82.
995 129. Kock R, Schaumburg F, Mellmann A, Koksal M, Jurke A, Becker K, Friedrich AW. 2013.
996 Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human
997 infection and colonization in Germany. PLoS One 8:e55040.
998 130. Bens CC, Voss A, Klaassen CH. 2006. Presence of a novel DNA methylation enzyme in
999 methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to
1000 uninterpretable results in standard pulsed-field gel electrophoresis analysis. J Clin Microbiol
1001 44:1875-6.
1002 131. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. 2011. Rapid PCR
1003 detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-
1004 modification system carrying *sau1-hsdS1*. J Clin Microbiol 49:732-4.
1005 132. Li S, Skov RL, Han X, Larsen AR, Larsen J, Sorum M, Wulf M, Voss A, Hiramatsu K, Ito T. 2011.
1006 Novel types of staphylococcal cassette chromosome mec elements identified in clonal
1007 complex 398 methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents
1008 Chemother 55:3046-50.
1009 133. Argudin MA, Tenhagen BA, Fetsch A, Sachsenroder J, Kasbohrer A, Schroeter A, Hammerl JA,
1010 Hertwig S, Helmuth R, Braunig J, Mendoza MC, Appel B, Rodicio MR, Guerra B. 2011. Virulence
1011 and resistance determinants of German *Staphylococcus aureus* ST398 isolates from
1012 nonhuman sources. Appl Environ Microbiol 77:3052-60.
1013 134. Hallin M, De Mendonca R, Denis O, Lefort A, El Garch F, Butaye P, Hermans K, Struelens MJ.
1014 2011. Diversity of accessory genome of human and livestock-associated ST398 methicillin
1015 resistant *Staphylococcus aureus* strains. Infect Genet Evol 11:290-9.
1016 135. Wulf MW, Sorum M, van Nes A, Skov R, Melchers WJ, Klaassen CH, Voss A. 2008. Prevalence
1017 of methicillin-resistant *Staphylococcus aureus* among veterinarians: an international study.
1018 Clin Microbiol Infect 14:29-34.
1019 136. Bisdorff B, Scholholter JL, Claussen K, Pulz M, Nowak D, Radon K. 2012. MRSA-ST398 in
1020 livestock farmers and neighbouring residents in a rural area in Germany. Epidemiol Infect
1021 140:1800-8.
1022 137. Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D. 2011. Persistence of livestock
1023 associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS One
1024 6:e16830.

138. van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, Kluytmans JA. 2011. Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol 49:1030-3.
139. Richter A, Sting R, Popp C, Rau J, Tenhagen BA, Guerra B, Hafez HM, Fetsch A. 2012. Prevalence of types of methicillin-resistant *Staphylococcus aureus* in turkey flocks and personnel attending the animals. Epidemiol Infect 140:2223-32.
140. Schaumburg F, Kock R, Mellmann A, Richter L, Hasenberg F, Kriegeskorte A, Friedrich AW, Gatermann S, Peters G, von Eiff C, Becker K, study g. 2012. Population dynamics among methicillin-resistant *Staphylococcus aureus* isolates in Germany during a 6-year period. J Clin Microbiol 50:3186-92.
141. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA. 2006. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J Bacteriol 188:1310-5.
142. Rooijakkers SH, Ruyken M, Roos A, Daha MR, Presanis JS, Sim RB, van Wamel WJ, van Kessel KP, van Strijp JA. 2005. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol 6:920-7.
143. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP, van Strijp JA. 2004. Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. J Exp Med 199:687-95.
144. McCarthy AJ, Witney AA, Gould KA, Moodley A, Guardabassi L, Voss A, Denis O, Broens EM, Hinds J, Lindsay JA. 2011. The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country. Genome Biol Evol 3:1164-74.
145. McCarthy AJ, van Wamel W, Vandendriessche S, Larsen J, Denis O, Garcia-Graells C, Uhlemann AC, Lowy FD, Skov R, Lindsay JA. 2012. *Staphylococcus aureus* CC398 clade associated with human-to-human transmission. Appl Environ Microbiol 78:8845-8.
146. Schijffelen MJ, Boel CH, van Strijp JA, Fluit AC. 2010. Whole genome analysis of a livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 isolate from a case of human endocarditis. BMC Genomics 11:376.
147. Yu F, Chen Z, Liu C, Zhang X, Lin X, Chi S, Zhou T, Chen Z, Chen X. 2008. Prevalence of *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes among isolates from hospitalised patients in China. Clin Microbiol Infect 14:381-4.
148. Corvaglia AR, Francois P, Hernandez D, Perron K, Linder P, Schrenzel J. 2010. A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical *Staphylococcus aureus* strains. Proc Natl Acad Sci U S A 107:11954-8.
149. Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH. 2005. Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. J Antimicrob Chemother 56:692-7.
150. van Duijkeren E, Wolfhagen MJ, Box AT, Heck ME, Wannet WJ, Fluit AC. 2004. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 10:2235-7.
151. Rutland BE, Weese JS, Bolin C, Au J, Malani AN. 2009. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 15:1328-30.
152. Loeffler A, Lloyd DH. 2010. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect 138:595-605.
153. Faires MC, Traverse M, Tater KC, Pearl DL, Weese JS. 2010. Methicillin-resistant and -susceptible *Staphylococcus aureus* infections in dogs. Emerg Infect Dis 16:69-75.
154. Morris DO, Lautenbach E, Zaoutis T, Leckerman K, Edelstein PH, Rankin SC. 2012. Potential for pet animals to harbour methicillin-resistant *Staphylococcus aureus* when residing with human MRSA patients. Zoonoses Public Health 59:286-93.

155. Deurenberg RH, Stobberingh EE. 2009. The molecular evolution of hospital- and community-associated methicillin-resistant *Staphylococcus aureus*. Curr Mol Med 9:100-15.
156. Weese JS, van Duijkeren E. 2010. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol 140:418-29.
157. van Duijkeren E, Moleman M, Sloet van Oldruitenborgh-Oosterbaan MM, Multem J, Troelstra A, Fluit AC, van Wamel WJ, Houwers DJ, de Neeling AJ, Wagenaar JA. 2010. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. Vet Microbiol 141:96-102.
158. Witte W, Strommenger B, Stanek C, Cuny C. 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg Infect Dis 13:255-8.
159. Seguin JC, Walker RD, Caron JP, Kloos WE, George CG, Hollis RJ, Jones RN, Pfaller MA. 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J Clin Microbiol 37:1459-63.
160. Morgan M. 2008. Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? J Antimicrob Chemother 62:1181-7.
161. Cuny C, Strommenger B, Witte W, Stanek C. 2008. Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. Microb Drug Resist 14:307-10.
162. Koop G, Vrieling M, Storisteanu DM, Lok LS, Monie T, van Wigcheren G, Raisen C, Ba X, Gleadall N, Hadjirin N, Timmerman AJ, Wagenaar JA, Klunder HM, Fitzgerald JR, Zadoks R, Paterson GK, Torres C, Waller AS, Loeffler A, Loncaric I, Hoet AE, Bergstrom K, De Martino L, Pomba C, de Lencastre H, Ben Slama K, Gharsa H, Richardson EJ, Chilvers ER, de Haas C, van Kessel K, van Strijp JA, Harrison EM, Holmes MA. 2017. Identification of LukPQ, a novel, equid-adapted leukocidin of *Staphylococcus aureus*. Sci Rep 7:40660.
163. de Jong NWM, Vrieling M, Garcia BL, Koop G, Brettmann M, Aerts PC, Ruyken M, van Strijp JAG, Holmes M, Harrison EM, Geisbrecht BV, Rooijakkers SHM. 2018. Identification of a staphylococcal complement inhibitor with broad host specificity in equid *Staphylococcus aureus* strains. J Biol Chem 293:4468-4477.
164. Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP. 1998. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol Microbiol 29:527-43.
165. Schaumburg F, Pauly M, Anoh E, Mossoun A, Wiersma L, Schubert G, Flammen A, Alabi AS, Muyembe-Tamfum J-J, Grobusch MP, Karhemere S, Akoua-Koffi C, Couacy-Hymann E, Kremsner PG, Mellmann A, Becker K, Leendertz FH, Peters G. 2015. *Staphylococcus aureus* complex from animals and humans in three remote African regions. Clinical Microbiology and Infection 21:345.e1-345.e8.
166. Senghore M, Bayliss SC, Kwambana-Adams BA, Foster-Nyarko E, Manneh J, Dione M, Badji H, Ebruke C, Doughty EL, Thorpe HA, Jasinska AJ, Schmitt CA, Cramer JD, Turner TR, Weinstock G, Freimer NB, Pallen MJ, Feil EJ, Antonio M. 2016. Transmission of *Staphylococcus aureus* from Humans to Green Monkeys in The Gambia as Revealed by Whole-Genome Sequencing. Appl Environ Microbiol 82:5910-7.
167. Koymans KJ, Vrieling M, Gorham RD, Jr., van Strijp JAG. 2017. Staphylococcal Immune Evasion Proteins: Structure, Function, and Host Adaptation. Curr Top Microbiol Immunol 409:441-489.
168. Loffler B, Hussain M, Grundmeier M, Bruck M, Holzinger D, Varga G, Roth J, Kahl BC, Proctor RA, Peters G. 2010. *Staphylococcus aureus* panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 6:e1000715.
169. Vrieling M, Boerhout EM, van Wigcheren GF, Koymans KJ, Mols-Vorstermans TG, de Haas CJ, Aerts PC, Daemen IJ, van Kessel KP, Koets AP, Rutten VP, Nuijten PJ, van Strijp JA, Benedictus L. 2016. LukMF' is the major secreted leukocidin of bovine *Staphylococcus aureus* and is produced *in vivo* during bovine mastitis. Sci Rep 6:37759.
170. Wilson GJ, Seo KS, Cartwright RA, Connelley T, Chuang-Smith ON, Merriman JA, Guinane CM, Park JY, Bohach GA, Schlievert PM, Morrison WI, Fitzgerald JR. 2011. A novel core genome-

encoded superantigen contributes to lethality of community-associated MRSA necrotizing pneumonia. PLoS Pathog 7:e1002271.

171. Smyth DS, Hartigan PJ, Meaney WJ, Fitzgerald JR, Deobald CF, Bohach GA, Smyth CJ. 2005. Superantigen genes encoded by the egc cluster and SaPIbov are predominant among *Staphylococcus aureus* isolates from cows, goats, sheep, rabbits and poultry. J Med Microbiol 54:401-11.

172. Fitzgerald JR, Monday SR, Foster TJ, Bohach GA, Hartigan PJ, Meaney WJ, Smyth CJ. 2001. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. J Bacteriol 183:63-70.

173. Jarraud S, Peyrat MA, Lim A, Tristan A, Bes M, Mougel C, Etienne J, Vandenesch F, Bonneville M, Lina G. 2001. *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J Immunol 166:669-77.

Figures and Tables

Table 1 Selected staphylococcal elements associated with specific hosts

MGE	MGE-associated determinants putatively involved with virulence/resistance/host specificity	Reference
Human		
Φ*Sa*3 (β-haemolysin converting phage)	*sea*, *sep*, *scn*, *chp* and *sak* genes encoding staphylococcal enterotoxin A and P, staphylococcal complement inhibitor, chemotaxis inhibitory protein and plasminogen activator staphylokinase, respectively	(43)
MGE	Type I restriction modification system	"
Ruminant		
SaPIbov	Staphylococcal enterotoxin C (*sec*-bovine) and L (*sel*), toxic shock syndrome toxin *tst* (TSST-1)	(171, 172)
Enterotoxin gene cluster	Gene cluster encoding 5 enterotoxins (*seg*, *sei*, *sem*, *sen*, *seo*)	(171, 173)
Not described	Superantigen-like proteins encoded by *ssl07* and *ssl08*	(54)
SaPIbov4	von Willebrand factor binding protein with ruminant-specific activity	(63)
non-*mec*¬ staphylococcal cassette chromosome	LPXTG-surface protein	(53)
SCC-*mec*C	*mec*C	(79)
Equine		
ΦSaeq1	Contains immune modulators with equine-specific activity	(163)
	scn gene encoding staphylococcal complement inhibitor (SCIN)	
	*luk*PQ genes encoding the bipartite leucocidin PQ	(162)
SaPIeq1	Encodes vWbp able to coagulate equine and ruminant plasma	(63)
Porcine		
Plasmid	SCC*mec*	(43)
Plasmid	Resistance to heavy metals	"
SaPI-S0385	Composite of SaPI5 and SaPIbov1	(146)
	Unique region at 3'-end encoding extracellular proteins with similarity to staphylococcal complement inhibitor (SCIN) and von Willebrand factor-binding protein (vWbp)	
Avian		
ΦAvβ (β-haemolysin-converting phage)	Putative ornithine cyclodeaminase 38% amino acid identity to ornithine cyclodeaminase made by *Bacillus cereus*	(40)
	HMM match to ornithine cyclodeaminase/mu-crystalin family (PF02423)	
	Putative membrane protease 27% amino acid identity to Plnl (membrane-bound protease of CAAX family) made by *Lactobacillus plantarum*; HMM match to CAAX amino terminal protease family	
ΦAv1	Ear-like protein	"
	ear previously identified in pathogenicity islands SaPI1, SaPI3, SaPI5 and SaPImw2	
	ear encodes β-lactamase-like protein	

SaPIAv	Putative virulence region Novel hypothetical proteins in accessory region A3 where virulence genes such as *tst* and *eta* located in other SaPI SAAV_0806: signal peptide, 1 transmembrane helix SAAV_0810: signal peptide, 4 transmembrane helices May suggest role as membrane transporter	"
pAvX	Thiol protease ScpA 99.5% amino acid identity to ScpA (GenBank accession no. AB071596) previously identified among chicken isolates from Japan Suggested role in poultry dermatitis Lysophospholipase 42% amino acid identity to a lysophospholipase encoded by *Bacillus clausii* Bacterial phospholipases are known virulence factors implicated in disease pathogenesis	"
pAvY	N/A	
pT181	Tetracycline resistance	(102, 108)
pT127	Tetracycline resistance	"
pC194	Chloramphenicol resistance	"
pC221	Chloramphenicol resistance	"
pC223	Chloramphenicol resistance	"
pUB112	Chloramphenicol resistance	"

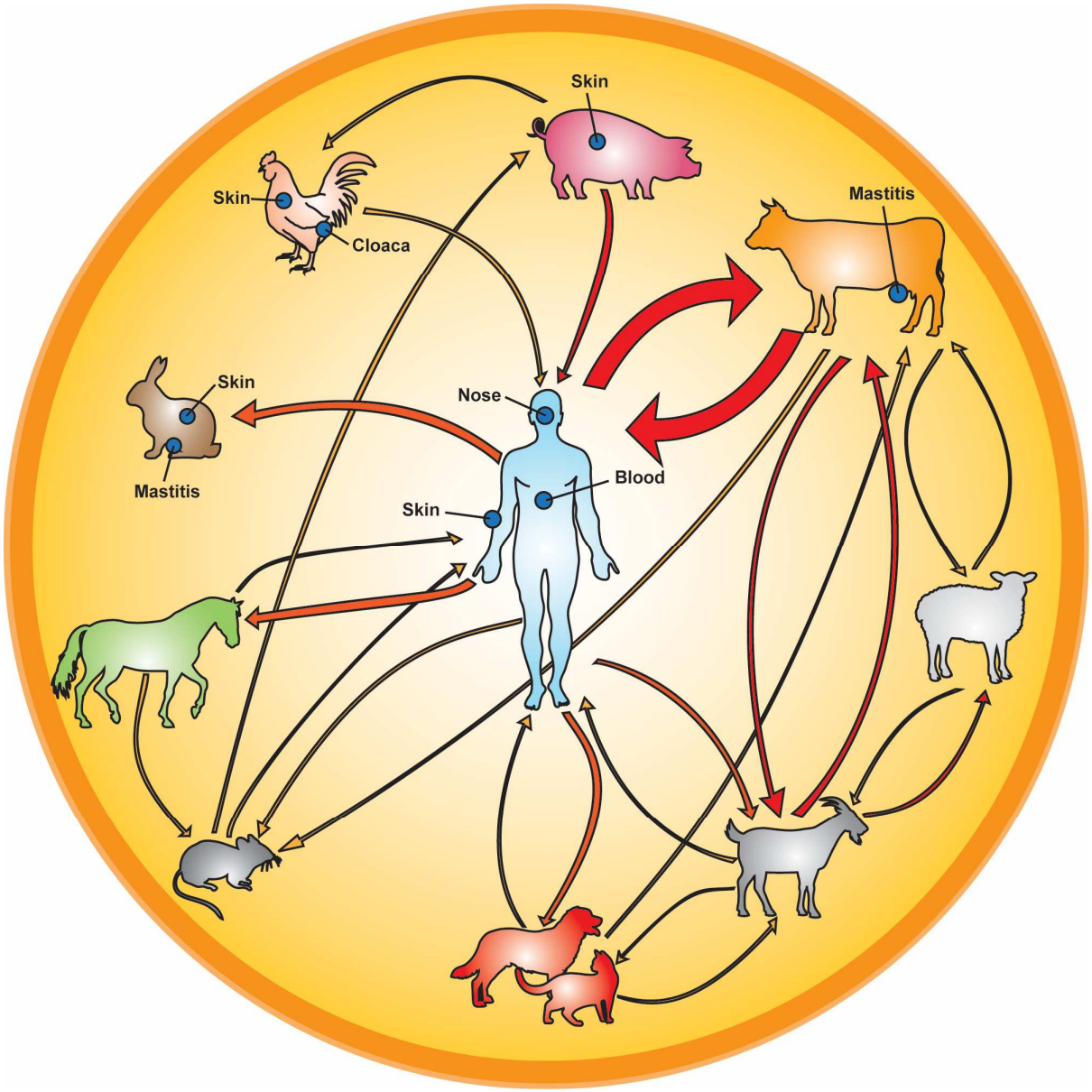

Figure 1 Humans act as a major hub for *S. aureus* host jumps. *S. aureus* has been isolated from a plethora of vertebra and has undergone multiple series of host jumps. A major exchange hub are humans that interact with domesticated livestock and companion animals. Arrow thickness indicates the frequency of host jumps with colours from yellow to red indicative of their likelihood. Figure adapted from (43).