REVIEW 10.1111/j.1469-0691.2012.03846.x

Livestock-associated *Staphylococcus aureus*

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Abstract

Staphylococcus aureus has long been associated with livestock. Livestock can be carriers of *S. aureus*, but can also become infected. The best-known infection is bovine mastitis. The discovery of methicillin-resistant *S. aureus* belonging to sequence type (ST)398 boosted interest in livestock-associated *S. aureus*. ST398 is pandemic. Whole genome sequencing and other genetic analyses have shown that livestock-associated strains are distinct from human-derived strains. However, there is also an exchange of strains between the reservoirs. Livestock-associated and human-associated strains share virulence factors, but have also distinct virulence factors that appear to be important in host adaptation. Exchange of genes encoding these virulence factors between strains may expand the host range and thereby threaten public health. Vaccination of animals may be a solution to this problem, but new avenues for vaccination need to be explored, because no vaccine is currently available.

Keywords: Livestock, MRSA, ST398, *Staphylococcus aureus*, virulence
Article published online: 19 March 2012
Clin Microbiol Infect 2012; **18:** 735–744

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Introduction

Staphylococcus aureus is a well-known commensal and pathogen of a large number of animal species, including humans. A wide variety of infections can be caused by *S. aureus*, from superficial skin and soft tissue infections to life-threatening septicaemia. *S. aureus* represents a serious public health burden in both hospital and community settings, as well as an economic and animal welfare problem in dairy farming. In this article, I focus on livestock-associated *S. aureus*, and define livestock as pigs, cattle, and poultry. Interest in livestock-associated *S. aureus* was renewed with the discovery of the methicillin-resistant *S. aureus* (MRSA) sequence type (ST)398 in pigs, and then in veal calves and poultry [1–3]. However, *S. aureus* was already a major problem in dairy cows, where it causes mastitis, and infections in chickens have also caused problems.

Pigs are often carriers, and are only rarely infected [4]. In chickens, several disease manifestations have been described, such as comb necrosis [5], bacterial chondronecrosis, which is a cause of leg weakness/lameness [6,7], and septicaemia [7]. These diseases may affect a significant proportion of a flock.

Staphylococcal mastitis is a major problem in dairy industry, affecting animal health and causing economic losses of up to €300 per cow per year. Although antibiotic treatment is an option for individual animals, it is unfavourable because of costs and the potential risk of the development of antibiotic resistance, and is unsuitable for addressing the problem of long-term persistence of pathogenic *S. aureus* in udder tissue. In addition, increased awareness of the use of antibiotics in husbandry and animal welfare is further contributing to the urgent need to address bovine mastitis in a different way. Vaccination would be a logical option.

Virulence Factors

Successful infection of both humans and animals depends on virulence factors produced by *S. aureus*. A wide spectrum of secreted and cell surface-associated virulence factors can be

expressed to promote adhesion to the host extracellular matrix components, damage host cells, and fight the immune system [8]. At least 25 different toxins, 15 microbial surface components recognizing adhesive matrix molecules, which are important for adhesion to tissues, 20 immune evasion molecules and several other virulence factors are known. The majority of these virulence factors have been identified in isolates of human origin, and only a few studies have investigated virulence genes in non-ST398 *S. aureus* from chickens and cows. However, some novel virulence factors have recently been identified in mastitis and livestock-associated *S. aureus* [9,10].

Data on the presence of virulence genes in livestock-associated *S. aureus* are summarized in Table 1. Smyth *et al.* investigated the presence of the superantigen genes *sae–see*, *seg–seo*, and *seq*, as well as the toxic shock syndrome toxin 1 gene, in isolates from a number of animals, including 15 chicken and 99 cow isolates (Table 1) [11]. The majority of the chicken isolates carried *seg*, *sei*, *sem*, *sen*, and *seo*, whereas, among others, the genes for the classical superantigens,

TABLE 1. Presence of virulence genes in *Staphylococcus aureus* isolates from different sources

		Smyth *et al.* [11]		Monecke *et al.* [99]	Ikawaty *et al.* [12]	Huber *et al.* [100]		Hallin *et al.* [101]
		No positive/%		No positive/%	No positive/%	No positive/%		No positive/%
Gene	Product	Chicken (*n* = 15)	Cow (*n* = 99)	Cow (*n* = 20)	Cow (*n* = 76)	Cow (*n* = 6)	Pigs (*n* = 10)	ST398 (*n* = 16)
tst	Toxic shock syndrome toxin 1	0/0	19/19	3/15	21/28	–	–	0/0
sea	Enterotoxin A	0/0	0/0	3/15	0/0	0/0	0/0	0/0
seb	Enterotoxin B	0/0	0/0	1/5	0/0	0/0	0/0	0/0
sec	Enterotoxin C	0/0	19/19	3/15	19/25	0/0	0/0	–
sed	Enterotoxin D	0/0	6/6	3/15	–	0/0	0/0	–
see	Enterotoxin E	0/0	0/0	0/0	–	0/0	0/0	–
seg	Enterotoxin G	13/87	22/22	9/45	58/78	0/0	0/0	0/0
seh	Enterotoxin H	1/7	0/0	1/5	1/1	0/0	1/17	–
sei	Enterotoxin I	13/87	32/32	9/45	61/80	0/0	0/0	0/0
sej	Enterotoxin J	0/0	6/6	2/10	–	0/0	0/0	0/0
sek	Enterotoxin K	1/7	0/0	0/0	0/0	0/0	0/0	0/0
sel	Enterotoxin L	0/0	18/18	3/15	15/20	0/0	0/0	0/0
sem	Enterotoxin M	13/87	32/32	9/45	52/68	0/0	0/0	0/0
sen	Enterotoxin N	13/87	32/32	9/45	55/72	0/0	0/0	0/0
seo	Enterotoxin O	13/87	32/32	9/45	0/0	0/0	0/0	0/0
sep	Enterotoxin P	–	–	–	0/0	–	–	0/0
seq	Enterotoxin Q	1/7	0/0	0/0	0/0	0/0	0/0	0/0
eta	Exfoliative toxin A	–	–	0/0	0/0	–	–	–
etb	Exfoliative toxin B	–	–	0/0	0/0	–	–	–
hla	α-Haemolysin	–	–	20/100	–	10/100	6/100	–
lukE	Leukocidin component E	–	–	–	15/20	0/0	1/17	0/0
lukF	Leukocidin component F	–	–	20/100	–	10/100	6/100	–
lukS	Leukocidin component S	–	–	20/100	–	10/100	5/83	–
lukM	Leukocidin component M	–	–	8/40	–	0/0	0/0	–
PVL	Panton–Valentine leukocidin	–	–	0/0	0/0	0/0	0/0	0/0
hlgA	γ-Haemolysin component A	–	–	20/100	–	10/100	6/100	–
chp	Chemotaxis inhibitory protein of *S. aureus*	–	–	–	1/1	–	–	–
scn	Staphylococcal complement inhibitor	–	–	–	1/1	–	–	–
sak	Staphylokinase	–	–	–	1/1	–	–	0/0
fnbA	Fibronectin-binding protein A	–	–	–	73/96	–	–	16/100
fnbB	Fibronectin-binding protein B	–	–	–	33/43	–	–	0/0
clfA	Clumping factor A	–	–	–	16/21	–	–	16/100
sdrE	Serine aspartate repeat protein E	–	–	–	25/33	–	–	–
cna	Collagen-binding protein	–	–	–	37/49	–	–	16/100
ebps	Elastin-binding protein	–	–	–	76/100	–	–	0/0
efb	Extracellular fibrinogen-binding protein	–	–	–	76/100	–	–	–
cap5A	Type 5 capsular polysaccharide	–	–	–	3/4	–	–	16/100
cap8	Type 8 capsular polysaccharide	–	–	–	73/96	–	–	0/0
icaB	Polysaccharide intercellular adhesin operon	–	–	–	64/84	–	–	–
icaC	Polysaccharide intercellular adhesin operon	–	–	–	63/83	–	–	–
icaD	Polysaccharide intercellular adhesin operon	–	–	–	65/86	–	–	–
sspA	Serine protease	–	–	–	76/100	–	–	–
sspB	Cysteine protease	–	–	–	76/100	–	–	–
slpA	Serine protease-like protein	–	–	18/90	–	–	–	0/0
slpB	Serine protease-like protein	–	–	18/90	–	–	–	0/0
map	MHC class 2 analogue protein	–	–	–	14/18	–	–	16/100
ACME	Arginine catabolic mobile element	–	–	–	0/0	–	–	–

sea–*see*, and also the toxic shock syndrome toxin 1 gene were lacking. Among cow isolates, no particular gene was dominant, but *sea*, *seb*, *see*, *seh*, *sek*, and *seq* were lacking in this collection. Ikawaty *et al.* studied a larger collection of virulence genes in 76 *S. aureus* isolates from clinical cases of bovine mastitis from all over The Netherlands (Table 1) [12]. This study also showed variation in the presence of the genes encoding the different superantigens. In addition, the presence of the genes for a number of additional virulence factors, including adhesins, proteases, and capsule type, was investigated. For the adhesins, the genes for fibronectin-binding protein A, elastin-binding protein and extracellular fibrinogen-binding protein were almost always present, as was the gene for capsule type 8 (96%). Only one isolate encoded staphylococcal complement inhibitor, chemotaxis inhibitory protein of *S. aureus*, and staphylokinase. This suggests that the isolate may have a human origin, because these virulence factors show activity only against the human innate immune system [13]. The *sec3*/*sel*/*tst* signature of the bovine staphylococcal pathogenicity island (SaPIbov) was present in only 20% of the isolates; 10% of the isolates lacked one of the genes, and thus appeared to contain an incomplete or variant SaPIbov. The isolates could be clustered into six major groups on the basis of their virulence gene content. This clustering agreed with typing performed with multilocus sequence typing and pulsed-field gel electrophoresis.

The whole genome sequence of bovine strain ET3 provides additional detail about the virulence factors [14]. In fact, SaPIbov was discovered in this strain [15]. Part of this island was duplicated, and so was part of a second island in this strain called SaPIbov3. In addition, several new other islands carrying genes previously unknown in *S. aureus* were present. These included *ν*SaBov and a phage. Another interesting but unexplained observation was that several adhesin-encoding genes are pseudogenes, owing to the introduction of stop codons. Examples of the proteins affected are clumping factor A and protein A. Mutations in genes involved in iron uptake suggest that iron metabolism, at least in this clone, may differ considerably from that of sequenced human strains. The differences in adhesins and iron uptake support, according to the authors, the idea that a transition to an intracellular lifestyle has been made by this strain [14]. It is well known that *S. aureus* can survive intracellularly, and this would constitute a perfect means of escape from phagocytes and antibiotics. However, evidence for the importance of intracellular survival for infection is still limited. For a review, see [16].

Population and genetic analysis of *S. aureus* in broiler chickens from different continents showed that the majority of the isolates are most likely derived from the transfer of a human ST5 isolate to chickens somewhere between 64 and 30 years ago. The whole genome sequence of one representative isolate showed that it became adapted to the new host. Several virulence factor genes, such as the human-specific immune evasion cluster and several other genes encoding human-specific proteases, are lacking, whereas at least five avian-specific mobile genetic elements have been acquired. These elements encode several proteins that may play a role in virulence in chickens. The clearest example is a protease that has been implicated in the pathogenesis of poultry infections. It should be noted that the second largest cluster of isolates belonged to a clonal complex (CC) that has not been detected in humans, and several sequence types linked to human isolates were also found [17]. Thus, chickens are not carriers of a single clonal lineage.

The whole genome sequence of an MRSA ST398 isolate obtained from a human case of endocarditis in an immunocompromised patient showed the complete absence of enterotoxin genes. The strain did contain a novel staphylococcal pathogenicity island (SaPI) that appeared to be a composite of sequences from SaPIbov, SaPI5, which is found in strain USA300 (the most important community-acquired clone in the USA), and previously undescribed genes. The novel part encoded a staphylococcal complement inhibitor variant and a variant of von Willebrand factor-binding protein [9]. The first protein may be active against complement from other species than humans, in contrast to the protein from human isolates, which is human-specific [13]. The von Willebrand factor-binding protein has been implicated in host specificity. Strains carrying SaPIbov2, which is widely disseminated among ruminant isolates, are able to coagulate ruminant plasma, in contrast to isolates lacking this pathogenicity island. The coagulation activity is attributed to the variant of von Willebrand factor-binding protein encoded on this island [10]. Therefore, SaPIpig (or SaPI-S0385) may also contribute to the spread of ST398 among different animal species. Although not directly involved in virulence, S0385 has two other interesting features. First, *ν*Sa*β* lacks the characteristic restriction-modification system. This system may protect an isolate from (unwanted) DNA that is introduced into the cell. The lack of this system may make it more prone to accept foreign DNA. This may include both virulence factor and antibiotic resistance genes. Second, the genome contains three integrative conjugative elements. One of them is located within SCC*mec*. Their precise function is not known, but most likely these elements can transfer DNA to other strains, and possibly also play a role in acceptance of foreign DNA. This could make these strains intermediates for the transfer of genes between different strains. Alternatively, the system could function as a protein secretion mechanism [9].

Panton–Valentine leukocidin (PVL) has been reported in several Chinese ST398 isolates [18,19]. This toxin is common among community-associated MRSA strains. For example, the dominant community-associated MRSA strains in the USA and Europe, USA300 and ST80, encode PVL, and it has been suggested that PVL contributes to the wide dissemination of these strains [20–23].

An additional level of complexity for many infections with *S. aureus*, possibly including mastitis, is the formation of biofilms [24]. Outside the laboratory, most bacteria grow as communities attached to surfaces known as biofilms. Biofilms consist of bacteria embedded in polysaccharides, proteins, extracellular DNA, and combinations of these compounds [25–27]. Biofilm formation is a potent immune evasion strategy, both through physical blocking of access [28,29] and through active immune evasion because of secreted immune evasion components [8]. Bacteria in biofilms are also less sensitive to treatment with antimicrobial agents [30–32]. This helps to maintain chronic infections.

The proteins involved in biofilm formation are microbial surface components recognizing adhesive matrix molecules, but other proteins play roles, as well as polysaccharide intercellular adhesin, the product of the *icaABCD* operon, also plays a role [33]. These proteins are of great interest for biofilm prevention and removal. Several livestock strains harbour the *ica* genes, which encode products involved in biofilm formation (Table 1). Also free DNA helps to build biofilms in combination with β-toxin encoded by the *hlb* gene, which acts not only as a toxin, but also as a DNA-binding protein in biofilm formation [34]. In human-derived strains, the *hlb* gene is often disrupted by phages encoding human-specific immune evasion proteins [13]. Therefore, it appears that, in human infections, the bacterium has to make a trade-off between expression of immune evasion factors by maintaining the phage encoding these factors in the *hlb* gene and biofilm formation using β-toxin by losing the phage and thereby restoring the *hlb* gene.

ST398 and ST398 Transmissibility

The first report on ST398 in pigs was by Armand-Lefevre *et al.* [1]. They found both methicillin-sensitive *S. aureus* (MSSA) and MRSA in pigs and humans, but ST398 isolates constituted only a minor proportion of the types found. Furthermore, they noted that pig farmers were more often carriers of *S. aureus* than non-pig farmers. MRSA ST398 was first recognized as a problem by Voss [35], and a follow-on study showed MRSA ST398 transmission between pigs and humans [36]. Since then, MRSA ST398 has been documented in many countries, and has become pandemic. The prevalence of MRSA ST398 varies widely from country to country. Prevalences of up to 85% for pigs, 70% for farms and 45% for personnel have been reported, but ST398 may also be absent from countries, or at least not found [37–40]. There are indications that, in some farms, the rates of MRSA may be lower, or that MRSA may even be absent [41]. It remains to be seen whether these lower rates result from different isolation methodology, or whether these farms mainly operate outside the mainstream of pig farming. Differences in year of isolation, sampling site, type of farm, time-point of production (age and location) and methods used for isolation could explain some of the differences found in the different studies [42]. In addition, antibiotic usage and the type of antibiotic used before sampling will also influence results. The type of pigs may play also a role [40].

ST398 proved to be not limited to pigs, but was also detected in healthy poultry [2], veal calves [3], and cases of bovine mastitis [43,44], and contact with these animals appears to be a risk factor for becoming a carrier [45]. Furthermore, MRSA ST398 has been shown to be a cause of bovine mastitis [43]. Mastitis caused by MRSA ST398 was detected in 10% of tested Belgian farms [46]. ST398 has also been isolated from horses and horse handlers [47,48], dogs [49], and rats. It was suggested that, considering the behaviour of rats, they may play a role in spread [50]. ST398 is not a homogeneous lineage, as shown by *spa* typing [51], SCC*mec* typing [52], pulsed-field gel electrophoresis typing [53,54], and microarray analysis [50].

In Southeast Asia, MRSA isolates belonging to CC9 appear to be more frequent than ST398. In Malaysia, ST9 with SCC*mec* type V was present in 1% of the pigs and in 5.5% of the pig handlers. No MRSA ST398 was found [55]. In Hong Kong, 16% of nasal swabs taken from carcases on markets were MRSA ST9-positive [56]. ST9 is also geographically widespread present in China. Sixty isolates from four provinces were recovered. These isolates all had *spa* type t899 and SCC*mec* type III, and belonged to only two resistance patterns. The isolates were obtained from animals and farm workers [57]. Although these isolates (and others) have been reported as having SCC*mec* type III, they were shown to most likely have SCC*mec* type V when they (as the above isolates were) were typed with the method of Zhang *et al.* [58], because this method associates a particular PCR product with SCC*mec* type III, but this PCR fragment is also obtained with SCC*mec* type V from ST398 [52]. Another study also found MRSA ST9 on Chinese farms, but also found MSSA, and both belonged to *spa* type t899. In addition, an MSSA ST398 isolate was found [59]. Besides ST398 and CC9 isolates, MRSA isolates belonging to other lineages

have been detected. These lineages were usually isolated from either humans or bovines [43].

An unexpected discovery was a novel type of *mecA* determinant called $mecA_{LGA251}$ in livestock. The novel determinant showed only 63% homology at the amino acid level and 70% at the DNA level. This determinant leads to MICs ranging from 0.75 to 32 mg/L. The new *mec* gene was initially detected in 15 isolates from dairy cows in England. The isolates belonged to three different lineages according to multilocus sequence typing, but it was also found in 51 human *mecA*-negative isolates showing resistance to methicillin. Most of them had the same *spa* type as found in cows [60]. The oldest isolate was from 1975, indicating that this *mec* gene has been present but unnoticed for a long time. It also suggests that its spread is slow, although it is present in three lineages. The main problem is detection. The MIC may be below the cut-off point for resistance. Its (apparently) low prevalence makes testing every isolate by PCR a costly proposition. The future evolution of this gene and isolates carrying it is impossible to predict, but, through mutation, these isolates may become more resistant or they may acquire additional virulence factors, making them better adapted to new hosts [61].

Trade between farms has been shown to be a possible transmission route [52]. As trading of pigs between farms (usually from farrowing farms to finishing farms) is a common practice, it may play an important role in MRSA transmission. Nevertheless, the spread of MRSA ST398 among livestock throughout the world within a few years is not understood. In general, it is well accepted that antibiotic use selects for resistant organisms, but this explanation is too simple. Also, no worldwide change in antibiotic policies appears to have occurred. Therefore, it is unlikely that antibiotic usage alone can explain the spread. Resistance to zinc has been suggested as a contributory factor. The zinc resistance gene *crzC* is found on SCC*mec* type V (and was first wrongly annotated as a copper resistance gene), and nearly all isolates with SCC*mec* type V display zinc resistance [9,62,63]. A controlled study with naturally contaminated piglets housed with ST398-negative piglets showed an increase in bacterial load in piglets receiving tetracycline or zinc as a food additive, but an effect on transmission could not be established. However, it should be noted that this was a small-scale study [64]. Nevertheless, antibiotic use correlated with MRSA carriership in veal calves, whereas farm hygiene correlated with a lower prevalence [3].

Transmission of MRSA ST398 has been investigated both in piglets and in humans. In piglets, transmission rates of 3.92–52.54 were found [65]. However, a controlled study where a mixture of four MRSA strains of the two dominant lineages, ST398 and ST9, was used showed that nasal and gastrointestinal inoculation of piglets did not result in stable colonization. In contrast, vaginal inoculation of a pregnant sow shortly before farrowing led to stable colonization, indicating that vertical transmission may be a more effective means of spread [66]. However, this is a model system in which four strains directly compete with each other, and also the mode of delivery of the bacteria during inoculation differs from natural conditions, so it cannot be excluded that nasal gastrointestinal colonization is much more effective under natural conditions.

In 2007, 30% of all human isolates sent to the National Institute of Public Health and the Environment in The Netherlands were MRSA ST398 [67], but it should be noted that The Netherlands is a country with low MRSA prevalence (<3%). However, in general, ST398 appears to account for only a small proportion of MRSA isolates from humans. Most findings have been from Austria, Belgium, Denmark, and The Netherlands [68]. In a Dutch study, the presence of animal contact was the most important risk factor. However, the rapidly decreasing MRSA prevalence during absence of animal contact indicates that CC398 isolates are poor colonizers of humans [69]. This suggests that the majority of carriage in animal handlers results from continuous exposurem and not stable colonization. Another factor that may contribute to the poor ability to colonize humans is the presence of alternative immune evasion molecules for some human-specific immune evasion molecules and the lack of most toxin-encoding genes. This may significantly broaden the host range as compared with most human-derived isolates [70,71].

For the same reason, MRSA ST398 does not appear to be highly infectious for humans, but several case reports have been published, e.g. skin lesions [72], bacteraemia [73], and endocarditis [74].

A Dutch study showed that MRSA ST398 isolates were nearly six times less transmissible than other types. This led to the suggestion that less stringent transmission control measures may be possible for MRSA ST398 [75]. This would only be true if nothing changed in the ability of the circulating strains to be transmitted between patients. However, the introduction of novel genes, e.g. immune evasion molecule-encoding genes, which seem to make these isolates better adapted to humans, may change this.

MSSA ST398 has been found to be commonly present in pigs but not in other livestock animals [76]. It is not clear whether is this is generally true. However, MSSA ST398 is not limited to pork, and has also been indentified among *S. aureus* isolates obtained from humans. It has been identified both in patients without contact with livestock [77] and in healthy humans [78].

S. aureus in Meat Products

Although contact with animals seems to be the most important risk factor for human ST398 carriage, meat products may also be a source. A recent Dutch study showed that 35% of 40 broiler flocks were MRSA-positive, with the majority of isolates belonging to ST398, but slightly more than one-quarter belonged to ST9 and a single *spa* type. Nearly 8% of individual broilers were MRSA-positive upon arrival in the slaughterhouse, but, during the day, this increased to 35%, owing to contamination. Twenty per cent of employees involved in hanging the animals on the slaughter line were MRSA-positive, as compared with 1.9% for other personnel in contact with the broilers, and 0.1% of the general population [79]. A study from the pre-MRSA era showed no evidence of human nasal carriage of *S. aureus* poultry types [80]. When this is true, it is suggestive that MRSA ST398 behaves differently from other *S. aureus* strains present in poultry.

Because of these findings, it is not surprising that meat is also contaminated with *S. aureus* and MRSA. Percentages reported for chickens vary between 0% and 68% [81–83], but the levels of *S. aureus* can be even higher. Almost two-thirds of chicken meat samples tested in a Japanese study were positive for *S. aureus* [84]. The majority of the MRSA isolates present in meat samples belong to ST398, but ST9 and more human-related sequence types have also been identified [82,83,85–87]. The human-related types in meat may be derived from livestock contaminated by these strains or during handling from slaughter to retail.

In pork, *S. aureus* rates vary greatly. Recovery in 5% of the samples has been reported [88,89], but more than half of all fresh pig meat samples have also been reported to be positive [90]. Varying rates of MRSA in pork have also been reported. The rates for beef seem to be lower. A Danish study reported only 1.4% MRSA-positive samples [89].

In Canada, pork, beef and chicken may all carry MRSA. However, these isolates all belong to the Canadian epidemic MRSA-2 (ST5) [86]. It should be remembered that *S. aureus* in meat products is not a recent phenomenon. It has always been a source of food-poisoning.

Co-resistance

MRSA isolates from livestock usually show also resistance to other antibiotics, and the resistance patterns of isolates can be highly variable. A German study found 22 different types [91]. ST398 isolates appear to be universally resistant to tetracyclines, owing to the presence of the *tet*(M) gene on a chromosomally located transposon, often in combination with the plasmid-encoded *tet*(K) gene [9,39]. However, high rates of resistance to other antibiotics have been reported for ST398 from pigs. For example, a Belgian study showed that, among 643 MRSA isolates, 97% were resistant to trimethoprim, 73% to lincosamides, and 32% to fluoroquinolones [39]. However, rates may vary according to several factors, including country, type of farm, and age of the animals.

Several novel resistance genes have been discovered in MRSA ST398. These include: the apramycin resistance gene *ampA* [92], *vga*(C), which encodes resistance to streptogramin A, pleuromutilin, and lincosamides [93], *dfrK*, which encodes resistance to trimethoprim [94], and *vga*(E), which encodes resistance to streptogramin A, pleuromutilin, and lincosamides. The last of these genes is integrated in the well-known transposon Tn*554* [93]. The *dfrK* gene was also seen as part of a new combination of plasmid-borne resistance genes that also included *erm*(T), which encodes macrolide–lincosamide–streptogramin A resistance, and *tet*(L), which encodes resistance to tetracycline [95]. It is likely that, sooner or later, these genes will also be transferred to human-specific lineages, thereby further compromising our ability to treat *S. aureus* infections.

Vaccination

The disease burden and the number of fatalities caused by *S. aureus* are high in both humans and livestock. Initially, the need for an *S. aureus* vaccine was mainly determined by the economic loss in dairy farming resulting from mastitis in cows. Infections could be readily treated with antibiotics, although a vaccine would be an important addition to the armamentarium. The problem of *S. aureus* infection has increased with increasing levels of methicillin resistance. Vaccination could help to reduce the burden of mastitis in cows. The need to clear MRSA ST398 from livestock is less clear. The disease burden resulting from ST398 appears to be low, certainly in pigs. Also, the danger to humans is rather limited. However, with the development of more human-adapted strains this may well change, in particular when these more human-adapted strains are also still well adapted to livestock and widespread among livestock. ST398 in livestock will then become a public health hazard, and vaccination of animals may then reduce this risk.

However, the development of a vaccine against *S. aureus* has proved problematic. Three decades of extensive research did not result in a protective vaccine on the market. Numerous vaccination studies have been initiated, with

a variety of different approaches. These include whole cells, individual proteins or polysaccharides, modified toxins, fusion proteins, and DNA vaccination. Many were tested in combination with adjuvants. The results of nearly all studies showed that an antibody response was obtained. A number of studies provided additional *in vitro* data, such as enhanced opsonophagocytosis by polymorphonuclear leukocytes, and T-helper and cytokine responses. However, challenge experiments have shown a less favourable response. The reasons why these prototype vaccines failed may be multiple, and different reasons may apply to different studies. Vaccination studies have been reviewed recently [24,96].

Concluding Remarks

There is a tendency to declare livestock-associated MRSA synonymous with ST398. However, multiple STs belonging to different CCs are livestock-associated, e.g. CC97 and CC151 in bovine mastitis [97]. These include both MRSA and MSSA isolates. In addition, human-associated isolates are also found among livestock, just as livestock-associated MRSA can be found among humans. Nevertheless, among livestock-associated MRSA, ST398 seems to dominate, followed by ST9. ST398 is not clonal, but consists of a highly variable set of strains.

The most important danger is when host-adapted strains acquire virulence factors that enable them to colonize and infect new hosts. The biggest threat in this respect is further adaptation of ST398 to humans, because of its pandemic nature and the huge reservoir of livestock animals. The first signs of potential adaptation are indicated by the acquisition of PVL, which is thought to be a major toxin in community-acquired MRSA, and phages encoding human-specific innate immune evasion factors. During the final preparation of this manuscript, Price *et al.* provided whole genome sequence-based evidence that ST398 originated in humans, adapted to humans, and is now adapting back to humans by the acquisition of phage carrying human-specific immune evasion factors [98].

The presence of increasing numbers of multiresistant isolates among livestock limits treatment options. Vaccination would be a logical solution, but three decades of research have not resulted in an effective vaccine on the market. New approaches have recently been suggested [96]. This may help to solve this issue and the problem of livestock-associated *S. aureus*.

Transparency Declaration

Conflict of interest: nothing to declare.

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