Animal and Human Health Implications of Livestock Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Historical background of MRSA in humans and animals

Staphylococcus aureus is a bacterium that commonly colonizes the skin and mucosal surfaces of many mammalian and avian species. The nasal cavity is the most epidemiologically important site for colonization of humans, and estimates of the prevalence of nasal carriage of *S. aureus* by people are of the order of 20-30%.[1,2]

S. aureus is also an opportunistic pathogen and a leading cause of bacterial infections of people, causing a broad spectrum of pathology ranging from minor skin infections to fatal invasive disease. Nasal colonization is considered a risk factor for developing clinical infections, but the link between colonization and disease is poorly understood.[3]

Prior to the availability of antibiotics, the fatality rate of cases of *S. aureus* bacteremia was of the order of 80%.[4] The introduction of penicillin reduced the case fatality rate of *S. aureus* bacteremia to around 20%, but was almost immediately compromised by the emergence of penicillin resistant strains. The later introduction into human medicine of methicillin, a semi-synthetic derivative of penicillin that is resistant to penicillinase enzymes, similarly was soon followed by the emergence of *S. aureus* resistant to that drug (MRSA). The capacity of *S. aureus* to acquire resistance to antibiotics has made multiple-resistant strains a major public health concern.[5]

MRSA became endemic in hospitals in the 1960s (hospital associated MRSA, or HA-MRSA). Later, in the 1990s, MRSA emerged worldwide to become a prevalent cause of infections in the general community (termed community associated MRSA, or CA-MRSA).[5] *S. aureus* is a 'clonal' organism, and epidemic waves have been described when particular *S. aureus* clones have predominated among human cases over particular time periods and places.[6] Clones associated with hospital infections have generally been different from clones involved in infections in the general community, and different clones have been predominant in different geographical regions.[7,8]

The mechanism of action of all beta-lactam antibiotics (penicillins, cephalosporins, carbapenems) is to bind to a protein involved in bacterial cell wall synthesis (penicillin binding protein, or PBP). MRSA contain the *mecA* gene which encodes for an altered protein (PBP-2) with low affinity for beta lactams, thus conferring resistance to these classes of compounds which do not bind effectively to the altered target molecule.[9]

The *mec*A gene is carried on a mobile chromosomal element (Staphylococcal Cassette Chromosome mec, or SCCmec) which may contain other genes conferring resistance to other antibiotics or heavy metals.[9] Therefore many isolates of MRSA display multiple resistance

profiles. Also, typing of SCCmec is one of the methods used to differentiate among MRSA strains and to study the evolution of these organisms. Since 2007, a homologue of *mec*A that is only 69% identical at the DNA level (63% at amino acid level) to *mec*A has been found in diverse *S. aureus* isolates from several animal species. Now denominated as *mec*C, this variant has been described in many European countries, but as yet has not been found in MRSA isolates from pigs, nor in the predominant lineage (ST398) of MRSA in livestock in Europe.[10]

Several methods have been employed for subtyping *S. aureus*, including phage typing, pulse field gel electrophoresis (PFGE), multilocus sequence typing (MLST), spa typing, and SCCmec typing (Table 1).[7,8,11] Although PFGE is the standard method used historically in medical laboratories, sequence based methods (MLST, spa typing, SCCmec typing) are increasingly used due to their greater reproducibility among laboratories and the availability of online databases that facilitate comparison of typing data from diverse geographic regions.

MRSA were first isolated from people in 1961, and from animals (cattle with mastitis) in 1972. However, prior to 2004 animals were not considered to have any significant role in the epidemiology of MRSA in humans. Increasingly frequent reports of MRSA in a range of pet, food animal, equine, and wildlife/exotic animals have raised concerns about animals as reservoirs of MRSA (Table 2).[12]

Livestock associated MRSA is the term applied to a group of closely related MRSA isolates first identified in three people with direct or indirect exposure to pigs in Holland.[13] These previously unrecognized strains were not typable using the standard PFGE method (sma1 enzyme) used to type MRSA in Holland and were found to belong to a new MLST sequence type, ST398.[14] A survey found 39% of pigs slaughtered in Holland were positive for ST398 MRSA, including three spa types within the ST398 lineage. This striking finding led to considerable research into ST398 lineage in animals and humans in Holland, and to surveys for MRSA in pigs and other livestock in many countries.[15] Apart from two reports of outbreaks of exudative epidermitis in pigs,[16,17] the organisms appear to be sporadic and opportunistic pathogens in pigs and not of major significance to swine health.[18]

Reports from several European countries have confirmed that ST398 MRSA is typically the predominant lineage detected in swine populations on that continent. Although ST398 MRSA have also been reported in pigs in Asia,[19] most studies in that continent have found MRSA of the ST9 lineage to be predominant.[20-23] Initial studies in North America confirmed ST398 MRSA is present in pigs in both Canada and the USA.[24,25] but there is growing evidence that MRSA of the ST5 lineage are also prevalent,[24,26,27] and also two reports of ST9 MRSA.[26,28] Ongoing studies of *S. aureus* in pigs and swine veterinarians across the USA have found that MSSA of ST398, ST9, and ST5 appear to be widespread in pigs and swine veterinarians in the US swine industry.[29]

The realization that people exposed to livestock were at greatly elevated risk of colonization with ST398 MRSA,[13] led to changes to patient screening practices in the Netherlands, a country with a low endemic MRSA incidence and where a “search and destroy” policy is used to control MRSA in hospital settings.[30] The decision to screen patients with occupational exposure to pigs or cattle in 2006 led to marked increases in positive screening results and subsequent costs in managing these patients.[30-32]

An extensive review of livestock-associated MRSA in Europe concluded that in some countries with low prevalence of human MRSA infection, livestock-associated isolates make a major contribution to the overall MRSA burden.[33] In 2007, 30% of all MRSA human isolates typed by a national institute in the Netherlands belonged to the ST398 lineage, although most isolates were from screening at risk patients on admission to hospitals rather than from actual clinical infections.[34] It is important to note that some reports from Europe do not distinguish between positive screening cultures and clinical infections when reporting 'case' numbers. However isolates of the livestock-associated lineage have been associated with both superficial and systemic infections of humans (see Supplement 1).[31,35-38]

Where ST398 is prevalent in livestock, people with occupational exposure to live animals are at greater risk of MRSA colonization, and therefore infection, than the general population.[13,39-41] There is considerable occupational risk of nasal colonization of pig farm workers, veterinarians, and abattoir workers.[40,42-44] Although isolates of the ST398 clonal complex have been predominant in most studies, this likely reflects the status of the swine populations. Individual studies have reported ST5 and ST9 isolates to be predominant in people exposed to pigs in North America and Asia respectively.[22,27]

Community-based studies in swine-dense rural areas of both Germany and Holland found that exposure risk appears to be limited to people having direct contact with pigs (farmers and veterinarians) and their immediate families, and did not extend to the adjacent communities.[39-41] One spatial analysis in the Netherlands reported a higher ratio of rural vs. urban residents among individuals positive ST398 MRSA than individuals positive for other MRSA and claimed elevated risk for rural residents without animal contact.[45] However, the study involved only a small number of isolates and had other methodological limitations that make the inference questionable.[46] A single ecological study at county level in Pennsylvania associated risk of HA-MRSA and CA-MRSA with spreading of animal manure,[47] but the observed effect was small (odds ratio <1.4 between lowest and highest quartile groups), no typing of isolates was conducted, and the validity of this weak association remains to be confirmed. There has yet to be a community based outbreak of ST398 infection reported from any country, and the evidence to date indicates any health risks associated with ST398 organisms of livestock origin are overwhelmingly concentrated among people with direct animal contact and, to a lesser extent, their immediate families.

The public health consequences of livestock associated MRSA are not well defined, and available information relates almost exclusively to the ST398 lineage. Due to lack of information about the potential public health implications of other MRSA lineages found in pigs (e.g., ST5, ST9), the following discussion is restricted to the ST398 MRSA. Some reports of severe or fatal systemic infections in people with ST398 *S. aureus* suggest that organisms of this lineage have the potential to be serious human pathogens.[35,36,48,49] Unfortunately, many European studies have not distinguished clearly between events of colonization and clinical infection,[34] and thus there is limited quantitative information about the actual clinical risks associated with livestock exposures and colonization with ST398 MRSA.

Globally, there have been only 2 ST398 MRSA fatalities reported over 9 years,[38,50] compared to an estimated 18,000 annual MRSA fatalities in the USA alone.[51] Population-based estimates (cases per 100,000 people per year) of the incidence of ST398 MRSA infections in pig dense areas where ST398 MRSA are prevalent are: 2 clinical infections, 0.38 invasive infections, and 0.04 bacteremia cases in the Netherlands;[31,52], and 0.25 clinical infections in Denmark (none invasive).[53] In contrast, the CDC estimates 31.8 invasive cases and 6.3 fatalities from MRSA per 100,000 people per year in the USA.[51] Although ST398 MRSA have been found in nasal swabs of USA livestock, farmers, and veterinarians, there has yet to be a case of clinical infection with ST398 MRSA reported in the USA. Future reports of ST398 MRSA infections, particularly minor skin infections or contaminated wounds, are almost certain to occur and will need to be assessed in the context of the substantial burden of MRSA infections due to human adapted strains in the USA.

Observations that some spa types (t571, t567) of ST398 appear to be more common among human clinical isolates than livestock isolates,[31,54-56] first suggested the possibility that ST398 variants may differ in their ability to colonize and/or cause disease in humans. It has now been established that there are distinguishable human and swine clades of ST98/t571 *S. aureus*,[57,58] and that the human clade is 'animal independent' or can persist in human populations without livestock reservoirs. PCR based tests that distinguish the human from livestock clades of ST398 *S. aureus* are now available.[59] Genetically distinguishable human and swine variants of ST1/t127 MRSA have also been described,[60] and it is feasible that increased use of more discriminating genotyping methods will reveal similar patterns of host adaptation within other lineages of livestock associated *S. aureus* (e.g., ST9, ST5).[61,62] Human infections with ST398 *S. aureus* cannot be automatically assumed to originate from livestock, particularly when the variants isolated have attributes that are not common in animal isolates and there is no history of animal contact.[54]

Observations on colonization, transmission and virulence of ST398 MRSA

Colonization

It has been consistently reported that people with regular contact with pigs colonized with ST398 MRSA are frequently culture positive (nasal and/or throat swabs) for the same organisms found in the animals. All studies have used multiple enrichment protocols and have reported prevalence of culture positivity. There are no studies reporting quantitative data on numbers of ST398 organisms present in swabs. As *S. aureus* organisms appear to be among the predominant organisms in bioaerosols of swine barns,[63] discriminating between transient contamination of airways or skin and true colonization of humans is problematic, particularly when workers have had recent animal contact where contamination risk is high.

Several studies indicate that culture positivity for ST398 MRSA is transient in most people exposed to colonized animals. Presence of MRSA in veal farmers was strongly related to duration of animal contact and was markedly reduced after periods without animal contact.[64] Among research workers sampling animals at pig farms with ST398 MRSA, 17% of exposures led to colonization of previously culture-negative workers, but all but one individual were culture negative after 24 hours.[65] Similarly, a study in Iowa found 22% of veterinary students had

culture positive nasal swabs following exposure to MRSA-positive pork farms, but all were culture negative by 24 hours post visit.[27] Further insight into *S. aureus* colonization and livestock exposure has come from longitudinal studies of in swine veterinarians in the Netherlands and USA.[44,66] These studies found comparable *S. aureus* prevalence of the order of 70%, but the MRSA prevalence in Dutch veterinarians (44% of samples) greatly exceeded that of US swine veterinarians (8%). Moreover, both studies found that similar minorities (14%, 21%) of veterinarians to be permanently colonized with the same *S. aureus* variants (MRSA or MSSA) while the majority were intermittently colonized or consistently culture negative. The apparently transient nature colonization following most events of exposure to *S. aureus* in animal populations is consistent with observations that culture positivity occurs predominantly among animal workers and their immediate families, but is very uncommon in the general community even in areas with a high density of pig farming. Further studies are necessary to better understand the temporal dynamics of exposure and colonization, including quantitative evaluation which is likely important for risk of person-to- person transmission and also risk of clinical infection.

Transmission (person to person)

Initial studies assessing the transmissibility of ST398 among people at hospitals consistently found lower transmission risk for ST398 compared with common human MRSA isolates, with estimates that transmission risk was reduced by approximately one quarter to one third.[30,67,68] Recently a much larger study involving 62 hospitals in the Netherlands also estimated that the risk of transmission of ST398 MRSA was 4.4 times lower (i.e. 23%) than for other MRSA.[69] The explanation for the apparently lower transmissibility of ST398 among humans is unknown. Possible mechanisms that could be investigated include lower risk of colonization of humans at the same exposure dose (if the ST398 organisms are less well adapted to humans), or lower exposure risk (due to lower numbers of organisms in colonized people). The conclusion from one study was that the transmission risk of ST398 may be too low to support an outbreak,[68]and it was proposed that the practice of preemptive isolation used to manage MRSA cases in the Netherlands may not be necessary for LA-MRSA due to the lower transmission risk.[69]

Virulence

S. aureus is considered unsurpassed among human pathogens with respect to its versatility of pathogenic strategies, numbers of virulence factors, and capacity to survive and multiply in a wide range of environments.[70] Numerous factors have been identified as virulence-associated factors in *S. aureus*, including toxins, adhesins, enzymes, and immune-modulators.[71] This vast range of virulence factors likely underpins the wide biological variability in clinical expression of staphylococcal infections. For example, significant differences were found in production of secreted virulence factors by CA-MRSA, HA-MRSA and community-associated MSSA.[72]

The epidemiological significance of specific virulence determinants is often uncertain. A small number of factors, termed superantigens, are known to be necessary for certain clinical manifestations of staphylococcal disease. Most notable are the toxic shock syndrome toxin-1 and enterotoxin genes (associated mostly with foodborne staphylococcal enterotoxicoses). The Panton-Valentin Leucocidin (PVL) gene is a virulence determinant of emerging concern due to

its prevalence among clones causing epidemics of community associated infections in many countries, although its importance remains uncertain.[73]

With respect to the virulence of ST398 MRSA, a German study found a significantly shorter length of stay in hospitals (7 days versus 13 days) for patients with ST398 MRSA versus other MRSA, and less likelihood of being admitted to intensive care.[74] The early studies of the presence of known *S. aureus* virulence factors in ST398 were generally consistent in reporting a low frequency of virulence factors.[75-78] A study of 52 ST398 isolates from pigs and humans in Holland found only one isolate of human origin was positive for PVL.[75] Other studies of small numbers of isolates from cases of skin infections linked to exposure to pigs also report a general absence of recognized virulence factors.[77] A Dutch study found only 3 of 1,738 ST398 MRSA (0.17%) to be PVL positive compared with 461 (19.2%) of 2,405 non-ST398 MRSA.[79]

Schijffelen et al (2010) conducted full genome sequencing of a single ST398 isolate (spa type t011) from a non-fatal case of human endocarditis in a transplant patient.[78] Although two virulence factors were detected, the isolate lacked several virulence factors (enterotoxins, and phage encoded toxins including PVL) and the authors suggested that the lack of major virulence factors may explain the relative infrequency of serious clinical infections with ST398 MRSA.

A larger study of 100 ST398 isolates from various sources (healthy carrier and diseased pigs, dust from pig farms, milk, and meat) in Germany examined 37 virulence and 31 resistance determinants.[76] A high number of resistance determinants and a low number of virulence factors were identified. The authors speculated that use of antimicrobials in livestock production could select for resistance determinants, and the lack of virulence determinants could be attributable to limited interaction of livestock adapted strains to more pathogenic bacteria common in hospitals.

A Belgian study comparing 18 ST398 isolates with CA-MRSA and HA-MRSA isolates from the same region found the ST398 isolates constituted a homogenous lineage distinct from the HA-MRSA and CA-MRSA strains.[80] In particular, the accessory genome content of ST398 strains lacked human-associated virulence and adhesion determinants. The authors also noted that the absence of enterotoxin genes among ST398 LA-MRSA strains pointed to their likely insignificance with respect to risk of foodborne enterotoxicosis.

Similar findings continue to be reported and loss of major virulence factors appears to be associated with host adaptation of *S. aureus* following events of interspecies transmission.[81-83] A comparative study of virulence genes among multiple lineages found in animals [sequence types ST398, ST5, ST8, ST15, ST22, and clonal complexes (CC) CC30, CC97, CC130, and CC151] found the ST398 lineage displayed the lowest content of virulence genes, and that all MRSA ST398 isolates lacked accessory virulence genes that were present in the other lineages.[84] The number of isolates evaluated in genomic studies remains modest, and sampling has been by convenience and heavily biased towards MRSA rather than MSSA from pigs. However, the relative scarcity of recognized virulence factors for humans among ST398 *S. aureus* isolates of porcine origin has been a consistent finding.

Public health risks associated with ST398 MRSA

Understanding of the emergence of ST398 MRSA and their human health implications is incomplete. A study of clinical infections with ST398 MRSA in a hog dense region of Denmark concluded 'we therefore face an infectious occupational exposure of huge quantitative dimensions but of unknown clinical importance'.[53] Although the risk of exposure is heavily concentrated primarily in people with occupational exposure to livestock (particularly pigs or cattle), as yet there are no data documenting elevated risk *of clinical infection* in these groups (e.g. farmers and veterinarians). However, there are case reports of skin and soft tissue infections in people working with pigs,[85-88] indicating that prompt treatment of skin wounds should be emphasized in producer education programs, as well as the prudence of seeking medical attention if signs of infection are evident.

There is yet to be any community outbreak linked to ST398 MRSA, and only two reported institutional outbreaks in a hospital and a nursing home. The hospital outbreak involved 10 subjects, but the report did not clearly differentiate colonization from infection, and all infected lesions appear to have been diabetic ulcers in which a primary etiologic role of the bacteria is unlikely (versus secondary infection or simply wound contamination).[89] The more recent 'outbreak' involved only 9 subjects and also described colonization rather than infection, apart from in the index case who had an infected wound.[90]

As noted above, there have been occasional reports of severe infections of humans with ST 398 *S. aureus*.[31,35,36,49] To date, there have been 6 reported fatalities associated with ST398 *S. aureus.* Two fatal cases were MRSA (spa type t011, spa type not reported) and only one involved contact with livestock (pigs). The 79 year old man suffered from lung carcinoma and chronic obstructive pulmonary disease, and the organism was isolated from pleural fluid.[38] The second fatal infection resulted from an infected catheter in an 84yo woman following thoracic surgery.[50] Among the remaining 4 fatalities, all were infections in people without known livestock contact and involved ST398/t571 MSSA (4 cases).[48,91,92], and therefore were likely the human clade than the pig clade of t571.[57] Other than infected bite wounds, reports of medically significant ST398 MRSA infections in healthy livestock workers remain remarkably scarce, and known livestock contact is a notably uncommon feature of invasive ST398 infections.

Studies assessing the relative importance of ST398 infection in human disease in Europe have found significantly lower incidence of systemic infections with ST398 cases. A survey of 24 laboratories in 17 countries in Europe in 2007 found ST398 MRSA accounted for only a small proportion of MRSA isolates from humans in 2007, with most cases identified in the Netherlands, Belgium, Denmark, and Austria.[93] Furthermore, ST398 isolates were significantly less likely to be found in cases of systemic disease compared with other MRSA. A larger study of 357 laboratories serving 450 hospitals in 26 countries collected 2,890 MSSA and MRSA isolates from patients with invasive *S. aureus* infection.[94] This study found no cases invasive infections with ST 398 MRSA. However, MSSA isolates of the ST398 lineage were found in 12 invasive cases. In Denmark, where MRSA is notifiable in people, detailed national reports (DANMAP) from 2009 to 2012 include a total of 45 bacteremic cases of ST398 infection, of which only 2 were MRSA. As in Holland and Germany, these cases constitute only a small percentage (around 1%) of human cases of human *S. aureus* cases in Denmark. Neither spa types nor contact with pigs were reported, therefore the proportion of the bacteremic cases attributable

to the human t571 clade cannot be determined. Overall these studies of clinical infections, plus evidence of the rarity of virulence factors in ST398 isolates from pigs, point to a relatively modest impact on public health of livestock associated MRSA in endemically infected regions.

The number of cases of ST398 MRSA infections recorded in Denmark has steadily increased between from 2007 and 2012.[95,96] Most of these cases are skin and soft tissue infections in people exposed to pigs. Similarly, an extensive retrospective study from 2008 to 2012 of MRSA isolates from 39 German hospitals in a swine dense region indicated an increasing proportion of ST398 MRSA among screening samples and among clinical cases, including bacteremia.[97] However, in both reports, case ascertainment or work up biases could be contributing to the observed increases in case numbers, and the absolute number of severe infections remains small (e.g., 7 cases of ST398 bacteremia over 18 months in Germany; 2 cases in Denmark in 2012). Therefore, although there is some indication of increasing prevalence among cases in Europe, the impact of ST398 MRSA on human illness in pig dense regions remains modest.

We have compiled data from 108 publications relevant to ST398 (MSSA or MRSA) infections in humans (Supplement 1). In some publications cases of clinical infection were not distinguished from cases of colonization or contamination (i.e. with no pathological process attributable the presence of the organism). For the 1,492 cases that could be identified as clinical infections, the associated illness or pathology was not specified for 527 cases. Among the 965 cases with some clinical description, the most common presentations were skin and soft tissue infections including wounds (451 cases, 46.7%); bacteremia (167 cases, 17.3%); and pneumonia (99 cases, 10.3%). Using a very inclusive definition of invasiveness (i.e., infections other than skin and soft tissue infections) a total of 400 reported ST398 infections were deemed to be invasive. For context, the sum of invasive infections due to ST398 S. aureus (MRSA and MSSA) reported globally from 2004 to 2013 equates to approximately 0.4% of the invasive MRSA cases estimated in the USA in 2005.[51]

Information on human disease associated with ST398 *S. aureus* is more sparse in North America than in Europe. There is yet to be a clinical infection with ST398 MRSA reported in the USA. A retrospective study of 3,687 MRSA clinical isolates in Canada identified only 5 cases with ST398 MRSA, 4 of which presented with skin or soft tissue infections.[98] The CDC has examined over 12,000 MRSA isolates and is yet to identify ST398 among US human clinical isolates (Dr. Brandi Limbago, personal communication 2011). Similarly, in the hog dense state of Minnesota, the MN Department of Health has tested over 7,000 clinical isolates of MRSA with sma1 PFGE (inability to type isolates with sma1 is a characteristic of ST398 lineage) and is yet to identify an 'untypable' isolate (Dr. Kirk Smith, personal communication,2013). Given the known presence of ST398 in the North American swine industry, and the sporadic reports of clinical infections in swine workers in Europe, it is likely that some clinical infections may have occurred in occupationally exposed individuals in the USA. The absence of reported cases until now in the USA is likely attributable to both a low incidence and low severity of ST398 infections.

In contrast, the human clade of t571 ST398 MSSA may be relatively common in the USA. Retrospective typing of 4,167 clinical *S. aureus* isolates from studies of inpatients and outpatients in the New York City found 8 MSSA isolates with ST398-associated spa types, of which 6 were t571. A subsequent study found the human clade of t571 in 5% of non-invasive

MSSA cases and 2.5% of MSSA bacteremias.[57] The same variant was also recently reported to be the most common MSSA variant colonizing detainees in a Texas prison.[99] To date, the only documented cases of clinical ST398 infections in the USA have had no known livestock contact and involved the human clade of t571 that is considered 'animal independent'.

There are numerous reports of detection of ST398 MRSA in meat products including pork,[100-102] with high prevalence (11% of pork; 35% of turkey meat) reported in one study in the Netherlands.[103] North American studies have generally used convenience sampling and results regarding prevalence and the subtypes isolated are variable.[26,104-108] The relative importance of pigs, compared with other sources (particularly people) in the abattoir and meat processing chain, in contamination of retail pork with MRSA remains uncertain. It is also likely to vary among countries. Foodborne staphylococcal enterotoxicosis ("food poisoning") is an important disease caused by S. *aureus* toxins ingested in food. However, staphylococcal food poisoning is not an infectious process, antimicrobial treatment is not indicated for treatment, and therefore the antimicrobial resistance of foodborne *S. aureus* is of no clinical relevance in this disease. Risk is determined by the ability of staphylococci to produce toxins. Current evidence indicates negligible foodborne risk due to ST398 *S. aureus* in meat because the typical absence of enterotoxin genes in ST398 isolates means these strains are unable to produce the toxins responsible for staphylococcal food poisoning. A Swiss study reported the complete absence of overlap of spa types causing staphylococcal food poisoning in people and spa types of isolates from pork carcasses or bovine mastitis, concluding that neither milk nor pork are common causes of staphylococcal enterotoxicosis.[109] Arguably the sole material concern with ST398 MRSA in the food supply relates to the potential risk of pork (or other animal products) as mechanical vehicles for transmission of MRSA to consumers handling the products.[110,111]

Although slaughter plant workers with live animal exposure are at elevated risk of colonization,[112] MRSA was not detected in professional meat handlers despite their exposure to contaminated product.[42]A report from the European Food Safety Agency concluded that the risk from contact with contaminated food appears to be small, and certainly much reduced from that associated with contact with live animals or humans.[33] The DANMAP 2010 report inferred that "the relatively frequent occurrence of MRSA in meat combined with very few cases in urban areas makes it safe to conclude that there is very little if any risk for meat being a risk for contracting MRSA CC398".[113] This scenario is unchanged in the most recent data from Denmark.[96] Relative to the high rates of exposure to ST398 for people occupationally exposed to live animals, the theoretical (but likely non-zero) risk of exposure of consumers via the food chain is arguably trivial. As suggested by Weese et al (2010), "standard recommendations for handling and cooking raw meat should greatly reduce if not eliminate the risk of transmission of MRSA, just as proper cooking and food handling should reduce or eliminate the risk of enterotoxin-associated gastroenteritis."[102]

Summary

The apparently recent emergence of ST398 MRSA in livestock populations in many countries is a valid cause of consternation, and the public health implications need to be better understood. However, almost a decade after being first recognized the burden on human health remains minor, although possible increasing in some regions. The risk of exposure to ST398 MRSA of

livestock origin is overwhelmingly concentrated in people with occupational exposure to livestock. Available data indicate that ST398 MRSA originating from livestock are less transmissible among people, and also less virulent, than other MRSA variants. Risks to people without direct livestock contact appears to be minimal, even in pig dense communities, and foodborne transmission also appears to be of negligible concern. The factors underlying the emergence of ST398 in animal populations are not understood, and likely are complex. Quantifying the occupational health risks in livestock workers, and education of these groups about proper management and treatment of wounds should be the main priorities in the immediate future.

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Table 1: Outline of methods for subtyping of MRSA

Method	Mechanism	Strengths/Limitations	Livestock MRSA
Pulsed Field Gel Electrophoresis (PFGE)	Cut bacterial DNA into large fragments with restriction enzymes (e.g. sma1). Separate fragments using pulsed electric fields and examine patterns in gels. Not sequenced based.	Most discriminative method. Relatively subjective. Interlaboratory variation Rapid and useful for outbreak investigation Sma1 enzyme widely used for typing human isolates	ST398 isolates untypable with sma1 enzyme. Other enzymes can be used with ST398 isolates.
Multilocus sequence typing (MLST)	DNA sequencing of 7 "housekeeping" genes that are highly conserved (limited variation) in the bacteria of interest. 450-500 bp of each gene sequenced and different sequences assigned as distinct alleles. Patterns of alleles used to define the sequence type (ST) of isolates. Easy interlaboratory comparison via international databases.	Expensive Less discriminating than PFGE/spa typing Better for studying long term evolution vs. outbreak investigation	ST398 isolates defined by MLST based on novel sequence type (398) not previously recognized in human isolates.
Spa typing	DNA sequence analysis of the protein A gene variable repeat region of S. *aureus*. Easy interlaboratory comparison via international databases. Two typing databases: egenomics (USA) and Ridom (Denmark), with Ridom database most often cited.	Relatively inexpensive Sequence based Spa types mostly but not always correspond with MLST types (same spa types can occur in different MLST types	(Ridom types) t011, t108, t034, t567, t899, t571, t2330, t2121,..etc (over 30 spa types within ST398)
Staphylococcal Cassette Chromosome typing (SCCmec)	DNA sequencing of the ccr gene complex (ccr) and mec gene complex (mec) that comprise the SCCmec cassette (containing g the mecA gene that confers methicillin resistance) Now 11 major types (I – XI), and many subtypes	Standardized methodology not yet established Useful for evaluation of bacterial evolution of methicillin resistance	ST398 isolates have varied SCCmec types. III, IV, V, Untypable, .. Type V appears most common

Note: other methods (e.g. multiple-locus variable-number tandem-repeat assay) are available but are less widely used.

Table 2: Timeline of reports of isolation of MRSA from non-human species (adapted from Leonard and Markey, 2007)[12]

Years	Cattle	Cat	Dog	Horse	Chicken	Sheep	Rabbit	Pig	Seal	Guin. Pig
70s	+									Turtle
80s		+	+							Bat
90-95			+							Parrot
96				+						
97				+						
98		+								
99			+	+						
00										
01										
02										
03	+ (milk)		+		+ (muscle)					
04			+	+		+	+			
05	+ (milk)	+	+	+			+	+	+	
06		+	+	+				+		
07	+	+	+	+			+	+		+

SUPPLEMENT 1: Summary of reports of clinical cases of ST398 *S. aureus* infections in humans

Author	Ref	Country	Year	Screen	Clin	UNS	SSTI	BT	EC	PN	RF	OM	UR	Misc	INV	FAT
Armand-Lefevre L	1	France	2005	6	0										0	
Aschbacher R.	2	Italy	2012		1			1							1	
Aspiroz C	3	Spain	2010	6	1		1								0	
Aspiroz, C	4	Spain	2012	5	3									3	0	
Bhat M	5	USA	2009	13	0										0	
Camoez M	6	Spain	2013		10			1		4				5	**6**	1
Chen H	7	China	2010		29	29									0	
Chlebowicz M	8	Holland	2010		2		1			1					1	
Chroboczek T	9	France	2013	0	89		29	22	7	12		6		13	41	
Cuny C	10	Germany	2009	146	0										0	
Cuny C	11	Germany	2013		6		3	2						1	2	
DANMAP 2007	12	Denmark	2007	10	9		4	**5**							0	
DANMAP 2008	13	Denmark	2008	45	22	16		**6**							0	
DANMAP 2009	14	Denmark	2009		50	40		10							10	
DANMAP 2010	15	Denmark	2010		120	109		11							11	
DANMAP 2011	16	Denmark	2011		74	63		11							11	
DANMAP 2012	17	Denmark	2012		105	92		**13**							**13**	
De Vries L	18	Denmark	2009		4			4							4	
Declercq P	19	Belgium	2008	0	1									1	0	
Denis O	20	Belgium	2009	47	1		1								0	
Deurenberg R	21	Europe	2009	12	0										0	
Donker GA	22	Holland	2009	4	0										0	
Drougka E	23	Greece	2012		1			1							1	
Edwards G	24	Scotland	2008	0	3	3									0	
Ekkelenkamp M	25	Holland	2006		1				1						1	
Fanoy E	26	Holland	2009	5	1		1								0	
Garcia-Grealls C	27	Europe	2012	16	0										0	
Golding G	28	Canada	2010		4		3							1	0	
Graveland H	29	Holland	2010	16	0		0								0	
Grisold A	30	Austria	2010		6		**4**					1	1		2	
Grundmann H	31	Europe	2010		12	12									12	
Hartmeyer G	32	Denmark	2010		3		**2**			1					1	
He W	33	China	2013		9			9							9	
Hetem D	34	Holland	2013		0										0	
Huber	35	Switzerland	2010	3	0										0	
Huisjdens X	**36**	Holland	2006		1								**0**	**1**	1	
Huisjdens X*	37	**Holland**	**2009**	**793**	**0**										**0**	
Ip M	38	Hong Kong	2005		3			3							3	

Jimenez J	**Ref**	Colombia	2011	0	1		1							0	
Author	42	**Country**	**Year**	**Screen**	**Clin**	**UNS**	**SSTI**	**BT**	**EC**	**PN**	**RF**	**OM**	**UR**	**Misc**	**INV**	**FAT**
Khanna T	39	Canada	2008	5	0										0	
Kock R	40	Europe	2009	73	0										0	
Kock R	41	Germany	2009	249	0										0	
Kock R	43	Germany	2011	149	18		6	2			4		1	5	7	
Kock R	44	Germany	2013	2255	**352**		200	16	0	48	15	0	18	55	113	
Krziwanek, K	45	Austria	2009	15	5									5	0	
Larsen A	46	Denmark	2009	0	6	6									0	
Lewis H	47	Denmark	2008	11	10		10								**1**	
Lozano C	48	Spain	2011	0	1		1								0	
Lozano C	49	Spain	2011	0	1									1	0	1
Lozano C	50	Spain	2011	1	1		1								0	
Lozano C	51	Spain	2012	9	26		10	2					1	13	4	
Luxner J	52	Austria	2013		1			1							1	
Mammina C	53	Italy	2010	0	1		1								0	
Mammina C	54	Italy	2010	0	1					1					1	
Mammina C	55	Italy	2012	0	3		2			1					1	
Mediavilla J	56	USA	2012		8		4	4							4	
Monaco M	57	Italy	2013	5	1									1	0	
Moodley A	58	Denmark	2008	4	0										0	
Mulders M	59	Holland	2010	21	0										0	
Omland L	60	Denmark	2012		3		2							1	0	
Pan A	61	Italy	2009	1	1									1	0	
Pomba C	62	Portugal	2009	1	0										0	
Pomba C	63	Portugal	2010	5	0										0	
Rasigade J	64	France	2010		1					1					1	1
Rijnders M	65	Holland	2009		5	5									0	
Ruhlmann C	66	Denmark	2008	0	2		1							1	0	
Ruimy R	67	French Guiana	2010	1	0										0	
Salmenlinna S	68	Finland	2010	6	4		3							1	0	
Schaumburg F	69	Germany		-	14		5	1	1	1	3		2	1	8	
Schijffelen M	70	Holland	2010		0										0	
Senneville E	71	France	2013		35							31		4	**31**	
Smith T	72	USA	2009	9	0										0	
Soavi L	73	Italy	2010		1									1	1	
Springer B[a]	**74**	**Austria**	**2009**	**21**	**0**										**0**	
Stegger M	75	Denmark	2010	2	2		2								0	
Tavares A[a]	**76**	**Portugal**	**2013**		**16**	**16**									**0**	
Tristan A	77	France	2012		7			2	5						7	
Uhlemann A	78	Carribean	2011	0	18		18								0	

Uhlemann A	**Ref**	USA	2012	14	12	4	4	4							4	
Author	42	**Country**	**Year**	**Screen**	**Clin**	**UNS**	**SSTI**	**BT**	**EC**	**PN**	**RF**	**OM**	**UR**	**Misc**	**INV**	**FAT**
Uhlemann A	79	USA	2013	6	61		40	4		9		3	2	3	18	
Valentin-Domelier A	80	France	2011		18			18							18	2
van Belkum	81	Holland	2008		13	1	**6**	3			1		2		6	
van Cleef B	82	Holland	2010	14	0										0	
van Cleef B	83	Holland	2010	14	0										0	
van Cleef B[a]	85	Europe	2011	46	113	113									0	
van Cleef B	86	Holland	2013		3			3							3	
van den Broek I	87	Holland	2009	33	0										0	
van der Mee-Marquet N	88	France	2011	0	4			1						3	1	1
Van Hoecke H	89	Belgium	2009		1							1			1	
van Loo I	90	Holland	2007	16	19		10			3				**6**	3	
van Rijen M	91	Holland	2008		3		1			1		1			2	
Vandendriessche S	92	Belgium	2011		13		5	2		5			1		7	
Vandendriessche S	93	Belgium	2012		8		3	1		3			1		5	
Verkade E	94	Holland	2012		3			3	0	0					3	
Verkade E	95	Holland	2012	9	2		2								0	
Voss A	96	Holland	2005	12	0										0	
Wassenberg M	97	Holland	2011	27	1		1								0	
Welinder-Olsson C	98	Sweden	2008		2		2								0	
Williamson D	99	NZ	2013		7		6				1				1	
Witte W	100	Germany	2007		9		**2**			7					7	
Wu D	101	China	2010		16	16									0	
Wulf M	102	Holland	2006	7	0										0	
Wulf M	103	Many	2008	31	0										0	
Wulf M	104	Holland	2008	9	3		3								0	
Wulf M	105	Holland	2012	262	30	2	22	1			3		2		6	
Yu F	106	China	2008		6					1	4			1	5	
Zarfel G	107	Austria	2012		1							1			1	
Zhao C	108	China	2012		28		28								0	
TOTALS				**4470**	**1492**	**527**	**451**	**167**	**14**	**99**	**31**	**44**	**31**	**128**	**401**	**6**

Legend: Screen = No. of screening isolates; **Clin** = No of clinical cases; **UNS** = unspecified; **SSTI** = skin or soft tissue infection; **BT** = bacteremia; **EC** = endocarditis; **PN** = pneumonia; **RF** = respiratory fluids (sputum); **OM** = osteomyelitis/mastoiditis; **UR** – urine; **Misc**. = other; **INV** = invasive; **FAT** = fatal

[a] Studies which do not distinguish numbers of screening and infection samples

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