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# Assessment of the Public Health significance of meticillin resistant *Staphylococcus aureus* (MRSA) in animals and foods[1]

## Scientific Opinion of the Panel on Biological Hazards

**(Question No EFSA-Q-2008-300)**

## Adopted on 5 March 2009

**PANEL MEMBERS**

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**SUMMARY**

The European Food Safety Authority (EFSA) asked its Panel on Biological Hazards to deliver a scientific opinion on: The assessment of the Public Health significance of meticillin resistant *Staphylococcus aureus* (MRSA).

There are different states of interaction between *S. aureus* and its host: infections, carriage or colonisation, and contamination. Meticillin resistant *S. aureus* (MRSA) can be persistently or intermittently carried by healthy humans, and colonisation is the major risk factor for infection. Infection can be mild to severe and, in some instances, fatal. MRSA are now a major cause of hospital acquired infection in many European countries, with large differences in prevalence and control policies. A limited number of lineages of MRSA tend to predominate in specific geographical locations. CC398 is the MRSA lineage most often associated with asymptomatic carriage in intensively reared food-producing animals. MRSA commonly carry enterotoxin genes but there has been only one report of food intoxication due to MRSA.

***On the question on what is the risk to human health posed by MRSA associated with food-producing animals, the Panel concluded that:***

Livestock-associated MRSA (LA-MRSA) represent only a small proportion of the total number of reports of MRSA infections in the EU. However, this proportion differs between Member States. In some countries with low prevalence of human MRSA infection, CC398 is a major contributor to the overall MRSA burden. In countries with high overall human MRSA prevalence, CC398 is considered of less significance for the public health. CC398 has, albeit rarely, been associated with deep-seated infections of skin and soft tissue, pneumonia and septicaemia in humans. Where CC398 prevalence is high in food-producing animals, people in contact with these live animals (especially farmers and veterinarians, and their families) are

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at greater risk of colonisation and infection than the general population. The risk to human health from different levels (dose response) of MRSA during carriage in animals (and in the environment) is not known.

***On the question of what is the importance of food, food-producing animals, and companion animals in the risk of human infection and/or food-borne disease caused by MRSA in both the community and hospital settings, the Panel concluded that:***

Food may be contaminated by MRSA (including CC398): eating and handling contaminated food is a potential vehicle for transmission. There is currently no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by CC398 both in the community and in hospital. MRSA (including CC398) can enter the slaughterhouse in or on animals and occurs on raw meat. Although it may become part of the endemic flora of the slaughterhouse, the risk of infection to slaughterhouse workers and persons handling meat appears to be low based on currently available data.

Where CC398 prevalence is high in food-producing animals, people in direct contact with these live animals (especially farmers and veterinarians, and their families) are at risk of colonisation and subsequent infection. The potential for CC398-colonised humans to contribute to the spread of MRSA in hospitals currently seem to be less than for hospital associated MRSA strains.

MRSA infections in companion animals are increasingly reported and in almost all cases, the strains causing infection in animals are the same as those commonly occurring in hospitals in the same geographical region. Humans are likely to spread MRSA to companion animals, and these can then be a reservoir for humans both in the community and in health care facilities. Horses can become colonised and/or infected with MRSA from humans or from other animal sources in their environment. There are sporadic reports of human disease, usually minor skin infections, attributable to an equine source.

***On the question of which animal species (and if appropriate, foods derived there from) represent the greatest risk to humans, the Panel concluded that:***

The primary reservoirs of CC398 in affected countries are pigs, veal calves, and broilers. CC398 has also been found in companion animals and horses on farms with colonised livestock. MRSA has now been reported from dogs, cats and horses with sporadic reports of isolation from wide range of other companion animals. There are no specific studies which examined the relative risk of different small animals and horses as sources of infection or colonisation in humans.

***On the question of which methods are best suited for the isolation and molecular typing of MRSA of animal origin, the Panel concluded that:***

There is a wide variety of methods available for the isolation of MRSA. MRSA can be identified using phenotypic (antimicrobial susceptibility testing) or genotypic methods. For diagnosis of infection, samples taken directly from a lesion, biopsy specimens or blood cultures are cultured onto non-selective and selective media. For detection of carriage or contamination, swabbing of noses (for individuals), dust (for herds or flocks), and sampling of food are used. Increased sensitivity is obtained when using selective liquid enrichment methods. *spa* typing is applicable for lineage detection in first line typing because of wide congruence with results of MLST and other typing methods.

The Panel recommended that further work should be performed on harmonising methods for sampling, detection and quantification of MRSA during carriage in both humans and animals,

as well as for detection of MRSA as a contaminant of food, and in the environment including from dust both in air and on surfaces.

***On the question of what control options (pre- and post-harvest) can be considered to minimize the risk of transfer of food-associated and animal-associated MRSA to humans, the Panel concluded that:***

Monitoring and surveillance are not control options as such, however these processes are essential for determining control strategies and for the evaluation of their effectiveness. Surveillance of MRSA in humans, including *spa* typing of a representative number of isolates is necessary in order to monitor the occurrence of different strains of MRSA including CC398 in people.

The Panel indicated that periodic monitoring of intensively reared animals in all Member States would provide trends in the development of this epidemic, and recommended carrying out systematic surveillance and monitoring of MRSA in humans and food producing animals in order to identify trends in the spread and evolution of zoonotically acquired MRSA.

Animal movement and contact between animals is likely to be an important factor for transmission of MRSA. In the absence of specific studies on the spread and persistence of MRSA, general control options on farms, in slaughterhouses and in food production areas are likely to be the same for MSSA as well as MRSA, and include good husbandry practices, HACCP, GHP, and GMP. Monitoring and subsequent restrictions on movement may reduce transmission. Since the most important routes of transmission to humans are through direct contact with live animals and their environments, the most effective control options will be at pre-harvest.

LA-MRSA carriers in hospital and other healthcare settings can be managed in the same way as HA- and CA- MRSA carriers in both staff and patients by screening and infection control measures. Strategies for screening (together with actions taken following their results) vary considerably between different MS's. Search and destroy policy seems to be the most effective, however its implementation is difficult when MRSA is already prevalent. The panel recommended that protocols for screening at admission to hospitals should be expanded to include humans exposed to intensively reared livestock.

Transfer of MRSA to humans from companion animals and horses is difficult to control. Basic hygiene measures are key, especially hand washing before and after contact, and if possible, avoiding direct contact with nasal secretions, saliva and wounds. Decolonisation of these animals is a potential control option but controlled studies are lacking.

The Panel recommended that intervention studies should be carried out in order to evaluate the effectiveness of control measures to reduce the carriage of CC398 in livestock. Such studies should be longitudinal over consecutive production cycles. In addition, the factors responsible for host specificity, persistence in different environments, transmission routes (including airborne transmission) and vectors, should be investigated. The panel also recommended that intervention studies should be carried out in order to evaluate the effectiveness of control measures to reduce the carriage of MRSA in companion animals and horses and their human contacts.

**Key words:** MRSA, meticillin, antimicrobial resistance, farm animals, pets, companion animals

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## BACKGROUND AS PROVIDED BY EFSA

*Staphylococcus aureus* is frequently present on the skin, in the nose or in the mouth of human hosts without causing illness. However, in some instances, *S. aureus* can cause disease when it enters wounds or damaged skin, and can cause abscesses, pneumonia, meningitis, endocarditis and septicaemia.

Meticillin-resistant *S. aureus* (MRSA) is considered to be resistant to virtually all available beta-lactam antimicrobials. This resistance is mediated by the *mec*A gene, chromosomally located in the staphylococcal cassette chromosome (SCCmec), which codes for a penicillin binding protein (PBP)2a with a low affinity for beta-lactams. MRSA first emerged in hospitals in the 1970s, and by the 1990s increased dramatically worldwide, becoming a serious clinical problem in hospital environments. In recent years a major change in epidemiology of MRSA has been observed, with the appearance of cases in the community affecting people having no epidemiological connection with hospitals. The strains isolated from such cases are referred to as Community-acquired or Community-associated MRSA (CA-MRSA). Isolates from these cases have clear pheno- and genotypic differences from the strains isolated from classically health-care associated MRSA cases.

The hazard of animal-associated MRSA has also been recently identified. In this case, it is important to distinguish between MRSA isolated from pet animals, and MRSA from animals used in food production.

Since the 1990s, an increasing number of studies have reported MRSA infections in pet animal patients at veterinary clinics and hospitals. Strains isolated from these cases are usually indistinguishable from those isolated from human contacts. It is generally accepted that pets become infected through contact with infected or colonised people, and that pets in turn pass MRSA back to humans. MRSA is not only carried by pet animals but can also cause clinical disease in a number of such animals. Most of the cases of MRSA in pets are reported in dogs and horses, and the majority of such clinical cases have been due to post-operative infections.

In the case of animals in food production, in addition to reports of sporadic cases in dairy cattle, a new specific clone (CC398) of unknown origin, appears to be emerging. This clone has been found in production animals in several countries including Austria, Belgium, Canada, Denmark, France, Germany, The Netherlands and Singapore. Further studies are underway, but it appears likely that MRSA CC398 is widespread primarily in the pig, but also in cattle and perhaps poultry populations, most likely in all European countries with intensive production systems. CC398 is mainly found to colonize animals, but, in a few isolated cases, has caused clinical infections in animals. The reason for the colonization by MRSA CC398 of pigs and other production animals, and the epidemiology of this clone are currently not known. The use of cephalosporins, tetracycline and other antibiotics may have a role in providing a niche for this clone; until further studies are carried out this is mere speculation.

With our current knowledge it is reasonable to assume that CC398 is a MRSA clone that can be transmitted from production animals to humans. Animals in food production and their products are therefore a potential source of community-acquired MRSA.

There is increasing concern about the public health impact of MRSA associated with food-producing animals. Accordingly, attention requires to be paid to the epidemiology, prevalence and virulence of food and animal derived MRSA strains.

## TERMS OF REFERENCE (TOR) AS PROVIDED BY EFSA

The Scientific Panel on Biological Hazards is requested:

1. To assess the risk to human health posed by MRSA associated with food-producing animals.
2. To assess the importance of food, food-producing animals, and companion animals in the risk of human infection and/or food-borne disease caused by MRSA in both the community and hospital settings.
3. To determine which animal species (and if appropriate, foods derived there from) represent the greatest risk to humans.
4. To identify which methods are best suited for the isolation and molecular typing of MRSA of animal origin.
5. To indicate what control options (pre- and post-harvest) can be considered to minimize the risk of transfer of food-associated and animal-associated MRSA to humans.

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## ASSESSMENT

## 1. Hazard Identification

### 1.1. MRSA disease, biology, genetics, toxin, virulence factors, antimicrobial resistance.

*Staphylococcus* is a genus of Gram-positive bacteria that are coccoid (spherical) and approximately 1 micrometer in diameter, have thick peptidoglycan cell walls, have a low G + C content, and grow in clusters similar to grapes (staphyl = grape). They are commensals (normal bacterial flora) of mammals, but can also survive in a variety of environments and survive desiccation (drying). There are over 30 species of staphylococci, the most pathogenic species for humans is *S. aureus* which can be differentiated from other staphylococcal species colonising humans (e.g. *S. epidermidis, S. haemolyticus, S. hominis, S. saprophyticus, S. capitis*) in the diagnostic laboratory by a positive coagulase reaction. *S. aureus* occurs in the human nose, and can also be found in the throat, auxilla, rectum, perineum or gastrointestinal tract. It is important to recognise different states of interaction between *S. aureus* (including meticillin resistant *S. aureus*, MRSA) and its environment. These can be defined as **infections** in both animals and humans where growth of the bacterium occurs together with overt or covert pathological changes indicating the presence of disease. **Carriage or colonisation** also occurs in both humans and animal where *S. aureus* (including MRSA) multiplies in the nares, throat or other superficial sites but without causing disease. **Contamination** occurs in humans, animals, food, the environment etc where *S. aureus* (including MRSA) is present due to exposure from another site (i.e an infected or colonised host or the environment such as dust). Animals or humans can be contaminated at external surfaces (skin, hair, fur, etc), and there is no multiplication of *S. aureus* and no clinical symptoms.

Up to 20% of healthy humans persistently carry *S. aureus* in their nose, with no symptoms, and are considered to be colonised, and another 60% are intermittent carriers with no symptoms (Peacock, S.J. *et al.*, 2001). *S. aureus* is a very common cause of minor skin infections in healthy people that usually do not require treatment. In hospitals, with immuno-compromised patients and frequent breaches of the skin due to wounds, surgery, catheters, injections, etc., *S. aureus* is the most common cause of hospital-acquired infection. Infection can be trivial to severe and, in some instances, fatal.

Antibiotics are widely used for prophylaxis and treatment of *S. aureus* infections, especially in immuno-compromised patients. The most useful antibiotics are the β-lactamase resistant penicillins of the meticillin (synonym methicillin) family which includes flucloxacillin, dicloxacillin, oxacillin, nafcillin, cloxacillin and meticillin. Due to the widespread use of antibiotics in hospitals, there is selective pressure for *S. aureus* to become resistant to antibiotics. Meticillin resistant *S. aureus* (MRSA) are those which carry the *mecA* gene and are resistant to all penicillins, cephalosporins and carbapenems.

MRSA is now widespread in hospitals in many European countries (see section 2.1) and is the most common cause of nosocomial infection: e.g. 40-70% of all staphylococcal infections in intensive care units are due to MRSA (Diekema, D.J. *et al.*, 2001; Sahm, D.F. *et al.*, 1999). In the USA there are increasing reports of community acquired MRSA that cause skin and soft tissue infections in otherwise healthy people which require treatment. However, meticillin susceptible *S. aureus* (MSSA) are still a common cause of infection, particularly in the community. MRSA infections are treated with the last remaining reliable class of antibiotics, the glycopeptides (vancomycin, teicoplanin), or with some new and expensive drugs that all have limitations (linezolid, tigecycline, daptomycin).

The *mec*A gene which confers resistance to meticillin encodes a variant penicillin-binding protein, PBP2a. Native PBP2 catalyses a key step in the synthesis of the bacterial peptidoglycan cell wall, and is bound and inactivated by penicillin-type antibiotics including meticillin. PBP2a is not inhibited by penicillins and can function instead of PBP2 (Pinho, M.G. *et al.*, 2001). The *mec*A gene is carried within a larger family of DNA sequences called *SCCmec*, which can also encode other antibiotic resistance genes, and inserts into a specific site on the *S. aureus* chromosome called *orfX* (Ito, T. *et al.*, 1999). SCC*mec* regions vary in size and the genes they carry, but are relatively stable in the genome, and have originated in other staphylococcal species (Ito, T. *et al.*, 2001; Katayama, Y. *et al.*, 2003). In relation to the large number of humans colonized with *S. aureus*, infections are infrequent and mainly affect patients with specific predisposing risk factors (see 4.2). *S. aureus* can infect any tissue of the body and therefore can be associated with a wide range of disorders (e.g. wound infection, pneumonia, bacteraemia, endocarditis, osteomyelitis, abscess, septic arthritis, osteomyelitis, conjunctivitis). Localised infections mainly affecting skin and soft tissue as well as sites to which the bacterium gets access by disruption of skin and other sites caused by injury such as iatrogenic procedures (e.g. postoperative wounds). Localised infections can lead to septicaemia with heamatogenous dissemination to other organ systems and generalized inflammation and intoxication. Pneumonia due to *S. aureus* can start via the airways (in particular in ventilated patients) as well as from heamatogenous dissemination. MRSA are of similar virulence as meticillin susceptible *S. aureus* (MSSA). Higher mortality rates due to MRSA occur in case of severe infections and are mainly due to suboptimal treatment because of multiresistance of the isolates. MRSA bloodstream infections have a mortality rate of 30-40%, with about 20% attributable directly to the organism (Gould, I.M., 2007).

*S. aureus* is a major cause of food poisoning, due to the production of heat resistant enterotoxins, which when consumed cause vomiting and diarrhoea(ICMSF, 1996). In 2006, *S. aureus* toxins were responsible for 48.8% of the 482 human foodborne outbreaks caused by bacterial toxins reported by EU Member States (http://www.efsa.europa.eu/EFSA/DocumentSet/Zoon_report_2006_en,0.pdf). A wide variety of enterotoxins can be produced, dependent on the genes found in the causative bacterium. MRSA commonly carry enterotoxin genes but there has been only one report of food poisoning due to MRSA (Jones, T.F. *et al.*, 2002). Food was also reported to play a role in disseminating MRSA during an outbreak in a Dutch hospital in the 1990s (Kluytmans, J. *et al.*, 1995). In both instances, MRSA were likely to have been of human origin. Many MRSA carry and express enterotoxin genes, including those most often associated with food poisoning (SEA, SEB, SEC, SED). Food intoxications due to other enterotoxins are rarely recognised (Le Loir, Y. *et al.*, 2003).

*S. aureus* populations can be divided into independent dominant lineages, and about ten are common in humans, while animals carry unique but related lineages (Lindsay, J.A. *et al.*, 2006; Sung, J.M. *et al.*, 2008). The lineages differ in their combinations of surface exposed moieties that are predicted to interact with their hosts. The *S. aureus* genome can also carry multiple mobile genetic elements (MGE) that encode a wide range of virulence and resistance determinants which are capable of horizontal transmission between strains (Lindsay, J.A. and Holden, M.T., 2004). The specific virulence factors necessary for typical opportunistic *S. aureus* disease are unclear however, some toxins cause specific syndromes such as toxic shock syndrome, scalded skin syndrome, haemolytic pneumonia or food poisoning (Tristan, A., Ferry, T. *et al.*, 2007). Resistance to all known antibiotics have been identified in *S. aureus*, although few strains have, to date, accumulated pan-resistance to all antibiotics (Dancer, S.J., 2008a).

The major lineages are named after the clonal complex (CC) determined by multi-locus sequence typing (MLST) (Enright, M.C. *et al.*, 2000). MLST analysis assigns each isolate a sequence type (ST) and related ST types are grouped into the dominant CCs. The major human lineages of *S. aureus* that have acquired *SCCmec* are CC1, CC5, CC8 (including ST239), CC22, CC30 and CC45. Because each lineage is genetically very distinct from the other lineages (Lindsay, J.A. *et al.*, 2006), they can be distinguished by several different typing methods, including spa typing. Multiple clones within each of these lineages have also been described, and one or two types tend to predominate in specific geographical location and show some host specificities (Cockfield, J.D. *et al.*, 2007). The recently described MRSA lineage associated with pigs is CC398 (Witte, W., Strommenger, B. *et al.*, 2007). CC398 has also been shown to cause invasive infections in humans such as deep seated infections of skin and soft tissue (Cuny, C. and Witte, W., 2008; Declercq, P. *et al.*, 2008; van Loo, I., Huisdens *et al.*, 2007), ventilator associated pneumonia, and septicaemia (Witte, W., Strommenger, B. *et al.*, 2007), and one case of multiple organ failure following orthopaedic surgery (Lewis, H.C. *et al.*, 2008). The distribution of toxins and other virulence-associated genes is both lineage and MGE dependent, and there is enormous variation between strains.

The cost of MRSA infection is to the hospital and healthcare service provider as well as directly to the patient and society (Cosgrove, S.E., 2006; Gould, I.M., 2006). The cost of increased care of an MRSA infected patient is estimated at between US$ 2,500 to US$ 90,000, depending on a variety of factors such as how ill the patient becomes, the healthcare system involved, and which control groups are used to calculate the additional cost (Gould, I.M., 2006)

*S. aureus* is intrinsically physically and chemically robust and will tolerate pH ranges from 4.5 to 9.0 and NaCl concentrations up to 9%. Resistance to heat is dependent upon the surrounding matrices. *S. aureus* suspended in 0.9% NaCl is rapidly inactivated at 46°C, however, when protected by proteins (such as in milk or in pus) it can survive for more then 50 min at 60°C. The resistance of *S. aureus* to desiccation is surface and matrix dependent, but can be up to several days (Beard-Pegler, M.A. *et al.*, 1988; Clements, M.O. and Foster, S.J., 1999; Rountree, P.M., 1963). Increased resistance to physical and chemical stress has not been demonstrated for HA-MRSA (Beard-Pegler, M.A. *et al.*, 1988; Farrington, M. *et al.*, 1992). *S. aureus* can acquire genes conferring resistance to specific classes of disinfectants such as cationic substances (e.g. quaternary amines, triclosan) by an efflux mechanism, which show cross-resistance to some antibiotics (Russell, A.D., 2002). However the recommended application concentrations of the corresponding disinfectants, overcomes this resistance under ideal conditions. The current evidence suggests that physical and chemical control strategies are likely to be equally effective against MSSA as well as MRSA, including CC398 although there are limited in-vitro data to confirm this supposition.

### 1.2. MRSA reservoirs and host specificity

MRSA clones have originated in at least three separate settings: human hospitals, human carriers outside of hospitals (community), and livestock animals. This has occurred at different times and in different geographical locations. The subsequent spread of these MRSA clones over time has led to some hospital isolates that are now found in the community and vice versa, and livestock strains that are increasingly found in humans. However, the reservoirs, distribution patterns and strategies for dealing with MRSA in each MS are different.

Hospital associated MRSA (HA-MRSA) emerged in the 1960s in some MS, but did not become widespread until the 1980s/1990s. Most MS have endemic MRSA and high rates of

infection, but some MS initiated comprehensive and effective infection control measures and have much lower rates of infection. The major lineages of HA-MRSA are CC5, CC8, CC22, CC30 and CC45, but most MS will have only one or two of these (Cockfield, J.D. *et al.*, 2007). Colonised patients and staff in hospitals are the major reservoir of MRSA, but the hospital environment can also become contaminated. Hospitalised patients are at risk of infection particularly if they are immunocompromised, and have breaches to their skin integrity (surgery, wounds, catheters, etc), have received antibiotics and are colonised (Salgado, C.P. *et al.*, 2003). In countries with endemic HA-MRSA, this bacterium is increasingly found outside hospitals, such as asymptomatic carriage in discharged patients or outpatients, in healthcare workers, and in companion animals (Barr, B. *et al.*, 2007; Loeffler, A. *et al.*, 2005; Thomas, S. *et al.*, 2007). These can become reservoirs for infection, and in some regions, a significant proportion of patients are now entering hospitals already infected with "HA-MRSA" which may have been acquired in the community (Miller, R. *et al.*, 2008).

Community associated MRSA (CA-MRSA) refer to clones of MRSA that have evolved outside of the hospital setting, and cause infections in patients that are not normally at risk of *S. aureus* infection. CA-MRSA are typically sensitive to most other antibiotics, and carry the gene for the Panton-Valentine leukocidin. They were first described in the USA, where they are a serious problem with large numbers of healthy people with severe skin and soft tissue infections due to MRSA entering hospitals through the accidents and emergencies department, and are a responsible for a significant number of paediatric mortalities. The strains are now becoming endemic in USA hospitals (Gonzalez, B.E. *et al.*, 2006; Klevens, R.M. *et al.*, 2006). The lineages involved are CC8 (known as USA300 and genetically different from CC8 HA-MRSA) and CC1 (USA400). The reservoir of CA-MRSA is likely to be the noses of healthy people who have been exposed to the strains; at present this includes diverse populations in specific geographical locations which include, children, those in or released from prisons, drug abusers and men who have sex with men (Diep, B.A. *et al.*, 2008; Farley, J.E. *et al.*, 2008). In Europe, these clones are not widespread (Tristan, A., Bes, M. *et al.*, 2007), but two rarer lineages, CC80 and CC59, are associated with low incidence European CA-MRSA.

The third significant emergence of MRSA has been in livestock animals in Europe (LA-MRSA). The lineage is CC398 which is rare in humans and predominates in pigs and veal calves. Determinants of host specificity of *S. aureus* lineages to specific mammalian hosts are poorly understood (see section 4). The reservoir are animal noses and other moist sites, as well as contaminated animal housing and surrounding environments. There is evidence of MRSA CC398 spread to humans and other animal species. LA-MRSA have been well characterised in countries with active search and destroy programs to reduce HA-MRSA, as the LA-MRSA have contributed significantly to increases in MRSA detected in human hospitals.

This Opinion focuses on HA-MRSA clones found in companion animals and LA-MRSA (CC398) found in livestock, and their public health significance in humans.

### 1.3. Sampling for diagnosis of MRSA infection and carriage in humans

#### 1.3.1. Diagnosis of infections in humans

Diagnosis of staphylococcal infection is identical regardless of whether infection is caused by MRSA or MSSA. Since this bacterium is a common component of the human commensal skin flora, sampling from clinical material may be complex to interpret when distinguishing between infection, colonisation and contamination from skin sites. Infection in normally sterile sites (CSF, pus, tissue aspirates, and blood) is invariably accompanied by non-specific

signs of sepsis and sampling using microbiological culture on non-selective media (usually blood containing agar) is performed. Quantification or semi-quantification of the bacterium from these sites is often performed, and results may take the form of 'heavy pure growth in pure culture' or the speed and proportions of blood cultures which become positive for the presence of the bacterium. Culture of *S. aureus* from other sites (such as urine and bronchial lavage) is more complex to interpret and quantification and results of repeated sampling, together with clinical information, is also frequently used.

### 1.3.2. Detection of carriage in humans

Screening for carriage of *S. aureus* in humans is typically done using cotton swabs, which may or may not be moistened with sterile saline. Nasal samples should be taken from the vestibulum nasi, which is the anterior nasal passage at the border between skin and mucosal tissue (Peacock, S.J. *et al.*, 2001). Nasal screening alone will identify about 80% of carriers, and the addition of sampling of other sites, particularly throat, may increase this to 92% (Grundmann, H. *et al.*, 2006). Swabs can be collected and transported to the diagnostic laboratory for processing. Diagnostic tests and testing strategies for detection of carriage by MRSA or MSSA may differ, see next section.

### 1.3.3. Diagnosis of food intoxications in humans

A diagnosis of staphylococcal food poisoning (vomiting 1-18 hours after consumption of toxic food) is most usually established by the detection of staphylococcal enterotoxin in food consumed by patients. The presence of enterotoxin together with large numbers of organisms *in vomitus* would also support a diagnosis, although this clinical sample is only very rarely available for analysis. In addition to the presence of enterotoxin, there are usually $>10^6$/g of an enterotoxin producing *S. aureus* present in implicated food. However, because of the stability of staphylococcal enterotoxins which can remain biologically active after cooking and other processes, the toxins can be present in food in the absence of viable organisms, since the latter may be killed during food processing by, for example, cooking or by reduction in pH as occurs during the manufacture of cheese. *S. aureus* strain may be present in the faeces of affected patients following intoxication, however this will only provide supportive evidence for intoxication since the presence of the bacterium may be as a result of nasal or skin carriage. Diagnosis of staphylococcal food poisoning is identical regardless of whether infection is caused by MRSA or MSSA.

## 1.4. Sampling for diagnosis of MRSA carriage and infection in companion and food-producing animals

Clinical samples for detection of staphylococcal (including MRSA) infection in animals are the same as those collected for the detection of other infections by bacterial culture and may include swabs taken directly from a lesion and submitted in transport medium, biopsy specimens or blood culture (Lloyd, D.H. *et al.*, 2007). These samples are identical to those used in human medicine.

Samples used for detection of colonization in MRSA in animals include swabs of the nose, skin, perineum and rectum. The most common method for detecting MRSA colonization in animals is through nasal sampling (Abbott, Y. *et al.*, 2006; Baptiste, K.E. *et al.*, 2005; de Neeling, A.J. *et al.*, 2007; Khanna, T. *et al.*, 2008; Rich, M. and Roberts, L., 2006; Vengust, M. *et al.*, 2006). In some studies, two or more samples are taken from each animal but the relative sensitivity of sampling from different sites is not known. (Rich, M. and Roberts, L., 2006) detected MRSA from the nose of 1 of 255 dogs and not from any of the throat and skin swabs collected from the same animals. (Baptiste, K.E. *et al.*, 2005) detected nasal carriage in

12% and skin carriage in 3% of 67 horses in an equine hospital. (Khanna, T. *et al.*, 2008) reported significantly different isolation rates from nasal and rectal samples collected from pigs; 16% of pigs were positive on nasal swabs only, 7.4% were positive on both nasal and rectal swabs and 1.4% were positive on rectal swabs only.

*S. aureus* is the most important cause of subclinical mastitis in dairy herds worldwide and MRSA has been isolated from mastitis samples (Juhasz-Kaszanyitzky, E. *et al.*, 2007; Moon, J.S. *et al.*, 2007). Hamann, J., in 2005 described bacteriological examination of the quarter foremilk on two occasions at an interval of at least one week as the best method for the aetiological diagnosis of mastitis, but observed that this is not necessarily practical in field situations. It was recommended however that culture of foremilk be carried out in symptomatic cases and on all cows in a herd once a year as part of a well managed mastitis control programme.

Although *S. aureus* can be recovered from both dogs and cats, *Staphylococcus intermedius* has long been considered the most common coagulase-positive species isolated from both healthy and diseased dogs and cats and may be detected in up to 90% of animals (Biberstein, E.L. *et al.*, 1984; Cox, H.U. *et al.*, 1988; Lilenbaum, W. *et al.*, 1998). Recent molecular characterisation of staphylococcal cultures from dogs and cats indicates that isolates formerly classified as *S. intermedius* belong to the species *S. pseudintermedius* (Sasaki, T. *et al.*, 2007).

A study is currently ongoing in the EU to establish the prevalence of MRSA in dust samples collected from the pens of breeding pigs in different production stages (Commission Decision C(2007) 6579, 2008/55/EC, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:014:0010:0025:EN:PDF). Approximately 500 $cm^2$ dorsal surfaces of pen partition walls are being sampled with dry swabs and data from this survey will be available in 2009.

### 1.5. Sampling for detection of MRSA in food of animal origin (meat and dairy products).

MRSA has been associated with food both through contamination from humans (Kluytmans, J. *et al.*, 1995) and due to colonisation of food-producing animals (VWA, 2007). Therefore, food sampling aimed at detecting this organism should focus on foods of animal origin (especially meat and dairy products) and ready-to-eat foods for which the production process involves significant handling. Foods involved in *S. aureus* intoxications have commonly included poultry products, cold cooked meats and cream-filled bakery products. In particular, salted meats such as ham and corned beef are a common vehicle for this organism, since it is relatively resistant to the elevated salt levels. Although the physiology of CC398 has not been systematically investigated, isolates from within this clonal complex are likely to be physiologically similar to other *S. aureus*, and able to grow and survive under similar conditions. However, since CC398 isolates are most likely to originate from raw ingredients, these would be killed by adequate cooking or pasteurisation processes. Therefore, the foods of greatest risk of contamination by CC398, are unpasteurised dairy products and meats that undergo minimal or no heat treatment.

Food samples, usually of approximately 100g, should be aseptically collected into sterile containers. If food-handling practices at retail or catering premises are being investigated, it may be appropriate to sample the food using the utensils that would normally be used for handling or serving the food. However, if the food is to be examined as supplied by the producer, the sample should be collected using sterile utensils. Samples should be stored between 0 and 8°C, and transported to the laboratory for testing within a maximum of 24 hours of collection, but preferably on the day of collection (ISO/FDIS 7218:2007.

Microbiology of food and animal feeding stuffs – general requirements and guidance for microbiological examinations).

### 1.6. Sampling for diagnosis of MRSA contamination in the environment

Moistened cotton-tip swabs have been used to sample the environment of companion animals (Loeffler, A. *et al.*, 2005; Weese, J.S. *et al.*, 2004) and dust samples from pig houses to detect the prevalence of MRSA in pig herds (Broens, E.M. *et al.*, 2008; EFSA, 2007). (Weese, J.S., 2007) discusses a number of additional possible methods for screening of the environment of animals for MRSA including the use of contact plates, swabs, electrostatic cloths, passive and active air sampling. These sampling methods were successfully used to detect MRSA in human environments (Asoh, N. *et al.*, 2005; Shiomori, T. *et al.*, 2001) however there is limited information on the effectiveness and sensitivity of these methods in the environment of production animals with very high loads of bacteria and dust. Other approaches included the inoculation of broth solutions with feed material or faeces and the preparation of suspensions of bedding material (Lee, J.H., 2003; Lu, J. *et al.*, 2003). These methods have not been evaluated to isolate and identify MRSA.

### 1.7. Methods for isolation, identification and molecular typing of MRSA.

*S. aureus* and MRSA are some of the most widely encountered organisms in the diagnostic microbiology laboratory, and occur in a variety of specimens associated with carriage as well as a wide range of infections. Many methods have been used to sample, speciate, determine resistance and to type these bacteria. There is limited consensus on the optimal methods from the wide range of those used, and their choice depends on specimen type, concentration of bacteria, history and training of staff, cost, convenience, accuracy, speed results are required, and the range of other pathogens also tested. In the following sections discussion is restricted to the most widely used and recommended methods. Other methods may be available and may have been proven to be equally useful.

#### 1.7.1. Methods for detection of MRSA in specimens intended for colonisation (carriage in humans or animals) or for environmental screening.

Swabs taken from the anterior nares (or other sites expected to be colonized) are inoculated onto mannitol salt agar with cefoxitin (or oxacillin), or a commercial plate such as CHROMagar MRSA (Bocher, S., Smyth, R. *et al.*, 2008) which prevents the growth of other contaminating organisms, and allows the easy identification of *S. aureus*. In cases where it is important to identify colonisation with very low levels of MRSA, and/or MRSA in the presence of a high background flora, the specimen can be pre-enriched in selective broth containing salt and cefoxitin/oxacillin before subculture onto solid media (Bocher, S., Smyth, R. *et al.*, 2008). Molecular methods (see section 1.7.6) are also suitable for detection of MRSA, and are likely to be more rapid and of greater sensitivity than the culture based procedure described although of greater cost (Boyce, J.M. and Havill, N.L., 2008). The selective methods outlined above have been used in baseline and environmental surveys to investigate prevalence of MRSA (see later text in section 1.7.8).

#### 1.7.2. Methods for detection of MRSA in clinical specimens from humans and animals

The method used for isolation of *S. aureus* and MRSA depends on the specimen taken and whether it is likely to be contaminated with other organisms. If the specimen is taken from an otherwise sterile site from a patient or animal with clinical symptoms of disease (e.g. blood, cerebrospinal fluid), it is important to use a general isolation method which can identify a range of potential pathogens. Similarly, if the specimen is taken from a normally non-sterile

site which contains a mixed bacterial flora (e.g. sputum, pus, wound, skin), only some of which may be pathogens, a selective method is required. If the specimen is from blood, there are typically only small numbers of pathogenic bacteria and they need to multiply in a blood culture bottle such as the BACTEC system (http://www.bd.com/ds/productCenter/BC-Bactec.asp) prior to isolation. Growth is detected in blood cultures by gas production and an alert system identifies the bottles to be subcultured onto agar. Sputum, wound and pus swabs, etc are cultured directly onto non-selective agars. Blood agar is used as a general non-selective medium and if *S. aureus* is suspected a selective medium such as mannitol salt and/or CHROMagar can be additionally used. After overnight growth, the identification of colonies with characteristic morphology and haemolysis is confirmed as described later.

### 1.7.3. Methods for isolation of MRSA from food.

A wide range of methods have been used to detect and enumerate MRSA in food products, and these include two basic approaches:

(i) Enumeration of *S. aureus* followed by determination of meticillin sensitivity: *Staphylococcus aureus* is enumerated in food samples by the inoculation of selective agar plates with a 1 in 10 homogenate of the food. For example, the international standard method, BS EN ISO 6888-1:1999 (http://products.ihs.com/Ohsis-SEO/345154.html) uses Baird Parker agar for this purpose. Following biochemical confirmation of colonies as *S. aureus*, representative isolates are retained for subsequent determination of meticillin resistance by antibiotic susceptibility testing and/or molecular analysis

(ii) Direct detection and/or enumeration of MRSA from food products: MRSA presence in the food sample is determined directly by inoculation of agar plates containing a suitable antimicrobial such as oxacillin or cefoxitin: chromogenic agars containing cefoxitin are available commercially. Enumeration of MRSA, with a detection limit of approximately 10 colony forming units per gram of food, can be achieved by inoculation of agar plates with a defined volume of a 1 in 10 dilution of the food. In order to detect lower levels of MRSA, one or more enrichment stages can be introduced prior to plate inoculation. (van Loo, I., van Dijk, S. *et al.*, 2007) reported increased MRSA recovery from food following the use of a single enrichment stage in Mueller-Hinton broth containing 6.5% NaCl and following the addition of a secondary enrichment in phenol-red mannitol broth with ceftizoxime (5 µg/ml) and aztreonam (7.5 µg/ml). The first approach is useful for the retrospective analysis of collections of *S. aureus* isolates from food, and makes use of a standard method used commonly in food microbiology laboratories. Direct detection of MRSA, as described in (ii) is more sensitive, particularly when an enrichment stage is used. The direct inoculation of agar plates can provide quantitative data. However, the use of an enrichment method would provide more sensitive determination of prevalence in food products.

### 1.7.4. Identification methods for *S. aureus* cultures

After 18-36 hours growth, colonies of MRSA on blood agar are yellowish and usually surrounded by a zone of haemolysis. On mannitol-salt agar they are generally yellow surrounded by a zone of yellow caused by the fermentation of mannitol. On CHROMagar MRSA they are rose to mauve and have a typical colonial morphology. Gram-stain must be performed to confirm the presence of gram-positive cocci arranged in clusters, which strongly suggests the genus *Staphylococcus*. The species *S. aureus* is usually identified by agglutination tests based on latex beads coated with immunoglobulin B (reacting with protein A), fibrinogen (reacting with the clumping factor, a surface protein of *S. aureus)*, or with monoclonal antibodies against the frequent capsular types 5 and 8 (van Griethuysen, A. *et al.*, 2001). Previous generations of agglutination tests which are only based on IgG or fibrinogen

coated particles are at risk that MRSA which lack or exhibit low expression of protein A and/or clumping factor are not correctly identified. When equivocal are obtained results, the tube test for coagulase or a heat stable DNase are used for confirmation. The tube coagulase test is specified in the ISO 6888 method for examination of food for detection of coagulase positive staphylococci.

Since 1990, automated systems for species identification based on metabolic phenotypes (particularly sugar fermentation patterns (e.g. Vitek or BD Phoenix systems), are more frequently used in clinical bacteriology laboratories.

Further confirmation of species by genetic methods is not routinely performed by diagnostic laboratories. However, a reference laboratory, or a study for publication may wish to confirm the species with a molecular method such as PCR for one of the following *S. aureus* specific DNA sequences encoding: the protein synthesis elongation factor (*tuf*, (Martineau, F. *et al.*, 1998)), the heat stable DNase (*nuc*, (Brakstad, O.G. *et al.*, 1992)), the coagulase factor (*coa*, (Schmitz, F.J. *et al.*, 1997)), the superoxide dismutase (*sod*M, (Valderas, M.W. *et al.*, 2002)), and the cell wall synthesis enzyme (*femA*, (Vannuffel, P. *et al.*, 1999)). To discriminate between staphylococcal species by means of DNA detection and sequencing polymorphisms, 16S rRNA genes are ideal (Becker, K. *et al.*, 2004) but the *hsp60* (Goh, S.H. *et al.*, 1997), *fem*A (Vannuffel, P. *et al.*, 1999), *sodA* (Poyart, C. *et al.*, 2001), *tuf* (Martineau, F. *et al.*, 2001), *rpoA* (Drancourt, M. and Raoult, D., 2002), *gap* (Yugueros, J. *et al.*, 2000).

### 1.7.5. Methods for antimicrobial susceptibility testing (AST) and confirmation of MRSA

Routine methods for AST use disk-diffusion, E-test, or microbroth dilution assays for measuring minimum inhibitory concentrations (MIC) against cefoxitin which represents the "gold standard" of phenotypic methods. AST by automated systems is based on MIC (e.g. the Vitek system) are also available.

Breakpoints for discrimination of susceptible, intermediate and resistant isolates can vary considerably between different national laboratory standards. Therefore it is highly advisable to rely either on guidelines produced by the Clinical Laboratory Standards Institute method (CLSI, 2005) which are used in North America and in many other parts of the world, or on the EUCAST standard which is based on a broad MIC profile data base (Kahlmeter, G., 2008). The agar diffusion (disk) assay is still in use in many laboratories because of easy performance and of low costs. It is affected by a number of external influences, such as depth of agar, and it is important to perform the test exactly as recommended.

Because of heterogeneous *in vitro* expression of meticillin-resistance (heteroresistance) in nearly all of currently disseminated MRSA clonal lineages, phenotypic AST needs particular care when using oxacillin as a test substrate. Heteroresistance can be either detected using high inocula as recommended by the CLSI standard, or by use of cefoxitin disks since this antibiotic is less affected by heterogeneous expression. Laboratory standards such as CLSI recommends an additional screening test based on spot inoculation onto NaCl and oxacillin-containing screening plates with a high inoculum (CLSI, 2005). A rapid phenotype identification of MRSA starting from a culture plate can also be performed by a latex-agglutination test based on monoclonal antibodies against PBP2a (Nakatoni, Y. and Sugiyama, J., 1998). Assays based on cefoxitin are particularly important for detection of low level oxacillin resistant MRSA (Witte, W., Pasemann, B. *et al.*, 2007).

Molecular methods for detecting meticillin resistance in *S. aureus* by targeting the *mecA* gene are accurate (Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H., Watanabe, S., 1991), and can be included into conventional 'block based' multiplex PCR assays

(Strommenger, B. *et al.*, 2003) and real time PCR assays (Stratidis, J. *et al.*, 2007). Other antibiotic resistance genes can be incorporated into in-house produced microarray analysis for AST (Strommenger, B. *et al.*, 2007; Zhu, L.X. *et al.*, 2007).

### 1.7.6. Molecular methods for rapid detection of MRSA

There has been increasing demand in recent years for more rapid detection methods for MRSA detection and molecular methods based on PCR for *mecA* have been developed. Since many other staphylococcal species carry the *mec*A gene but are not considered clinically relevant, it is important to make the test specific for *S. aureus*. Such methods are now commercially available (Paule, S.M. *et al.*, 2007; Warren, D.K. *et al.*, 2004), notably BD GeneOhm MRSA and Cephaid Xpert™ MRSA. The target are the SCC*mec* elements, large pieces of DNA that carry the *mecA* gene that have moved horizontally into *S. aureus* on multiple occasions but always insert into a single specific location on the *S. aureus* chromosome, the *orfX* gene. PCR amplification of the SCCmec junction region is achieved using a left primer located on the right hand end of the SCCmec element and the right primer on the stable integration site in *S. aureus orf*X (Ito, T. *et al.*, 1999). Since there are several types of SCCmec, there are several variants of the left primer in the PCR reaction mixture. In a small number of instances, SCC elements that do not encode *mec*A have been reported (Holden, M.T. *et al.*, 2004), contributing to false positives. The procedure for using a molecular method typically involves swabbing a suspected MRSA colonised site, dispersing the bacteria into a buffer, extracting the DNA using a commercial kit, adding the DNA to a disposable cartridge with pre-prepared reagents and placing it into a real-time PCR machine which automatically completes amplification, detection (using a fluorescent beacon) and interpretation. In one system, the user only adds the swab to a cartridge and places the whole cartridge into the machine where all subsequent steps are performed automatically. The test takes around 2 hrs to perform, and the cost of a fully functioning system is currently in the region of €20-30 per test. Molecular methods are considered to be accurate, but identify more positives than by culture; there is some debate whether the molecular methods of increased sensitivity than culture, or if false positives are due to "dead" bacteria (Paule, S.M. *et al.*, 2007; Warren, D.K. *et al.*, 2004).

### 1.7.7. Molecular *typing*

Molecular typing of MRSA is performed to identify clones with known epidemiology and pathogenic characteristics, or to define the source and scope of an outbreak so as to prevent further spread and infection. MRSA typing is currently undergoing a period of change, due to increased knowledge of populations of the bacterium and how they vary as well as improved technology. Ideally a typing method will identify lineage and mobile genetic elements, be reproducible, rapid and inexpensive. At the present time, the best single method for identifying MRSA lineage is *spa* typing because it is less costly and time consuming than MLST and gives equivalent discrimination. The method involves extraction of DNA from a pure culture, PCR amplification of the variable region of the protein A (*spa*) gene, sequencing the PCR product in both directions, and comparison using a publicly available database (www.spaserver.ridom.de) (Harmsen, D. *et al.*, 2003). MLST is similarly as useful but involves sequencing seven instead of one gene (Enright, M.C. *et al.*, 2000): the MLST database is at www.mlst.net. The *spa* website is particularly useful as it has databases comparing some of the spa and MLST types to the dominant lineages. It is important to note that both methods are susceptible to point mutations or recombinations of the target genes, leading to a change of spa or MLST type that represents a minor variant within a lineage. The importance of such variation is unclear, although some are able to be used as epidemiological

markers. For example, the lineage of LA-MRSA is commonly known as CC398 or ST398, but single locus variants of MLST type include ST621, ST752, ST753, ST804 and ST1067 and spa types belonging to this lineage include t011, t034, t108, t567, t899, t1197, t1451, t1939 (van Duijkeren, E. *et al.*, 2008; Witte, W., Strommenger, B. *et al.*, 2007). Since lineages are continuing to evolve and new MLST and spa types will emerge, if a unique MLST type is obtained, it can be compared to previously described MLST types and placed in the corresponding lineage using a phylogenetic algorithm called eBURST ((Feil, E.J. *et al.*, 2004) www.mlst.net). Similarly, unique spa types can be clustered using a similar algorithm called BURP (Mellmann, A. *et al.*, 2007). Both spa and MLST methods are suitable for typing all of the described animal and human MRSA strains, unlike pulse field gel electrophoresis (PFGE) which has been widely used but cannot identify CC398 strains (Bens, C.C. *et al.*, 2006). A new PCR based approach called the RM test can identify the major human MRSA lineages very quickly and cheaply (Cockfield, J.D. *et al.*, 2007), and will be expanded in the future. Mobile genetic element (MGE) detection is more difficult and the best method, microarray, is currently too expensive and technically difficult for routine use (Lindsay, J.A. *et al.*, 2006; Monecke, S. *et al.*, 2008), although this is expected to change in the next few years. In the meantime, simple antibiotic resistance profiles and/or SCCmec typing (Kondo, Y. *et al.*, 2007; Oliveira, D.C. and de Lencastre, H., 2002) combined with PCR for toxin genes (e.g. PV*luk, tst, sea, seb, sec, sed, sek, sep*) can be useful for typing (Diep, B.A. *et al.*, 2006; Tristan, A., Ferry, T. *et al.*, 2007). In conclusion, *spa* typing is recommended for *S. aureus* lineage detection, including those from animals. In order to discriminate between isolates of the same lineage, it is best combined with a method for MGE detection.

### 1.7.8. EU study of MRSA in samples of dust from the pens of breeding pigs

The ongoing study in the EU to establish the prevalence of MRSA in samples of dust collected from the pens of breeding pigs in different production stages (Decision 2008/55/EC, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:014:0010:0025:EN:PDF) utilizes a combination of methods outlined above. Five dust swabs are pooled, and inoculated into 100ml of Mueller-Hinton broth supplemented with 6.5% NaCl and incubated at 37ºC for 16-20 hrs. One ml of the broth is transferred into 9ml of Tryptone Soy broth with 3.5 mg/l cefoxitin and 75 mg of aztreonam and incubated for a further 16-20 hrs at 37ºC which is then subcultured onto chromogenic MRSA agar. Any presumptive colonies are subcultured onto non-selective agar, confirmed as *S. aureus* and tested for *mec*-A by multiplex PCR. Those colonies identified as MRSA are further tested by *spa*-typing and selected isolates tested for antimicrobial susceptibility and MLST typing.

## 1.8. Specificity and Sensitivity issues.

For the laboratory detection of MRSA, the sensitivity and specificity of the tests are important prerequisites for interpretation including that for surveillance (Kelly, H. *et al.*, 2008). The entire methodological process is influenced by the prevalence of MRSA, starting with the conditions prior to sampling such as hygiene procedures at the point of sampling or treatment of patients, the sites sampled (Robicsek, A. *et al.*, 2008) and the type of sampling procedure used (e.g. dry or wet swabs, storage and temperature conditions and the duration prior to analysis in the laboratory). The efficiency of recovery of MRSA is likely to be influenced by the heterogeneity of *S. aureus* populations including the presence of MRSA as well as MSSA (Ornskov, D. *et al.*, 2008) as well as *mec*A variants which will be detected poorly by some genotypic methods (Desjardins, M. *et al.*, 2006).

Data produced on the occurrence of MRSA is generally qualitative. Whilst further work is required in optimising methods for future surveillance of MRSA (including from food), a combination of both direct inoculation and enrichment will generate both qualitative and semi quantitative data. To increase sensitivity, some methods include a pre-enrichment step during isolation, however there is uncertainty as to the significance of the results obtained both after enrichment, and the public health risk associated with low levels of MRSA in the original specimen. Because of the methodological differences, interpretation and comparisons between studies must take into account the strength and weaknesses of different isolation procedures. The use of standardized methods is therefore required for meaningful comparisons both over different periods and between regions.

## 2. Hazard Characterisation

### 2.1. Occurrence of MRSA in humans in the EU.

The occurrence of MRSA varies widely but is most dependent on geographical region. Some EU countries report a high prevalence of MRSA, such as the UK, while others have medium prevalence such as Germany, and others low prevalence such as the Netherlands. The reasons for the difference are likely due to the level of screening, isolation and monitoring of patients and staff in hospitals, with the Dutch having the most pro-active system over the last two decades. Hospitals have traditionally been the reservoir of MRSA due to the use of antibiotics, and there are geographical differences which are likely to be due to the dissemination of newly evolved strains and different antibiotics prescribing regimes.

#### 2.1.1. Carriage rates in hospital staff

Carriage rates in hospital staff vary widely depending on the geographical region. In a recent review of 127 investigations worldwide, an overall prevalence rate of MRSA colonisation in hospital staff was 4.6 % (Albrich, W.C. and Harbarth, S., 2008). While the rates in Europe are low compared to most other continents, there is substantial variation between different Member States. Although carriage studies in staff are rarely performed in endemic countries (Albrich, W.C. and Harbarth, S., 2008), in the UK (a high incidence country) a study of staff in an ITU in London revealed an 8% carriage rate (Edgeworth, J.D. *et al.*, 2007), and in the West of Ireland the carriage rate in medical general practitioners was 8% (Mulqueen, J. *et al.*, 2007). In low incidence countries screening of staff is a key component of 'search and destroy' policies to eradicate MRSA in hospitals. Staff carriers are removed from work, decolonised, and do not return until clear. Staff carriage rates in these countries are very low. In a recent study, 0.15% of healthcare workers in the Netherlands were carriers, although this rose to 1.7% in those that had direct contact with pig and veal calves, and this risk group made up 3% of the population (Wulf, M.W., Tiemersma, E. *et al.*, 2008).

#### 2.1.2. Carriage rates in the community

There is generally a shortage of quality data investigating carriage rates in the community. Nevertheless, this appears to vary substantially between countries. Furthermore, various risk factors in the community contribute to carriage rates. For example, in a high endemic country such as the UK, carriage rate in the normal population is around 1%, but, is higher (around 8%) in patients admitted to hospital accident and emergency departments (Gopal Rao, G. *et al.*, 2007): probably because this community group is frequently exposed to the healthcare sector and to antibiotics. Similarly, patients in the community visited by a district nurse had a carriage rate of 6.6% (Thomas, S. *et al.*, 2007), while those visiting a medical assessment unit

had a carriage rate of 21% (Thomas, S. *et al.*, 2007), and those in nursing homes had a carriage rate of 22% (Barr, B. *et al.*, 2007).

In a low prevalence country such as the Netherlands, the carriage rate in the community is probably very low. In 2003, the carriage at routine admission to hospitals excluding high-risk individuals was 0.03% (Wertheim, H.F. *et al.*, 2004). With such a low background rate and regular screening, any group with an enhanced carriage incidence is readily detected.

In the Netherlands, those identified at high risk of MRSA carriage are veterinarians, pig and veal farmers and slaughterhouse workers. Dutch pig farmers have a carriage rate of >20% (Wulf, M. and Voss, A., 2008). Dutch veterinarians had a carriage rate of 4.6% (Wulf, M.W., Sorum, M. *et al.*, 2008), predominantly due to CC398. Denmark is also a low prevalence country, but the carriage rate amongst veterinarians is 3.9% (Moodley, A. *et al.*, 2008). At an international veterinary conference, the carriage rate was 10-12% (Anderson, M.E. *et al.*, 2008; Wulf, M.W., Sorum, M. *et al.*, 2008). Participants positive for MRSA originated from Belgium, Canada, Denmark, France, Germany, Italy, The Netherlands, Spain and Thailand. In the UK, veterinarians also have a high carriage rate (18% in one small animal clinic), and the predominant clone is typical of UK hospitals and infections in companion animals, CC22 (Loeffler, A. *et al.*, 2005).

CA-MRSA is a serious problem in some countries, notably the USA, and is usually associated with *S. aureus* clones that have evolved independently of hospitals, and are positive for the Panton-Valentine leukocidin toxin, especially USA300, USA400, ST80 and ST59. These clones are typically associated with outbreaks of severe skin and soft tissue infection, particularly in communities of people in close contact, such as schoolchildren, the military, prisons, sports teams, and men who have sex with men. While all of these clones are seen in Europe, the incidence is relatively low compared to that in the USA, and low compared to hospital associated MRSA in endemic countries. There is little information about carriage rates of these clones compared to other types of MRSA. However, as the incidence of infection with these strains is rising dramatically in low incidence countries (Larsen, A.R. *et al.*, 2009), and the carriage rate is also likely to increase. Rates of CA-MRSA infection are probably also increasing in MRSA endemic countries, but it is masked by the high rates of infection with hospital MRSA clones.

### 2.1.3. Contamination of the environment

#### 2.1.3.1. Hospitals

The primary reservoirs of MRSA in hospitals are the noses, groins, armpits and hands of colonised patients, staff and visitors. Colonised people shed MRSA into their environment, which is disseminated by shedding skin cells or touching the environment. Faeces can also be a source of MRSA in the hospital environment (Klotz, M. *et al.*, 2005). The environment can include medical equipment, beds, mattresses, bedding, clothing, curtains, other soft furnishings, tables, floors, bathrooms, door handles and the air (Dancer, S.J., 2008b). MRSA are most often found near carriers. (Gehanno, J. *et al.*, 2009) found MRSA of the same genotype in infected and colonised patients in a hospital and in the air of the room of these patients.

MRSA can survive in the environment for months or even years. Hospital cleaning reduces MRSA contamination, but probably does not eliminate it. There is a shortage of evidence about whether hospital cleaning plays a major role in reducing MRSA infection (Dancer, S.J., 2008b), especially in an endemic environment.

2.1.3.2. Domestic and other settings outside hospitals and other health care facilities

There are fewer studies investigating the frequency of environmental contamination of MRSA in the home compared to those documenting the occurrence of MRSA in the environment of hospitals or nursing homes. Nevertheless there are some reports which document repeated re-colonization of hospital staff associated with contamination of the home environment (Allen, K.D. *et al.*, 1997; de Boer, H.E. *et al.*, 2006). A recent report from the USA found MRSA in 26% of 35 homes which had no history of MRSA infections but all households had a child in nappies and either a cat or a dog (Scott, E. *et al.*, 2008). The study reported a significant association between the presence of a cat and the isolation of MRSA from household surfaces.

A small number of studies examined the contamination of the environment in animal housing and veterinary hospitals. (Van Den Broek, I. *et al.*, 2008) reported MRSA isolation from pigs or pig dust in 28/50 farms investigated and human carriage was found only on farms in which pigs or dust samples collected from pig houses were positive. Human carriage was found on one farm in which pigs sampled were negative but dust samples were positive. There was a significant association between the intensity of contact with pigs and likelihood of MRSA carriage.

Loeffler, A. *et al.* in 2005 found MRSA in 3 of 30 environmental samples collected in a small animal veterinary hospital: MRSA was detected in veterinary staff and in dogs during the same sampling period. (Weese, J.S. *et al.*, 2004) conducted environmental sampling in an equine veterinary hospital during a period when MRSA-positive horses were present and found 25 of 260 (9.6%) sites were contaminated, mostly sites within stalls which housed MRSA-positive horses. These data suggest that MRSA-contamination of the environment outside the human hospital setting are an important source of colonisation for human occupationally acquired carriage such as pig farmers or veterinary personnel. In addition, pets including cats may serve to disseminate MRSA contamination in the household and act as secondary reservoirs of MRSA when they acquire human MRSA strains following contact with human carriers (Scott, E. *et al.*, 2008).

### 2.1.4. Occurrence in patients

The occurrence of MRSA varies widely among EU countries. The European Antimicrobial Resistance Surveillance System (EARSS) provides data on the percentage of MRSA among S. aureus isolates from invasive infections (mostly bloodstream infections) in Europe. In 2007, the median of these percentages in the EU was 19%; however, there were large differences between countries, from less than 2% in Denmark, Finland, the Netherlands and Sweden to more than 25% in Cyprus, France, Ireland, Italy, Malta, Portugal Romania, Spain and UK [EARSS Annual Report 2007, (http://www.rivm.nl/earss/Images/EARSS%202007_FINAL_tcm61-55933.pdf); EARSS Interactive database (http://www.rivm.nl/earss/database/)].

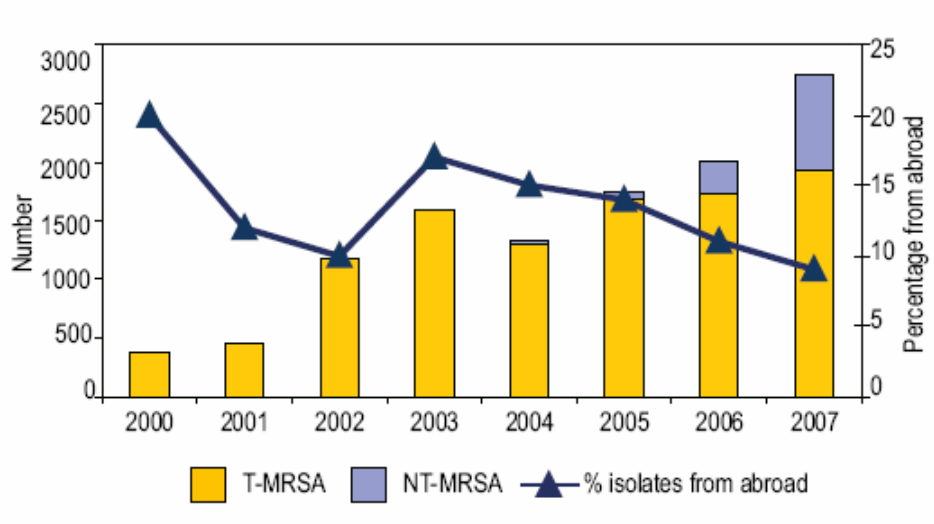
These inter-country differences have been relatively stable since the start of EARSS in 1999. Inter-country differences in MRSA prevalence are likely due to better infection control, i.e. level of screening, isolation and monitoring of patients and staff in hospitals, hand hygiene, decontamination of the environment, as well as a more prudent use of antibiotics. In recent years, several countries with high or average proportion of MRSA have reported a decreasing trend (EARSS Annual Report 2007). This is likely due to increased efforts to control MRSA in hospitals and other healthcare settings.

During the same period, other countries (namely the Netherlands and Denmark) with traditionally very low MRSA have reported an increase in percent MRSA. In Denmark, the

increase has been attributed to an increase in CA-MRSA infections that were commonly associated with young age, skin and soft tissue infections, and of foreign origin (Larsen, A.R. *et al.*, 2009). It is possible that CA-MRSA is slowly increasing throughout Europe, although this increase is currently more noticeable in countries with low MRSA prevalence.

In the Netherlands, a large part of the recent increase in MRSA prevalence in humans is due to CC398 LA-MRSA strains (Dutch Foundation of the Working Party on Antibiotic Policy (SWAB), 2008, http://www.swab.nl/swab/swabcms.nsf/(WebFiles)/E32F6709B7DB7F2EC125744F002ACAA5/$FILE/NethMap_2008.pdf), (Wannet, W.J.B. *et al.*, 2007)) (Figure 1).

Figure 1. **Number of typeable MRSA (T-MRSA) and of CC398 MRSA (NT-MRSA) in the Netherlands, 2000-2007.**




A recent survey initiated by ECDC was performed to obtain information on the proportion of LA-MRSA strain CC398 among human isolates in the EU. Preliminary results from 12 countries (Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Greece, Hungary, Italy, Ireland, the Netherlands and Sweden) show that, in 2007, the median percentage of CC398 isolates among typed MRSA isolates was 0.7%. The country with the highest percentage of MRSA CC398 was the Netherlands, where this strain represented 12% of typed clinical isolates, followed by Belgium (5%, clinical and active screening isolates), Austria (3%, clinical and active screening isolates) and Denmark (2%, clinical isolates)[2]. LA-MRSA colonisation and/or infection in humans was also found to be more common in areas of The Netherlands where pig farming was more prevalent (van Loo, I.H. *et al.*, 2007).

In conclusion, LA-MRSA strains seem to represent only a small proportion of the total number of reports of MRSA infections in the EU. However, this proportion is unevenly distributed among countries, and is much higher e.g. in Denmark, The Netherlands and Belgium.

The following sections provide examples of 3 EU Member States selected for low, medium and high HA-MRSA prevalence:

[2] Data from a recent survey initiated by ECDC. **Manuscript in preparation** by Brigitte A.G.L. van Cleef, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

### (i) Data from The Netherlands

In the Netherlands, the prevalence of HA-MRSA is low, and a search and destroy policy is implemented to prevent MRSA from becoming endemic in hospitals. The Dutch guidelines of the Working Group for Infection Prevention (WIP-www.wip.nl) distinguish four categories (1–proven carriage, 2–high risk, 3–intermediate risk, 4-no increased risk) of patients with respect to MRSA carriage.

Patients with an increased risk of being MRSA carrier (categories 1 and 2) are isolated at hospital admission until screening cultures for MRSA prove negative. In case of MRSA carriage, individual are kept in isolation and treated to eradicate MRSA. Combined with a restrictive policy on prescribing antibiotics, this has controlled the prevalence of MRSA to low levels. In 1999-2003, only 0.6% of all clinical isolates of *S. aureus* were resistant to meticillin (Tiemersma, E.W. *et al.*, 2004). This increased to 1.1% from 2004-2007, still well below the EU median of 19% (www.rivm.nl/earss/database). In 2003, the carriage at routine admission to hospitals excluding high-risk individuals was only 0.03% (Wertheim, H.F. *et al.*, 2004).

Category 2 includes certain patients who were treated in foreign hospitals, or from Dutch hospitals or nursing homes with a non-controlled MRSA epidemic, or contacts with known MRSA carriers.

The first, livestock-associated, non-typeable (NT)-MRSA isolate was found in the collection of the national reference centre for MRSA at RIVM in 2003, and the percentage of isolates in this group had increased to 14% in 2006. This was partly related to the start of a focused survey in the 2$^{nd}$ half of 2006, but there is also reflects an overall increase in the numbers of CC398 infections.

Based on these data, the WIP decided in 2006 that pig farmers, veterinarians and slaughterhouse workers were assigned to category 2. At that time, case-control studies had also indicated that there was an increased risk of MRSA carriage among veal farmers, but these were still in category 3 (screening but no isolation). New data (Graveland, H. *et al.*, 2008) led to the inclusion of veal farmers and others who have contact with live veal calves in category 2. Currently, all persons who have contact with live pigs or veal calves, irrespective of whether this is professional or not and irrespective of the location, are included in category 2. This implies an increased workload and costs for hospitals, especially those in rural areas (van Rijen, M.M. *et al.*, 2008).

### (ii) Data from Germany

Germany is a country with medium prevalence of MRSA as expressed as frequency among all *S. aureus* from systemic infections (EARSS 2007 Annual report, (http://www.rivm.nl/earss/Images/EARSS%202007_FINAL_tcm61-55933.pdf). The proportion of MRSA CC398 among all MRSA from infections treated in German hospitals in 2006-2007 was 0.22% (8 of 3,944 isolates analysed by the German National Reference Centre for Staphylococci) (Cuny, C. and Witte, W., 2008).

### (iii) Data from the UK

The UK is an example of a European country with a high incidence of MRSA. Forty percent of all *S. aureus* infections in hospitals are due to MRSA (Johnson, A.P. *et al.*, 2001). Each year, approximately 7,000 cases of MRSA bacteraemia are reported through mandatory surveillance in English hospitals (HPA, 2008). To date no report of LA-MRSA in animals exist, with the exception of 3 human cases of CC398 in Scotland in 2007 (two umbilical

swabs from infants and an orthopaedic wound) with no apparent connection to pigs (http://www.hps.scot.nhs.uk/giz/wrdetail.aspx?id=38084&wrtype=2).

In summary, the public health significance of LA-MRSA depends on the overall prevalence of MRSA infections in the country. In countries with low MRSA prevalence and an active search and destroy policy, LA-MRSA may contribute significantly to the pool of MRSA infections and to the costs of MRSA control.

#### 2.1.5. Differences in antimicrobial resistance in HA- CA- and LA- (including CC398) MRSA

Options for empirical treatment of HA-MRSA have long been limited to vancomycin since these strains have the potential to be resistant to virtually all other systemic antimicrobials. Newer agents (e.g. linezolid, daptomycin, tigecycline) are expanding the agents available for treatment but all have their limitations (Moreillon, P., 2008). In contrast to HA-MRSA, typical CA-MRSA strains are seldom multi-resistant and still susceptible to clindamycin, tetrayclines, trimethoprim-sulphonamides (cotrimoxazole), and fluoroquinolones (Dietrich, D.W. *et al.*, 2004). The resistance pattern of MRSA CC398 typically shows 100% resistance to tetrayclines and the majority also resistant to trimethoprim (but not cotrimoxazole). Resistance in CC398 also occurs to macrolides (erythromycin), lincosamides (clindamycine), aminoglycosides (kanamycin) but these vary (Van den Eede, A. *et al.*, 2009). Some reports describe 100% susceptibility to fluoroquinolones in CC398 isolates as susceptibility (de Neeling, A.J. *et al.*, 2007), however a Belgian study revealed about 35% resistance in isolates from pigs (Denis, O. *et al.*, 2008).

### 2.2. Occurrence of MRSA in animals

#### 2.2.1. Companion animals

*S. aureus* colonises less than 10% of healthy dogs and cats but is commonly isolated from clinical infections in horses, over 60% of staphylococcal isolates from horses were identified as *S. aureus* by (Biberstein, E.L. *et al.*, 1984). *S. aureus* causes a wide range of opportunistic infections in companion animals, including superficial skin infections, post-operative wound infections and occasionally life-threatening bacteraemia.

The first report of meticillin-resistant *S. aureus* (MRSA) in animals was in mastitic cows (Devriese, L.A. *et al.*, 1972) but it was not until the late 1980s and early 1990s that MRSA in companion animals was recorded. (Scott, G.M. *et al.*, 1988) suggested that a cat was the source of a MRSA outbreak in a geriatric ward and oxacillin-resistant coagulase-positive staphylococci (presumed MRSA) were isolated from post-operative wound infections in dogs by (Smith, M.M. *et al.*, 1989). Other reports of MRSA isolation from pets and horses followed (Anzai, T. *et al.*, 1996; Hartmann, F.A. *et al.*, 1997; Lilenbaum, W. *et al.*, 1998; Seguin, J.C. *et al.*, 1999; Tomlin, J. *et al.*, 1999). MRSA has now been reported from a wide range of companion animals apart from dogs, cats and horses with reports of isolation from rabbits, guinea pigs, a turtle, a bat and a parrot (O'Mahony, R. *et al.*, 2005; Walther, B. *et al.*, 2008).

Although there are some recent data documenting the occurrence of MRSA in clinically affected and healthy companion animals, data are frequently derived from small numbers of samples and definitive surveys on the prevalence of MRSA are lacking. Prevalence of MRSA in clinical infections in dogs was 13% in Ireland (Abbott, Y. *et al.*, 2006) and 9% (both clinically affected and healthy) dogs in London (Loeffler, A. *et al.*, 2005). Selected reports on

the prevalence of MRSA infection and carriage in companion animals are summarized in Table 1.

The clinical conditions caused by MRSA in animals appear to comprise mainly skin infections, post-operative and other wound infections and respiratory infections, with cases of sepsis being relatively rare (Lloyd, D.H. *et al.*, 2007; O'Mahony, R. *et al.*, 2005; Walther, B. *et al.*, 2008; Weese, J.S., Rousseau, J. *et al.*, 2006).

There are no specific studies which examined the relative importance of small animals and horses as sources of infection or colonisation in humans. The data in Table 1 present a wide range of isolation rates in both pets and horses with no definite trend for higher isolation rates in any particular species. However, data on the prevalence of MRSA colonisation in large animal veterinary personnel in contact with horses (15.6% colonised) compared to small animal personnel (4.4% colonised) (Hanselman, B.A. *et al.*, 2006) suggested that contact with large animals may present a greater risk to the handler than contact with small animal pets.

Table 1. **Prevalence of MRSA infection and carriage in companion animals and horses used for recreation according to some recently published reports.**

| Year | Country | Species/ healthy or clinical disease | Method | Prevalence of MRSA | MRSA characterization | Reference |
|---|---|---|---|---|---|---|
| 2004-2005 | Ireland | Dogs /clinical | Direct plating on blood agar and NaCl 7.5% broth enrichment | 13% | AR typing, similar to human hospital strains | (Abbott, Y. *et al.*, 2006) |
| Not given | UK | Dogs /both | Enrichment in TSB + 10%NaCl and plating on BA and mannitol salt agar | 9% | PFGE, strains indistinguishable or closely related to EMRSA-15 | (Loeffler, A. *et al.*, 2005) |
| 2004-2005 | Ireland | Dogs /clinical | Direct plating on blood agar and NaCl 7.5% broth enrichment | 8% | AR typing, similar to human hospital strains | (Abbott, Y. *et al.*, 2006) |
| 2003-2004 | Germany | Dogs /clinical | Direct plating on BA and Chrom agar | 7.5% | ST22, SCC*mec* IV and ST239, SCCmec not determined | (Walther, B. *et al.*, 2008) |
| 2003-2004 | Germany | Cats /clinical | Direct plating on BA and Chrom agar | 10% | ST22, SCC*mec* IV | (Walther, B. *et al.*, 2008) |
| 2001-2003 | Korea | Dogs /clinical | Direct plating on BA | 2% | ST5, SCC*mec* II | (Kwon, N.H. *et al.*, 2006) |
| Not given | Hong Kong | Dogs /unclear | Isolation method not given | 0.7% | AR typing indicated both HA and CA strains, SCCmec III and IV types detected | (Boost, M.V. *et al.*, 2007) |
| Not given | Australia | Dogs /clinical | Selective enrichment in Gioliti-Cantoni broth and plating on mannitol salt agar | 1.4% | AR typing, mecA detection. Isolates similar to human strains | (Malik, S. *et al.*, 2006) |
| 2004-2005 | Ireland | Dogs /healthy | Direct plating on blood agar and NaCl 7.5% broth enrichment | 0.6% | AR typing, similar to human hospital strains | (Abbott, Y. *et al.*, 2006) |
| 2003-2006 | UK | Dogs /healthy | Not given | 0.4% | Not done | (Rich, M. and Roberts, L., 2006) |
| Not given | Slovenia | Dogs /healthy | Direct plating on mannitol salt agar with 2µg/ml oxacillin and enrichment in broth with 7.5% NaCL | 0% | NA | (Vengust, M. *et al.*, 2006) |
| Not given | Australia | Dogs /healthy | Selective enrichment in Gioliti-Cantoni broth and plating on mannitol salt agar | 0% | AR typing, mecA detection. Isolates similar to human strains | (Malik, S. *et al.*, 2006) |

| Year | Country | Species/ healthy or clinical disease | Method | Prevalence of MRSA | MRSA characterization | Reference |
|---|---|---|---|---|---|---|
| Not given | UK | Horses /clinical | Direct inoculation onto mannitol salt agar with aztreonam and oxacillin-resistance screening agar | 4.4% | PFGE – unrelated to human hospital strains, most SCC *mec* IV | (Baptiste, K.E. *et al.*, 2005) |
| Not given | UK | Horses /both | Direct inoculation onto mannitol salt agar with aztreonam and oxacillin-resistance screening agar | 16% | PFGE – unrelated to human hospital strains, most SCC *mec* IV | (Baptiste, K.E. *et al.*, 2005) |
| 2003-2005 | Austria | Horses /clinical | Direct plating on BA | 1.2 to 5.5% | PFGE, unlike common HA and CA strains, ST254, SCC *mec* IVd | (Cuny, C. *et al.*, 2006) |
| 2000, 2002, 2003 | Canada | Horses | Initially (2000) direct plating on mannitol salt agar with 2µg/ml oxacillin and on Mueller-Hinton agar with 4%NaCl and 6µg/ml oxacillin<br>2002-2003 Enrichment in 7.5%NaCl broth and plating on mannitol salt agar with 2µg/ml oxacillin | 4-8% | Most strains PFGE type CMRSA-5, SCC*mec* IV, ST 8, *spa* type 7 | (Weese, J.S., Archambault, M. *et al.*, 2005; Weese, J.S., Rousseau, J. *et al.*, 2006) |
| 2003 | Canada | Horses /not stated, apparently healthy | Direct plating on mannitol salt agar with 2µg/ml oxacillin and enrichment in 7.5%NaCl broth followed by plating on mannitol salt agar with 2µg/ml oxacillin | 0-45% | PFGE, all CMRSA-5 | (Weese, J.S., Rousseau, J. *et al.*, 2005) |
| 2004 | The Netherlands | Horses | Mueller-Hinton agar, 4% saline, 5% blood and 6 mg/ml oxacillin. | 0% | NA | (Busscher, J.F. *et al.*, 2006) |
| 2007 | Belgium | Horses presenting at veterinary hospital | Enrichment in brain-heart infusion agar containing colistin and naladixic acid followed by plating on MRSA selective agar | 10.9% | SCC*mec* IVa or V, *spa* type t011 or t1451, ST398 | (Van den Eede *et al.*, 2009) |

### 2.2.2. Food-producing animals

MRSA CC398 was first isolated from several family members living on a pig farm, and the pigs on this farm (Voss, A. *et al.*, 2005). Subsequent studies on the prevalence of this clone in food-production animals showed its presence in pigs in several countries in Europe, Canada and the US and Asia (Table 2). The clone is also widely spread in veal calves in the Netherlands (Graveland, H. *et al.*, 2008). In poultry, a recent observation on the presence but not yet on prevalence of MRSA in Belgium was published (Nemati, M. *et al.*, 2008). In dairy cattle systematic monitoring is lacking in Europe but initial reports suggest a low prevalence. (Armand-Lefevre, L. *et al.*, 2005) reported a meticillin susceptible variant of CC398 isolated from pigs in France in 2002. CC398 has also been found in companion animals and horses but the primary reservoir is in food production animals (Nemati, M. *et al.*, 2008). Case control studies in humans identified working with live pigs and cattle as a risk factor for testing positive for MRSA CC398 (van Loo, I., Huisdens *et al.*, 2007). In a limited study on pig farms the use of preventive oral antimicrobial medication was a risk factor for MRSA carriage (van Duijkeren, E. *et al.*, 2008).

Table 2. **Prevalence of MRSA in food producing animals**

| Country (region) | Year | Animal species | Prevalence | Type | Reference |
|---|---|---|---|---|---|
| Germany (Lower Saxony, North Rhine - Westphalia) | 2007 | Pig (fattening) | 13% (85/678) | CC398 | (Meemken, D. *et al.*, 2008) |
| The Netherlands | 2006 | Pig (various) | 11% (35/310) | CC398 | (van Duijkeren, E. *et al.*, 2008) |
| The Netherlands | 2005-2006 | Pig (slaughter) | 39% (209/540) | CC398 | (de Neeling, A.J. *et al.*, 2007) |
| Canada (Ontario) | NK | Pig | 25% | CC398 | (Khanna, T. *et al.*, 2008) |
| US | NK | Pig | 70% | Untypeable (CC398) | (Smith, T.C. *et al.*, 2008) |
| Singapore | 2005 | Pig | Case study | CC398 | (Sergio, D.M. *et al.*, 2007) |
| Denmark | 2005 | Pig | 10% | CC398 | (Guardabassi, L. *et al.*, 2007) |
| The Netherlands | 2007-2008 | Calves | 88% veal calf farms, 28% calves | CC398 | (Graveland, H. *et al.*, 2008) |
| Spain | NK | Sheep | 1/38 mastitis isolates | Phenotypic resistance, *mec*A unconfirmed | (Goni, P. *et al.*, 2004) |
| Belgium | NK | Chickens | Case study | CC398 (t011 and t567) | (Nemati, M. *et al.*, 2008) |
| The Netherlands | NK | Poultry manure | Case study | CC398 | (Leenders, A.C. *et al.*, 2007) |
| Korea | 1997-2004 | Dairy cows | 19/696 mastitis isolates | NK | (Moon, J.S. *et al.*, 2007) |
| Hungary | 2002-2004 | Dairy cows | 27/595 mastitis samples | 4 isolates: ST1-t127,SCC*mec*IVa | (Juhasz-Kaszanyitzky, E. *et al.*, 2007) |

NK = not known

An ongoing study in all EU-Member States is currently determining the occurrence of MRSA in swine breeding herds and will provide an estimate of the presence and to some extent the prevalence of MRSA in the different Member States. This study will not give any information on the occurrence or prevalence of MRSA in other animal species or in slaughter pig herds, where studies from The Netherlands and Belgium have shown a higher prevalence compared to breeding farms (Denis, O. et al., 2008).

As can be seen in tables 2 and 3, MRSA has been isolated from most food-producing animals and from most meats as well as from milk. Since the emergence of CC398 in Europe and elsewhere, reports of MRSA in farm animals are increasing. The occurrence of MRSA in raw food is generally low with the highest prevalence reported in The Netherlands where MRSA was detected in 11% of meat samples (de Boer, E. *et al.*, 2008). This was the only study which quantified the levels of MRSA present; levels were low, less than 10cfu/g.

In conclusion, CC398 has recently emerged in food production animals and this clone has spread over many countries in Europe and North America.

### 2.3. Occurrence of MRSA in foods

*S. aureus* is routinely enumerated in a wide variety of ready-to-eat foods, as part of general microbiological safety checks. Moreover, Regulation (EC) No. 2073/2005 (on microbiological criteria in foodstuffs, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF) specifies maximum limits for coagulase-positive staphylococci in food, to be complied with by food business operators during processing of cheese, milk powder and whey powder, and staphylococcal enterotoxins must be absent in these products placed on the market during their shelf-life. However, the presence of MRSA in food is not routinely investigated, and data are only available from a small number of studies.

The results of studies that have investigated the occurrence of MRSA in food are summarised in Table 3. In ten separate surveys carried out between 1999 and 2006 in Korea, Hungary, France, Japan, Italy, and Jordan, the frequency of MRSA isolation was low when different types of food were tested, with results ranging from 0 to 1.2% (Kaszanyitzky, E.J. *et al.*, 2004; Kaszanyitzky, E.J. *et al.*, 2003; Kerouanton, A. *et al.*, 2007; Kitai, S. *et al.*, 2005; Kwon, N.H. *et al.*, 2006; Kwon, N.H. *et al.*, 2005; Lee, J.H., 2003; Normanno, G. *et al.*, 2007; Quddoumi, S. *et al.*, 2006; Shimamura, Y. *et al.*, 2006). A similar percentage was found in a recent study carried out in Spain (1.3%) in food samples of animal origin (Lopez, M. *et al.*, 2008; Lozano, C.[3]). However, higher rates of contamination with MRSA were observed in Pakistan (10.4%) in raw milk (Farzana, K. *et al.*, 2004), and in two recent studies in the Netherlands (2.5% and 11.9%) or in the United States (5%) in raw meat at retail (turkey, veal, chicken, beef, lamb, fowl, and game) (de Boer, E. *et al.*, 2008; Pu, S. *et al.*, 2009; van Loo, I.H. *et al.*, 2007; VWA, 2007). In general, foods from which MRSA were isolated included raw meat (including pork, beef, lamb, chicken, turkey and on one occasion rabbit), dairy products (milk and cheese) and, in one instance, pancakes. The methodology used in all these studies varied considerably as can be seen in Table 3 and this fact could contribute to the different prevalences of MRSA detected among the different studies.

Several studies carried out the typing of the MRSA isolates recovered from food samples. Two studies performed in the Netherlands have identified CC398 in one isolate recovered from raw food of pork origin (van Loo, I.H. *et al.*, 2007), and in 85% of MRSA food isolates

[3] Lozano, C., López, M., Gómez, E., Somalo, S., Ruiz-Larrea, F., Torres, C., and Zarazaga, M. Departamento de Agricultura y Alimentación, Universidad de La Rioja, Logroño, Spain. Detection of MRSA ST398 and ST125 in food samples of animal origin in Spain. **Manuscript submitted for publication.**

from different origins (chicken, turkey, pork, beef, veal, lamb and fowl) (de Boer, E. *et al.*, 2008). In Spain, MRSA CC398 was obtained in a meat pork sample (Lozano, C.[3]). Other sequence types (ST5 and ST125), associated with human infections, have been detected in other studies (Kwon, N.H. *et al.*, 2006; Kwon, N.H. *et al.*, 2005, Lozano, C.[3]). Where biotyping was carried out, human biotypes were identified in two studies (Kerouanton, A. *et al.*, 2007; Kitai, S. *et al.*, 2005), and animal biotypes in one study (Normanno, G. *et al.*, 2007). When SCCmec characterization has been carried out with MRSA isolates of food origin, the SCCmec III and IV (including a new subtype SCCmec IVg) were identified in isolates recovered from cows' milk and chicken (Kitai, S. *et al.*, 2005; Kwon, N.H. *et al.*, 2006; Kwon, N.H. *et al.*, 2005).

In conclusion, it is difficult to compare the risks contributed by different food types given the wide variety of methodologies used, and the lack of quantitative data on MRSA levels in the majority of studies. It has been shown that MRSA can frequently be detected on raw meats in low numbers.

Table 3. **Summary of published reports of MRSA detection in food**

| Year | Country | Food type | Method | Proportion of MRSA contamination | ST-*spa*-SCC*mec* typing | Other typing | Reference |
|---|---|---|---|---|---|---|---|
| 1992 | Pakistan | Raw milk | Enumeration of *S. aureus* on Baird-Parker agar plates, followed by disk diffusion testing | 8/77 isolates (10.4%) From 50 milk samples | | | (Farzana, K. *et al.*, 2004) |
| 1999, 2000, 2003 | Korea | Cows milk | Direct streaking of samples on 5% sheep agar plates and later sampling on Baird-Parker agar plates | 14 MRSA and 1 silent *mec*A$^+$-MSSA<br><br>(isolation ratio 0.18%). | 14 MRSA: ST5- SCC*mec*-IVg-PVL$^+$<br><br>1 silent *mec*A$^+$ MSSA: ST580-PVL$^+$ non-typeable | | (Kwon, N.H. *et al.*, 2005) |
| 2001-2003 | Korea | Foodstuffs from slaughterhouses and retail (pork, chicken and beef) | Not given | 0/159 pork samples (0%)<br>0/75 beef samples (0%)<br>2/620 chicken samples (0.3%) | ST5- SCC*mec* III<br><br>silent *mecA*$^+$-MSSA ST5 non typable | | (Kwon, N.H. *et al.*, 2006) |
| 2001-2003 | Korea | Samples from cattle, pigs and chickens | Enrichment in *Staphylococcus* broth or TSB plus 70 mg/ml NaCl and later sampling on Baird Parker plates | 0/126 specimens from beef cattle (0%)<br>12/1022 from dairy cattle (1.2%)<br>0/469 from pigs (0%)<br>3/296 from chickens (1.0%) | | AP-RAPD: six food isolates similar to those of humans. | (Lee, J.H., 2003) |
| 2001 | Hungary | Foods | Typing of *S. aureus* isolates | No MRSA detected | | | (Kaszanyitzky, E.J. *et al.*, 2003) |
| 1981-2002 | France | Various foods from Food-borne outbreaks | Typing of *S. aureus* isolates from 31 outbreaks | 2/178 strains (1.1%) From sliced pork and pancakes | | 2 isolates: human biotypes | (Kerouanton, A. *et al.*, 2007) |
| 2002-2003 | Japan | Retail raw chicken meat | Not given | 2 /444 samples (0.45%) | 2 isolates: SCC*mec*IV | Human biovar | (Kitai, S. *et al.*, 2005) |

| Year | Country | Food type | Method | Proportion of MRSA contamination | ST-*spa*-SCC*mec* typing | Other typing | Reference |
|---|---|---|---|---|---|---|---|
| 2002-2004 | Japan | Desserts (Japanese and Western style) | Enumeration of *S. aureus* on Manitol salt agar | 0 /368 samples | | | (Shimamura, Y. *et al.*, 2006) |
| 2004 | Hungary | Foods | Typing of *S. aureus* isolates | 5/1,921 strains (0.26%)<br>All from dairy herds | | | (Kaszanyitzky, E.J. *et al.*, 2004) |
| 2003-2005 | Italy | Foods | Typing of *S. aureus* isolates | 6/1,634 samples (0.37%)<br>4 milk, 2 cheeses | | 3 isolates: ovine biovar<br>3 isolates: non-host specific biovar | (Normanno, G. *et al.*, 2007) |
| 2006 | Jordan | Meat samples (sheep, cow, camel, poultry) | | 15/157 strains from 1,260 meat samples (1.2%) | | | (Quddoumi, S. *et al.*, 2006) |
| 2006 | Netherlands | Raw meat at retail<br>(pigs and cattle) | Two enrichment broth cultures and later sampling in chromogenic MRSA-ID agar | 2/64 pork samples (3.1%)<br>0/15 beef samples (0%) | 1 isolate:<br>CC398- t108<br>1 isolate:<br>T024-US300 clone | | (van Loo, I.H. *et al.*, 2007) |
| 2007-2008 | Netherlands | Raw meat at retail | Two enrichment broth cultures and later sampling in chromogenic MRSA-ID agar | 42/395 beef samples (10.6%)<br>39/257 veal samples (15.2%)<br>20/324 lamb/mutton samples (6.2%)<br>33/309 pork samples (10.4%)<br>83/520 chicken samples (16.0%)<br>41/116 turkey samples (35.3%)<br>4/118 fowl samples (3.4%)<br>4/178 game samples (2.2%)<br>Total 264/2217 (11.9%)<br>Low plate counts: <10cfu/g in positive MRSA samples | CC398<br>85% isolates (ST398 animal origin)<br>15% (other ST's possibly human origin) | | (de Boer, E. *et al,* 2008) |

| Year | Country | Food type | Method | Proportion of MRSA contamination | ST-*spa*-SCC*mec* typing | Other typing | Reference |
|---|---|---|---|---|---|---|---|
| 2008 | Spain | Food samples of chicken, beef, pigs, rabbit and lamb | Enrichment on BHI broth with 6.5% NaCl and sampling in chromogenic MRSA-ID agar | 3/254 samples (1.3%)<br>1 of chicken<br>1 of rabbit<br>1 of pork | 1 isolates: CC398-t011<br>2 isolates: ST125- t067 | | (Lopez, M. *et al.*, 2008)<br>Lozano, C.[3] |
| 2008 | United States | Retail meat (pork and beef) | Enrichment on TSB broth with 10% NaCl-1% sodium pyruvate and sampling in Baird-Parker supplemented with cefoxitin (4 μg/ml) | 5/90 of pork (5.5%)<br>1/30 beef (3.3%) | Pork: ST8-t*008*-SCC*mec*IVa-PVL+ USA300<br><br>Pork- beef: ST5-*t002*-SCC*mec*II- USA100: | | (Pu, S. *et al.*, 2009) |

## 2.4. Occurrence of MRSA in the environment

### 2.4.1. Concentrations of micro-organisms in animal houses, amounts of emissions, air-borne transmission and safe distances around farms

Modern animal production is increasingly regarded as a source of air pollutants which can be both aggravating and environmentally harmful (Seedorf, J. and Hartung, J., 2002). The air in animal housing contains gases, odours, dust particles and micro-organisms which may potentially be discharged by way of the ventilation system into the environment. The concentrations of airborne micro-organisms has been used to give an overview of the microbiological status of the air in animal houses in Germany (Seedorf, J. *et al.*, 1998). The highest bacterial concentrations have been detected in broiler houses where about 80 % of the airborne bacteria are *Staphylococcus* spp. (Schulz, J., 2007).

Bacteria emitted from animal houses can travel several hundreds meters from the source. (Muller, W. and Wieser, P., 1987) calculated distances between 150 and 250 m. Recently *Staphylococcus* spp. were detected at levels of about 4000 cfu/m³ nearly 500 m down wind of a broiler barn (Schulz, J., 2007; Seedorf, J. *et al.*, 2005).

### 2.4.2. Airborne MRSA in animal production environments

The relevance of the general findings described above to the transmission of MRSA CC398 from pigs and veal calves is unknown. MRSA can be readily detected in dust from infected herds (EFSA, 2007) and can undoubtedly be inhaled by workers in these environments. but colonisation is highest in people with direct contact with animals (29% positive) and prevalence of infection is much less in those living on farms with no animal contact (2%) (Van Den Broek, I. *et al.*, 2008). This suggests that although a role for airborne transmission of MRSA from animal houses cannot be discounted, it is of less importance than direct contact with live animals. Recently published data suggest that direct handling of animals may be more important than exposure to dust as workers on farms with sows had higher levels of MRSA colonisation compared to those working on farms with only fattening pigs (Van Den Broek, I. *et al.*, 2008). The closer and more prolongued contact with sows and their piglets than with finishing pigs may be one reason for higher colonisation rates in workers on farms with sows.

### 2.4.3. MRSA in the environment of abattoirs, cutting plants, food production environments

There are no published data available on the prevalence of MRSA in the environment of abattoirs and food production premises. However, it is likely that MRSA will behave similarly to MSSA if these become established in slaughter and processing plants. The origin of *S. aureus* found on carcasses at slaughter may be the animals entering the abattoir or the human handlers. The organism may become endemic in the environment of the slaughterhouse (Borch, E. *et al.*, 1996) and several reports suggest that human *S. aureus* may become established as part of the endemic flora of food handlers, with subsequent contamination of carcasses and meat. (Vanderlinde, P.B. *et al.*, 1999) used macrorestriction analysis of the DNA of coagulase-positive staphylococci isolated from minced beef and from workers' hands and concluded that the primary source of contamination was the hands of people working in the slaughterhouse. (Desmarchelier, P.M. *et al.*, 1999) reported increased levels of staphylococcal carcass contamination within 72h of chilling and these authors also suggested that workers' hands were the primary source of contamination for carcasses. Schlegelova, J., in 2004 found that 7.5% of beef carcass swabs were contaminated with *S.*

*aureus* immediately after evisceration compared with 24.3% of meat samples. Based on genotyping results, (Schlegelova, J., 2004) also concluded that the animals were not the source of the contaminating strains. A study in poultry (Dodd, C.E. *et al.*, 1988) used plasmid profiling to characterise the staphylococci isolated and concluded that the major contaminating flora on incoming birds' carcasses derived from strains endemic to the poultry, but after plucking, were endemic to the chicken pluckers. A study of pig carcasses (Nitzsche, S. *et al.*, 2007) however showed that *S. aureus* strains collected from carcasses were specific to pigs.

Numbers of *S. aureus* on poultry carcasses have been estimated in a few studies: 10 to $10^5$ organisms/g skin (Notermans, S. *et al.*, 1982) and 2.3 $\log_{10}$ cfu/g neck skin on exiting the chill (Whyte, P. *et al.*, 2004). Numbers on pork carcasses were 10 to $10^3$ organisms / $cm^2$ on the rind of 89% of uncured hams (Schraft, H. *et al.*, 1992) and 0.57 $\log_{10}$cfu/$cm^2$ of carcass (Yeh, K.S. *et al.*, 2005).

Since MRSA CC398 enters slaughterhouses in or on its host animal, it is possible that it could become part of the endemic flora of the slaughterhouse and slaughterhouse workers as occurs with other strains of *S. aureus,* although this has not yet been investigated.

## 3. Exposure assessment

### 3.1. Vectors for transmission of MRSA

Transmission of infectious agents can take place by direct contact between individuals or through the environment by indirect contact with contaminated surfaces. Transmission via the air, water, food and feed or by mechanical or biological vectors also occurs. Vectors can be viable such as mosquitoes, fish, birds or mammals or inanimate for example equipment or particles, which may all transmit infectious agents. *S. aureus* is transmitted from human to human, animals to human and vice versa and through viable or inanimate vectors (Roberson, J.R., 1999; Scalcini, M.C. and Sanders, C.V., 1980).

The transmission routes of MRSA are probably similar to those of MSSA (Kawada, M. *et al.*, 2003)but there are likely to be differences in the efficiency of host colonization following exposure. Factors responsible for whether a person becomes a persistent nasal carrier or not are poorly understood (Peacock, S.J. *et al.*, 2001), for further discussion see section 4. Furthermore, some lineages of *S. aureus* show host specificities, and are therefore associated with animals more than humans and vice versa (Sung, J.M. *et al.*, 2008).

The origin of MRSA strains which colonize or infect a specific vector is often not clear. Skin to skin contact is probably the main route of transmission between humans, humans to animals, animals to human and between animals, however contaminated materials, surfaces, food or dust can play also a role in transmitting the agent (Asoh, N. *et al.*, 2005; Lee, J.H., 2003). This is true for both HA-MRSA and CA-MRSA. Conditions such as overcrowding and understaffing in hospitals can contribute to high colonization pressure and increase the transmission risk (Clements, A. *et al.*, 2008; Merrer, J. *et al.*, 2000). MRSA control programmes and the application of strict hygiene measures can help to reduce transmission (Ben-David, D. *et al.*, 2008; Eveillard, M. *et al.*, 2006).

CA-MRSA is transmitted within groups such as families, sport teams, prisoners, drug addicts or men who have sex with men (Allen, U.D., 2006; CDC, 2003; Cook, H.A., Furuya, E. Y., Larson, E., et al., 2007; Diep, B.A. *et al.*, 2008; Lindenmayer, J.M. *et al.*, 1998). Crowding, frequent skin-to-skin contact, compromised skin, contaminated surfaces and shared items and lack of cleanliness are assumed to contribute to the transmission of MRSA inside these groups. The increasing number of cases of CA-MRSA presents a problem for MRSA control

in hospitals also as transmission of CA-MRSA in hospitals followed by infection has been reported in recent years (Saiman, L. *et al.*, 2003).

Airborne bacteria in livestock buildings are commonly attached to dust particles which originate from the animals (e.g. epithelial cells, hair, and feathers), the feed, the litter and the faeces. These particles are emitted from the animal house by the ventilation system together with the exhaust and can contaminate soil and plants close to the building or can be transmitted by the airborne route to residential dwellings or other farms in the vicinity. Gibbs, S.G. *et al.*, in 2006 found a range of bacterial species, including *S. aureus,* 150 m down wind of a confined pig farm. Many of the bacteria were resistant to at least two of the antimicrobials tested, which comprised tetracycline, β-lactams, macrolides and lincosamide. It was not possible to identify MRSA with the techniques employed. The authors concluded that the high concentrations of multi-resistant bacteria in the air at distances of (at least) 150 m could have a potential effect on human health for those who live in close proximity to these facilities. Distances up to 500 m are reported by (Schulz, J., 2007; Seedorf, J. *et al.*, 2005) for the spread of *Staphylococcus* spp. from broiler barns. It is known that *S. aureus* (Müller, W. and Gröning, K., 1981; Webb, S.J., 1965) has a relative high persistence in the airborne state. There is no evidence to suggest that this persistence is lower for MRSA than MSSA.

Dust is identified as a vehicle for the airborne transmission of *S. aureus* and may play a role in the spread of infections (Shiomori, T. *et al.*, 2001), therefore the presence of MRSA in air of a positive farm is likely. The degree of transmission and distance of spread is generally determined by the resistance of the organism outside the host and by its adaptability to changing environmental conditions (Clements, M.O. and Foster, S.J., 1999). The concentrations in air will strongly depend on the concentration in the animal house and on the emission rate as well as on the meteorological conditions such as temperature and relative humidity; the dispersion depends on the wind direction, speed and turbulence as shown by Schulz (2007) who measured and modelled the travel distance of *Staphylococcus* spp. on the downwind side of two broiler farms.

As already outlined, both farm and companion animals are potential reservoirs of MRSA affecting humans (de Neeling, A.J. *et al.*, 2007; Lee, J.H., 2006; Weese, J.S., Rousseau, J. *et al.*, 2005). The origins of these strains are unknown, however there is evidence for international spread of specific clones (Wulf, M. and Voss, A., 2008). The transmission of MRSA from animals to humans is evident and people with direct contact with animals such as pet owners, farmers, stockmen, veterinarians and the staff at slaughterhouses showed a higher prevalence of colonisation compared to unexposed people (Khanna, T. *et al.*, 2008; Meemken, D. *et al.*, 2008; Wulf, M.W., Sorum, M. *et al.*, 2008; Wulf, M.W., Tiemersma, E. *et al.*, 2008). As with other zoonotic agents, people in contact with animals colonised or infected with MRSA may become transiently contaminated, become carriers or they may become infected and develop disease. Clinical disease has been reported in humans following acquisition of MRSA infection from a number of animal species. Serious infections including pneumonia have been reported in people infected with MRSA CC398 (Witte, W., Strommenger, B. *et al.*, 2007). This clone was also responsible for a case of human endocarditis (Ekkelenkamp, M.B. *et al.*, 2006). Skin lesions in three people in close contact with a foal infected by Canadian epidemic MRSA-5, ST8 were reported by (Weese, J.S., Caldwell, F. *et al.*, 2006). Probable transmission of MRSA from a cat which resulted in multiple deep abscesses in an otherwise healthy woman was reported by (Sing, A. *et al.*, 2008). The woman's cat was colonised with the same MRSA strain which caused infection in its owner. The strains were characterised and found to be PVL positive (*lukS-PV* and *lukF-PV)* and of *spa*-type t131. The cat was considered the probable source of infection since *spa*-type (t131) is extremely rare in humans.

It is difficult to assess or quantify the risk to people of acquiring infection from animals colonised or infected with MRSA as few data are available. A recent report found a strong association between the intensity of contact with colonised pigs and the frequency of nasal colonisation in occupationally exposed humans. Of 678 pigs which were sent in for post mortem examination from 347 farms in two regions in the north-west of Germany 85 animals (18 %) from 62 (9.1 %) farms tested positive for CC398 by nasal swabs. Sucklers (20 %) and weaners (44 %) showed the highest prevalence followed by fatteners (25-40 kg, 12 %) and finishers (up to 110 kg, 9 %). None of the 39 investigated breeding sows was positive. Of 86 individuals, 20 persons (23 %) tested positive for CC398: 10 (36 %) pig practitioners, 7 (14 %) pig meat inspectors in abattoirs and 3 (100 %) members of a pig herd health service who had regular contact with numerous pig farms. All 5 employees who carried out the dissections or analysed material of the respective pigs were negative. The routes of transmission remained unclear and need systematic investigation (Meemken, D. *et al.*, 2008).

There are no published reports of eating contaminated food leading to throat or nasal colonisation.

### 3.2. Risk factors for the stages of: (i) contaminated, (ii) carrier and (iii) disease in humans and animals.

As outlined in Section 3.1, serious disease has been reported with the livestock-associated CC398 strain (Ekkelenkamp, M.B. *et al.*, 2006; Witte, W., Strommenger, B. *et al.*, 2007), however the process risks to becoming contaminated, developing carriage or developing disease after contact with MRSA positive animals are poorly understood. Recent data reported by (Van Den Broek, I. *et al.*, 2008) documented a carriage rate of 29% in persons who worked regularly with pigs, 12% in those entering pig houses at least once per week but not working with pigs and carriage in only 2% of those with no contact with pigs but living on positive farms. In Denmark, MRSA CC398 was isolated from 31 persons between 2003 and 2007 (Lewis, 2008). Further information was obtained on 21 cases, of which 10 had clinical symptoms, mainly skin and soft tissue infections. A case-control study and a case-case study (comparing with cases from which other MRSA were isolated) indicated that living or working on farms with animals was an independent risk factor for CC398 (matched odds-ratios 35.4 in the case-control study and 14.5 in the case-case study). A history of hospital admission in the 12 months before the diagnosis was associated with an increased risk (odds-ratio 11.4) only in the case-control study. As previously outlined, apart from host adaptation, it is unknown if there are physiological differences between MRSA strains in animals, particularly MRSA CC398, and 'human' strains, which might influence transmission between humans. The low carriage rate in those without pig contact suggests that person to person transmission of the CC398 strain occurs infrequently. However, using data from several studies, (Safdar, N. and Bradley, E.A., 2008) concluded that colonization with MRSA in humans was associated with a four-fold increase in the risk of developing infection. Considerations on the development of disease in healthcare workers colonised with MRSA other than CC398 may be helpful in assessing the risk of disease in colonised animal-associated personnel.

### 3.3. Transmission routes between animals and within the food chain.

The highest reported prevalence of CC398 positive swine was found during an investigation in 50 Belgian fattening farms where 68% (n=34) was found to be positive. A German study demonstrated that within MRSA-positive herds (18%, n=63), there were more CC398-negative than positive animals (Meemken, D. *et al.*, 2008). A marked difference in the number of MRSA positive animals between open (94%) and closed farms (56%) was

demonstrated in the Belgian survey (Denis, O. *et al.*, 2008). This difference might be the result of CC398 transmission within the production chain, e.g. from multiplier to finisher farms as indicated by findings in a Dutch survey (van Duijkeren, E. *et al.*, 2008). Both in the Belgian (Denis, O. *et al.*, 2008) and the German study (Meemken, D. *et al.*, 2008), breeding pigs tend to have a lower carriage rate compared to weaned and finishing pigs. Future studies should investigate to what extent the environment and contact with other vectors (rodents, insects, etc) are involved in the epidemiology.

In veal calves, a recent Dutch investigation found 88% of investigated herds positive for this livestock associated MRSA (Graveland, H. *et al.*, 2008). Little is known about the persistence of MRSA in veal calves over consecutive production rounds. In dairy cattle, the number of MRSA among mastitis isolates is very low. However, where carriage in a herd occurs, up to 15% of lactating cows can be positive for CC398 (Vicca, J. *et al.*, 2008).

In poultry, the highest percentage of CC398 positive animals was also found in Belgium; with 2 of 14 randomly selected broiler farms (14.3%) being positive (Persoons, D. *et al.*, 2009). Layer farms were found to be negative.

## 4. Risk Characterisation

### 4.1. Carriage versus disease.

Carriage of *S. aureus* in the nose, throat, axillae, perineum or gastrointestinal tract of humans and animals is asymptomatic. Disease caused by *S. aureus* varies widely, but often involves a breach of the skin or mucosal membranes followed by inflammation and neutrophil recruitment, fever and the generation of pus. Many infections in healthy hosts are minor and may not even be recognised or need treatment. At the other end of the spectrum of disease, shock, multi-organ failure and death can occur. Isolation of *S. aureus* is not sufficient to prove causation of disease, although isolation from an otherwise sterile site with symptoms of disease is significant. However the symptoms associated with most *S. aureus* infection are relatively non-specific and can also be caused by a range of other pathogens: indeed the disease will often be referred to on the basis of symptoms rather than by the causative organism, e.g. sepsis, pneumonia, bacteraemia, etc and in cases, such as chronic pneumonia in ventilated humans, defining when a patient is infected versus colonised with *S. aureus* is extremely problematic.

There are two main factors determining colonization and/or infection with *S. aureus* (including MRSA) in different animal species and humans, and these are: the degree of host specificity on part of the bacterium and host susceptibility on the part of the host.

The anterior nares are the main site which is colonized by *S . aureus*, therefore it represents the main reservoir for dissemination. In humans about 20% of the healthy population are permanently colonized (Kluytmans, J. *et al.*, 1997). There is evidence for a genetic basis for permanent carriage (Peacock, S.J. *et al.*, 2001) although the host factors and mechanisms leading to nasal carriage are poorly understood but likely to be multifactorial. Nasal colonization in humans and in pigs is usually with one clone of *S. aureus* (Nouwen, J. *et al.*, 2004). The basis for the mutual exclusion of colonization is unknown. It is likely that antibiotic use is also important e.g. fluoroquinolones clears carriage of susceptible *S. aureus* and allows colonisation with MRSA which are often resistant to this antibiotic. In a study on human volunteers, non-carriers quickly eliminated *S. aureus* following nasal inoculation, however persistent carriers selected their original resident *S. aureus* from the inoculated mixture (Nouwen, J. *et al.*, 2004).

### 4.2. Host specificity.

*S. aureus* is not only a colonizer of the mucosa of the upper respiratory tract of all mammals but it has also been found in natural population of birds, including industrially raised poultry. Recent studies suggest that factors relating to the ability of the organisms to colonize nasal epithelium (Quinn, G.A. and Cole, A.M., 2007) and host genotype may be important in determining whether colonisation occurs (Emonts, M. *et al.*, 2008). Characterization of MSSA isolates suggest the existence of host adapted lineages including humans, cattle, sheep, and chicken (Devriese, L.A., 1984). This hypothesis was later confirmed by MLST which identified *S. aureus* clonal lineages preferentially associated with cattle (MLST – ST151, ST97, ST126, ST464), sheep and goats (ST133, ST522), chicken (ST385), and pig (CC398) (Hata, E. *et al.*, 2008; Kapur, V. *et al.*, 1995; Rabello, R.F. *et al.*, 2007). However animal lineages do cause some human infections and *vice versa*. The basis for the host adaptation is poorly understood.

### 4.3. Conditions predisposing humans to infections with *S. aureus*.

In comparison to the wide-spread distribution of this bacterium as a colonizer, infections are rare events. For humans, the following conditions have been identified to predispose to *S. aureus* infections:

- exposure to the organism, including from colonised individuals (including patients and staff in hospitals) .
- exposure to the organism from the patients own colonisation sites.
- disruption of skin as an external barrier by injuries, catheters, surgery, eczema or psoriasis.
- immunodeficiency, especially neutrophil deficiency (e.g. Chronic granulomatous disease), age (neonates and the elderly), immunosuppressive therapy, diabetes, injecting drug users.

These factors predispose to HA-MRSA, as well as specific antibiotic usage (Tacconelli, E. *et al.*, 2008)which is more likely to lead to infection with MRSA. A recent history of hospitalisation as well as being hospitalised in specialised care units such as intensive care and burn units have also been identified as risk factors for HA-MRSA infection. In intensive care units, MRSA colonisation and a higher severity of illness at admission are also risk factors for infection. In long-term care facilities, risk factors for infection include persistent MRSA colonisation, dialysis, diabetes mellitus and peripheral vascular occlusive disease (Hartstein, A.I. *et al.*, 2004).

### 4.4. Conditions predisposing the humans to infections with CA-MRSA.

In the USA, a clone of MRSA called USA300 (CC8, PVL positive) has spread in the healthy human community and causes severe skin and soft tissue infection (SSTI) at high incidence. Although the clone is found in Europe, it is less common than in the USA. In Europe some clones of CA-MRSA (CC80, CC59 both PVL positive) also cause SSTI but are not as widespread as USA300 in the USA. Risk factors for developing USA300 infection are associated with over-crowding. In particular outbreaks have been associated with prisons, schools, day-care, nursing homes, military, MSM, IV drug abusers, athletes and gym users. Suboptimal hygiene probably contributes to transmission of MRSA in these settings. Recent antibiotic exposure is a risk factor for CA-MRSA (Tacconelli, E. *et al.*, 2008), as well as recent influenza-like illness and/or severe pneumonia, concurrent skin and soft-tissue infection, history of colonisation or recent infection with a CA-MRSA strain, close contact in same household with a person colonised and/or infected with MRSA, as well as pig farmers,

veterinarians and pet owners (Boucher, H.W. and Corey, G.R., 2008). In countries with low MRSA prevalence such as Denmark where importation is often the source for MRSA, foreign origin has been identified as a risk for CA-MRSA infection (Bocher, S., Gervelmeyer, A. *et al.*, 2008).

Epidemiological data showed that direct handling of sows is of greater importance for carriage than work with fattening pigs and exposure to dust (Van Den Broek, I. *et al.*, 2008) and therefore suggests that there is a dose response with exposure. However the risk to human health from different levels (dose response) of MRSA during carriage in animals (and in the environment) is not known.

### 4.5. Conditions predisposing to colonization and infection with MRSA in animals.

The relationship between antimicrobial usage and occurrence of LA-MRSA in food production animals has been addressed by a recent EMEA reflection paper (EMEA/CVMP/SAGAM 68290/2009: *"MRSA in companion and food producing animals in the European Union: Epidemiology and control options for human and animal health"*).

Within pig farms, younger animals tend to have a higher prevalence of MRSA compared with adult swine and sows (Denis, O. *et al.*, 2008; Smith, T.C. *et al.*, 2008). A Belgian survey also found open farms to have a higher prevalence of positive animals (94%) versus closed farms (56%) (Denis, O. *et al.*, 2008). A Dutch survey showed transmission of LA-MRSA from multiplier to finisher farms (van Duijkeren, E. *et al.*, 2008). Piglets in these multiplier farms can be colonized by different routes or vectors, and longitudinal studies are needed to indicate if the environment, e.g. feed or dust or the sows are the primary source of colonization (de Neeling, A.J. *et al.*, 2007).

There are few published data on the risk factors for occurrence of MRSA colonisation and/or disease in companion animal species. A recent study by, (Lloyd, D.H. *et al.*, 2007) suggested that contact of pets with a human MRSA carrier increased the risk that the animal will acquire MRSA rather than MSSA by 6-fold. Faires, M. and Weese, S., 2008b, reported that the presence of a urinary catheter, joint infection and prior administration of fluoroquinolones were risk factors for MRSA infection in pets in America, with (Loeffler, A., Soares, -.M., R. *et al.*, 2008) reporting similar risk factors in dogs and cats in the UK. This suggests the risk factors for pets developing MRSA infection are similar to those in humans.

Investigation of the risk factors for acquisition of MRSA has also been performed in horses on admission to a veterinary hospital (Weese, J.S. and Lefebvre, S.L., 2007). The following factors were found to be associated with increased risk of colonization: testing positive for MRSA previously, coming from a MRSA-positive farm, administration of antimicrobials and admission to the "Foal Watch" programme (approximately equivalent to intensive care). Farm size has been identified as a risk factor for colonization in horses (Weese, J.S., Rousseau, J. *et al.*, 2005) and prior colonization was a major risk factor for development of MRSA infection in horses (Weese, J.S., Rousseau, J. *et al.*, 2006). It is therefore likely that risk factors for MRSA colonisation and infection in pets and horses are similar to those identified in humans. However, (Weese, J.S. and Lefebvre, S.L., 2007) highlight the differences between human and equine healthcare, suggesting that equine stud farms may more closely mimic human healthcare settings than equine veterinary hospitals.

### 4.6. Risk of transmission of CC398 and other MRSA from farm animals to humans including within hospitals and other healthcare environments.

It has been demonstrated that LA-MRSA is able to colonize the nasal cavity of farmers, pig attendants and their family members veterinarians veterinary students, laboratory personnel and meat inspectors who have been exposed to colonized or infected animals as well as to the dust from animal houses (Armand-Lefevre, L. *et al.*, 2005; Aubry-Damon, H. *et al.*, 2004; Huijsdens, X.W. *et al.*, 2006; Loeffler, A. *et al.*, 2005; Meemken, D. *et al.*, 2008; Moodley, A. *et al.*, 2008; Weese, J.S., Rousseau, J. *et al.*, 2005; Wulf, M.W., Tiemersma, E. *et al.*, 2008). Such occupational groups can become carriers of CC398. Voss, A. *et al.* in 2005 reported that 23% of exposed pig farmers were shedders of this clone, and their likelihood of carriage was more than 760 times higher than the general Dutch population. However it is not known as to the duration of this carrier state. Although most human carriers of CC398 have no clinical symptoms, CC398 can cause severe systemic infections in humans (endocarditis, ventilator-associated pneumonia and wound infections) following contact with animals (Cuny, C. and Witte, W., 2008; Declercq, P. *et al.*, 2008; van Loo, I., Huisdens *et al.*, 2007; Witte, W., Strommenger, B. *et al.*, 2007; Wulf, M. and Voss, A., 2008). In addition, transmission between hospitalized patients by CC398 has been reported from Germany (Witte, W., Strommenger, B. *et al.*, 2007), the Netherlands (Wulf, M. *et al.*, 2008), and also from China where a cluster of MRSA CC398 with capacity to produce Panton-Valentine leukocidin has been reported (Yu, F. *et al.*, 2008). Spread of LA-MRSA can also occur in long-term care facilities (Fanoy, E. *et al.*, 2009). However, the basis of epidemicity of particular MRSA clones with respect to spreading in hospitals is poorly understood, although recent evidence indicates that LA-MRSA spreads less effectively in hospital than HA-MRSA (Wassenberg, M.W.M. *et al.*, 2008), and therefore the potential for CC398 to spread in hospitals is likely to be low unless changes in the host adaptation of this clonal complex occur in the future (see section 4.9).

### 4.7. Risk of human disease through food handling or consumption.

The risk from contact with contaminated food appears to be small, and certainly much reduced from that following contact with live animals or humans. Experience in countries with a high prevalence of MRSA, such as the UK, shows that hospital environments are often contaminated with MRSA. The major MRSA reservoirs in hospitals are the noses and hands of patients as well as staff and visitors, equipment, clothing, bedding, curtains and floors (Cimolai, N., 2008). An unpublished study[4] found 0/89 professional meat handlers colonized with MRSA, although the sample size examined here was inadequate to generate firm conclusions. Two food handlers in both a hospital kitchen and the community were reported to be carriers of an MRSA (clone III::B:A) in Brazil (Soares, M.J. *et al.*, 1997), although the original source of the MRSA was not established and these were not associated with disease.

There are however, descriptions of 2 outbreaks of foodborne disease due to MRSA. In the first, three family members who shared a meal of pork and coleslaw became ill with nausea, vomiting and stomach cramps. The same strain of MRSA (with an indistinguishable PFGE pattern) was isolated from the three family members, the coleslaw and a food handler at the convenience market where the food was purchased (Jones, T.F. *et al.*, 2002). In the second outbreak which affected 27 patients and 14 hospital workers in the Netherlands (Kluytmans, J. *et al.*, 1995), routine testing of food prepared for patients resulted in the detection of MRSA

[4] Rob de Jonge, National Institute for Public Health and the Environment, Bilthoven, the Netherlands; Jesse E. Verdier, Erasmus Medical center, Rotterdam, the Netherlands; A. Havelaar, Utrech University, the Netherlands. Manuscript submitted for publication: Prevalence of MRSA amongst professional meat handlers.

in a piece of banana, leading to the screening of all food handlers. One worker who had prepared food for patients at the start of the outbreak was found to be colonised with MRSA which was the same strain as that recovered from food sample and from the infected patients. This study suggests that food contaminated by the health worker food handler was likely to have caused the first case of MRSA septicaemia which was subsequently transmitted to other patients in the surgical unit by a colonized nurse (Kluytmans, J. *et al.*, 1995).

### 4.8. Risk of human infection through contact with companion animals and horses.

MRSA infections in pets is increasingly reported, and in almost all cases, the strains causing infection in animals were the same as those commonly occurring in hospitals in the geographical regions (Baptiste, K.E. *et al.*, 2005; Loeffler, A. *et al.*, 2005; Malik, S. *et al.*, 2006; Strommenger, B. *et al.*, 2006; van Duijkeren, E. *et al.*, 2004; Weese, J.S., Caldwell, F. *et al.*, 2006). This suggests that transfer of organisms occurs between people and their pets but does not prove the direction of transfer (Baptiste, K.E. *et al.*, 2005). Because the isolates from pets cannot be distinguished from the common human MRSA strains, it is impossible to differentiate human cases of colonisation or infection acquired from pets from those acquired from human sources. Therefore, the contribution of MRSA in pets to human infections is difficult to determine. However, there are some reports which present information strongly suggesting that pets may serve as reservoirs for MRSA infection (Manian, F.A., 2003; Scott, G.M. *et al.*, 1988) or colonization (Cefai, C. *et al.*, 1994; Weese, J.S., Caldwell, F. *et al.*, 2006) in humans. More recently, (Faires, M. and Weese, S., 2008a) found that amongst 18 households in which a pet had been diagnosed with a MRSA infection, colonization in one or more household members was detected in 5 of the households (28%).

Strains of MRSA which colonize and infect horses are frequently different from common human strains (Cuny, C. *et al.*, 2006; O'Mahony, R. *et al.*, 2005; Weese, J.S., Archambault, M. *et al.*, 2005). Although it may be possible to quantify the proportion of human disease attributable to these strains in the future, there are only sporadic reports of human disease, usually minor skin infections, attributable to equine MRSA strains (Weese, J.S., Caldwell, F. *et al.*, 2006).

### 4.9. Future risk of new zoonotic MRSA types emerging.

*S. aureus* are constantly evolving. Often this is due to the horizontal transfer of MGE, particularly those encoding antibiotic resistance, into new lineages and successful clones, which are then selected by antibiotic use (Lindsay, J.A. and Holden, M.T., 2004). There is increasing evidence that the SCCmec cassette is mobile and has moved into *S. aureus* repeatedly (Nubel, U. *et al.*, 2008), although the exact mechanism has not been determined. It is somewhat surprising that multi-drug resistant *S. aureus* are not more widespread. A genetic mechanism based on restriction modification called *Sau*1 that controls the spread of DNA between *S. aureus* lineages, as well as reducing the incidence of DNA transfer from other species and genera into *S. aureus* has probably played an important role in preventing horizontal gene transfer (Waldron, D.E. and Lindsay, J.A., 2006). However, one lineage of *S. aureus* found in cattle (ST151) is known to be deficient in *Sau*1, and susceptible to transfer of plasmids from enterococci (Sung, J.M. and Lindsay, J.A., 2007). The vancomycin resistance gene *vanA* is found on conjugative plasmids in enterococci (Flannagan, S.E. *et al.*, 2003), although the incidence of VRE (vancomycin-resistant enterococci) in cattle has slowly decreased due to the banning of the vanomcyin-related growth promoter avoparcin in the EU in 1997 (Witte, W., 2000). Fully vancomycin resistant *S. aureus* would have a highly detrimental impact on human healthcare, as these antibiotics are widely used to prevent and treat MRSA infections in humans. At this stage, only nine such cases have been documented,

all in the USA (Sievert, D.M. *et al.*, 2008). The susceptibility of CC398 to horizontal transfer is currently unknown. Resistance to a new antibiotic in human medicine, linezolid, can be due to the cfr gene which also confers resistance on the PhLOPSA family of antibiotics (Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A). cfr is reported on a composite transposon or plasmid in other staphylococcal species, and there is now evidence that the use of florfenicol in swine can lead to *crf* transfer and selection in CC398 (Kehrenberg, C. *et al.*, 2009). Resistance to virtually all types of antibiotics has been described in *S. aureus*, and virtually all resistance mechanisms can be transferred horizontally, especially given appropriate antibiotic pressure. In summary, the use of antibiotics in the food production sector, provides opportunities for the further evolution, selection and spread of increasingly resistant *S. aureus* clones adapted for a widening range of environmental niches.

## 5. Control options

In comparison to other zoonotic pathogens such as *Salmonella* and *Campylobacter*, MRSA (including LA-MRSA) has some unique characteristics that require consideration of different control options. This is partly because exposure to, and colonization by, MRSA does not normally lead to disease in healthy humans, however severe infection can occur.

At-risk human populations particularly occur in hospitals and other health-care settings, and in these settings, risks are related to introduction of MRSA by human carriers. LA-MRSA carriers can be managed in the same way as other MRSA carriers by screening and infection control measures. However strategies for screening (together with actions taken following their results) vary considerably between different MS's. In the Netherlands and in Denmark, search-and-destroy policies are implemented which appear to be effective for LA-MRSA as well as for other MRSA. In the Netherlands and in Germany it is recommended that persons in contact with live pigs or veal calves are screened for MRSA on admission to the hospital and nursed in isolation until screening tests demonstrate the absence of MRSA. For example, as discussed in chapter 2.1, a new policy was published in July 2006 in the Netherlands. By January 2007 the MRSA-policy had been adopted in 64% of Dutch hospitals, the number of screening tests increased by 15%, and the number of detected MRSA carriers increased by 44% (Wassenberg, M.W.M. *et al.*, 2008). There are indications however that LA-MRSA spreads less effectively in hospital than HA-MRSA (Wassenberg, M.W.M. *et al.*, 2008), hence it is possible that in the future the search-and-destroy policy may be relaxed. However, in absence of definitive evidence and considering the likelihood that the bacteria may continue to evolve, the current controls in health care facilities should be based on the assumption that LA-MRSA has similar potential to spread in the hospital as other lineages.

Colonization by LA-MRSA outside hospitals is acquired through direct contact with animals, through the environment contaminated by animal reservoirs or by secondary transmission from human carriers originally exposed to animal reservoirs. Transmission of infection by food products appears to be very rare, and based on current data does not warrant specific control measures. Since the most important routes of transmission are through direct contact with live animals and their environments, the most effective control options will be at pre-harvest. The need for additional controls of LA-MRSA in primary production and the food chain should be decided in close collaboration between medical and veterinary authorities and the epidemiological situation and prevalence of MRSA in different MS with regard to HA- and CA-associated MRSA should be taken into account. This may lead to different choices of control options being made in different MS.

LA-MRSA is one aspect of the wider problem of antimicrobial resistance related to the use of antimicrobials in veterinary medicine, and should be addressed as part of an integrated strategy to reduce resistance. At the European level, options for harmonized control are available in the domain of food safety, whereas infectious disease control in humans is mainly the responsibility of individual MS.

Strategies for control of MRSA in companion animals and individual horses are necessary because they reduce the risk of transfer of MRSA back to humans (EMEA/CVMP/SAGAM 68290/2009: *"MRSA in companion and food producing animals in the European Union: Epidemiology and control options for human and animal health"*).

## 5.1. Monitoring and surveillance of MRSA

Monitoring and surveillance are not control options as such, however these processes are essential for determining control strategies and for the evaluation of their effectiveness. The complex epidemiology of MRSA makes monitoring and surveillance necessary at local, regional, national and European levels and should inform policy decisions at all these levels. Currently, monitoring of *S. aureus* or MRSA in humans, food or animals, is not mandatory under Community provisions, with the exception of the baseline study on swine husbandry (see chapter 1.4).

Surveillance of human MRSA infections are carried out locally, regionally, nationally and at European level. There is a wide variation in the surveillance systems. At European level the EARSS system only provides data on the proportion of *S. aureus* that is MRSA causing blood stream infections in hospitals and it is not currently possible to differentiate between CA-HA- and LA-MRSA. Data are only available in some MS's on the incidence of MRSA and the type distribution of human strains from hospitalized and non-hospitalized patients. Therefore, it is not possible to assess the relative importance of animal reservoirs at European level.

Monitoring and molecular typing dedicated surveillance networks covering *S. aureus* (including MRSA) are in operation (Cookson, B., 2008; Friedrich, A.W. *et al.*, 2008), however as with the EARSS system, integration of this data with other risk factors is not currently possible.

There is a need for active surveillance and monitoring at pre-harvest in all MS since there is uncertainty about the current epidemic and its future course. Pre-harvest data should complement, and be harmonised with, that for human surveillance. The more the epidemic is allowed to spread among livestock in Europe, the more difficult and expensive it will be in future to implement effective controls. Furthermore, strains within the CC398 complex may acquire additional resistance genes, with increased risks of treatment failure or transmission to other lineages. In addition the *mec*A genes may be transferred to other, potentially more human-adapted strains in animal reservoirs. Therefore, even if the current CC398 LA-MRSA clones are less epidemic among humans than other MRSA-clones, it cannot be excluded that in future more transmissible and virulent clones arise.

Periodic monitoring of all farm animals, especially those in intensive systems, in all MS would provide trends in the development of this epidemic. At the present time, data that would be comparable with the ongoing on farm base line study in breeding pigs would be useful in countries where the problem already exists, and may be extended to fattening pigs, veal calves and poultry. The preferred sampling method would be the collection of dust samples.

However, in countries or with a low or zero prevalence, studies at the abattoir level may be sufficient and more convenient to detect the emergence of LA-MRSA. Although the preferred sampling method has not yet been established, nasal swabs are most likely to detect colonisation.

Monitoring of staphylococcal food poisoning in the EU are carried out as part of the Directive 2003/99/EC (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF) and data is available in the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union. MRSA can not be distinguished from other coagulase positive *Staphylococci*, however this is of limited importance since control measures for all staphylococcal food poisoning are identical.

### 5.2. Selective pressure for MRSA by the veterinary use of antimicrobial agents.

Although reducing the use of antimicrobials in veterinary medicine is a potential control option for reducing the selective pressure for MRSA colonization in animals, the effectiveness of such a measure is unknown (for more information on this topic refer to EMEA/CVMP/SAGAM 68290/2009: *"MRSA in companion and food producing animals in the European Union: Epidemiology and control options for human and animal health"*).

### 5.3. Husbandry interventions, management and organization of animal and food production

Animal movement and contact between animals are likely to be important factors for transmission of MRSA. In the absence of specific studies on the spread and persistence of MRSA, general control options on farms, in slaughterhouses and in food production areas are likely to be the same for MSSA as well as MRSA, and include good husbandry practices, HACCP, GHP, and GMP. Monitoring and subsequent restrictions on movement may reduce transmission. Since the most important routes of transmission to humans are through direct contact with live animals and their environments, the most effective control options will be at pre-harvest

For indoor farming systems, options to control airborne emissions (which is likely to include MRSA in dust) should be controlled by generic biosecurity measures which are applied at farm level (Hartung, J., 2005). In order to avoid airborne transmission of MRSA from farm buildings, sufficient "safe distances" to neighbouring farms and residential areas should be achieved. Technical devices such as biofilters or bioscrubbers can be used in order to reduce or to eliminate bacterial emissions from farms (Seedorf, J. and Hartung, J., 1999). However, data on the effectiveness of these techniques for MRSA control are not available.

LA-MRSA may also be introduced by contaminated or colonised humans. Reduction of the number of visitors (Ribbens, S. *et al.*, 2008) and implementation of control measures such as shower-in, dedicated clothing and other measures for employees and visitors who are allowed entry may help to prevent introduction of MRSA into a herd/flock, or food production area. The wearing of protective clothing and breathing masks are possible options to reduce the risk of contamination and carriage in humans. However, Denis, O. *et al.* in 2008 reported that the use of barrier precautions such as aprons, gloves or masks are associated with increased risk of colonization in pig farmers. The reasons for this are not clear and require further investigation but may include inappropriate preparation and handling of the personal protective equipment as experiences in other fields such as biocomposting plants (Schappler-Scheele, B. *et al.*, 1999). Testing of potential employees for MRSA carriage and exclusion is

one possible control measure, however the ethical implications of this are outside the remit of this opinion.

Little is known about the environmental contamination by manure from farms where MRSA carriers occur. MRSA can be excreted from the gastrointestinal tract of humans (Klotz, M. *et al.*, 2005), and animals. It was reported by (de Boer, E. *et al.*, 2008) that MRSA has been found in chicken droppings and in cattle faeces in the Netherlands: quantitative data on MRSA levels and persistence in manure are not available. Spreading of MRSA-containing manure on land may pose a risk of transmission to wild or farm animals and may lead to a contamination of feed, vegetables and fresh produce. Preventive measures in order to avoid MRSA transmission via manure may include long storage times, composting, heat treatment or digestion[5]. However, it has not yet been demonstrated how efficient such treatments are at reducing MRSA..

### 5.4. Control options for human food-borne staphylococcal intoxications

Although a variety of animals carry coagulase positive staphylococci, human carriers are the main reservoirs for *S. aureus* (including MRSA and MSSA) responsible for human food-borne staphylococcal intoxication. Control of staphylococcal food poisoning involves temperature and time control as well as the hygienic behaviour of food handlers, and will be identical for all coagulase positive staphylococci, including MRSA and MSSA. Direct handling of cooked foods should be avoided and suitable utensils used wherever possible. Food handlers with septic lesions should be excluded until treated successfully. Where hand contact is unavoidable, hands should be cleaned thoroughly and disposable gloves used where practical. Foods should be refrigerated, displayed for time periods in line with current European food safety legislation, and disposed after such time has elapsed. Finally, food processes should be controlled to prevent the growth of *S. aureus* in raw materials, and in the fermentation and maturations stages of foods such as sausages and cheese.

### 5.5. Options for control of transfer of MRSA from companion animals to humans

Vulnerable patients (including the immunocompromised, recently hospitalised, elderly, post-surgical patients, and known MRSA carriers) that have contact with small animals, especially those who may be infected or colonised with MRSA or have received antibiotics recently, should be educated about potential zoonotic transfer and hygiene. The risk to human health is likely to be equivalent to having a suspected colonised or infected family member. If companion animals are colonised with MRSA it will be found in the nose, as well as other moist areas. Infected animals may have lesions that can potentially disseminate high levels of MRSA. Transfer of MRSA to and from humans is potentially easy, and difficult to control. Basic hygiene measures are key, especially hand washing before and after pet contact, and if possible, avoiding direct contact with nasal secretions, saliva and wounds. In a study of equine veterinary personnel, hand washing between animals and between farms was associated with a reduced risk of MRSA colonisation, emphasizing the importance of this control measure (Anderson, M.E. *et al.*, 2008).

[5] For further information on heat treatment refer to *EFSA Journal* (2005) 265, 1-16. Opinion on the Scientific Panel on Biological Hazards on: Biological safety of heat treatment of manure (www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/biohaz_op_ej265_manure_opinion_en1.pdf?ssbinary=true**).** The Regulation 1774/2002 on animal by-products (http://eur-lex.europa.eu/LexUriServ/site/en/consleg/2002/R/02002R1774-20060401-en.pdf) foresees that manure can be directly applied to land, used or transformed in biogas or composting plants but provided that the competent authority does not consider it to present a risk of spreading any serious transmissible disease.

Control of MRSA in companion animals is covered in the reflection paper EMEA/CVMP/SAGAM 68290/2009: *"MRSA in companion and food producing animals in the European Union: Epidemiology and control options for human and animal health"*).

### 5.6. Decolonisation of humans / production animals / companion animals, and re-colonisation risk (environmental re-exposure).

(i) Considerations for decolonization in humans

The main site colonized by *S. aureus* in healthy humans is the anterior nares and therefore nasal carriage is the most important reservoir for autogenous infections by *S. aureus*/MRSA (von Eiff, C. *et al.*, 2001). Occurrence of *S. aureus* on other sites such as on healthy, non-injured skin is usually transient. MRSA colonization usually precedes MRSA infection (Rubinovich, B. and Pittet, D., 2001). Thus, the reasons for MRSA decolonization are to reduce the risk of MRSA infections in individual carriers and to prevent further dissemination in health care facilities and in the community. In addition, decolonization of *S. aureus*/MRSA carriage by treatment with mupirocin ointment is effective in preventing reinfection in recurrent furunculosis (West, S.K. *et al.*, 2007; Wiese-Posselt, M. *et al.*, 2007). Decolonization of people who are likely to become recolonised again within a short time presents a problem. Personnel working on livestock farms on premises where MRSA CC398 is widespread and those working in the healthcare industry in countries with a high MRSA incidence are likely to be reexposed to infection soon after decolonisation. Decolonisation of people who are continually exposed is only recommended prior to hospitalisation.

(ii) Decolonization procedures in humans

Decolonization of nasal carriage of MRSA by topical mupirocin treatment is however not always successful (Harbarth, S. *et al.*, 1999), probably due to recolonization from sites other than the anterior nares. This situation particularly applies to patients with diseased skin (e.g. eczema) and patients with diabetic foot ulcer. In addition, vaginal carriage may occur in young, sexually active women. Investigation of these other sites can easily be carried out by more complex screening using samples from nose, throat, ingual area, perineal area, vagina, and wounds. Decolonization regimes which take all these sites into account can be effective (Buehlmann, M. *et al.*, 2008).

Decolonization can be difficult in patients with chronic infections of skin and soft tissue (e.g. recurrent furunculosis) and topic treatment as mentioned above may be unsuccessful. In these cases, a second cycle should be started as outlined above in combination with systemic oral administration of rifampicin plus cotrimoxazol.

The throat has been shown to be an additional site for persistent MRSA colonization (Mertz, D. *et al.*, 2007). Difficulties in decolonization of MRSA from the throat may be due to internalization by epithelial cells; although rifampicin and cotrimoxazole are active against intracellular *S. aureus* (Yamaoka, T., 2007). However there are cases refractory to this treatment regime. Tonsillectomy has been successful in some cases (Gebhardt, D.O., 2003).

Resistance of MRSA to mupirocin is rare in most European countries. An alternative to mupirocin is bacitracin (West, S.K. *et al.*, 2007).

(iii) Decolonisation in animals

Routine decolonization of colonised animals is not recommended but may be required in some circumstances such as when there is a reinfection hazard for an animal or its owner. There have been no controlled studies on decolonisation regimes in pets. Lloyd, D.H. *et al.* in 2007 reported the anecdotal use of 1% fusidic acid on mucosal sites in dogs coupled with the

use of antimicrobial shampoos and systemic antimicrobial therapy. These treatments successfully removed MRSA but the period of time for which the patients remained free of MRSA was unknown. The topical agents recommended for use include chlorhexidine and benzoyl peroxide (Loeffler, A., Baines, S.J. *et al.*, 2008). Weese, J.S. and Rousseau, J. in 2005 reported the use of repeated nasal screening coupled with cohorting of positive and negative animals and improved infection control procedures in attempted eradication of MRSA colonisation on two horse farms. These procedures were successful in the case of all but 3 horses. Nebulized amikacin eradicated colonization in one horse, but was unsuccessful in a second horse given the same treatment. Oral chloramphenicol was also unsuccessful in the second horse. The third horse was not available for sampling for a sufficient period to evaluate whether colonization ceased. Containment strategies, including nasal screening and barrier precautions and without the use of antimicrobials, were also successful with pets as reported by (Weese, J.S. and Lefebvre, S.L., 2007).

# CONCLUSIONS AND RECOMMENDATIONS

## CONCLUSIONS

### General

- There are different states of interaction between *S. aureus* (including MRSA) and its host. These can be defined as: infections, carriage or colonisation, and contamination.
- Meticillin resistant *S. aureus* (MRSA) can be persistently or intermittently carried in the nose by healthy humans, and can also be found in the throat, axilla, rectum, perineum or gastrointestinal tract. Colonisation is the major risk factor for infection.
- MRSA are now widespread in hospitals in many European countries and is a major cause of hospital acquired infection. Infection can be mild to severe and, in some instances, fatal. There are large differences in prevalence and policies to control MRSA in different MS.
- There are major lineages within *S. aureus* (including MRSA), some of which show host specificity to humans or animals. A limited number of lineages of MRSA tend to predominate in specific geographical locations.
- CC398 is the MRSA lineage most often associated with asymptomatic carriage in intensively reared food-producing animals.
- MRSA commonly carry enterotoxin genes but there has been only one report of food intoxication due to MRSA. At present, CC398 has not been associated with staphylococcal foodborne intoxication.

**ToR 1. To assess the risk to human health posed by MRSA associated with food-producing animals.**

- LA-MRSA (CC398) represent only a small proportion of the total number of reports of MRSA infections in the EU. However, this proportion differs between Member States and is much higher in Denmark, The Netherlands and Belgium where active control policies and implemented.
- In some countries with low prevalence of human MRSA infection, CC398 is a major contributor to the overall MRSA burden. In countries with high overall human MRSA prevalence, CC398 is considered of less significance for public health.
- CC398 has, albeit rarely, been associated with deep-seated infections of skin and soft tissue, pneumonia and septicaemia in humans.
- Where CC398 prevalence is high in food-producing animals, people in contact with these live animals (especially farmers and veterinarians, and their families) are at greater risk of colonisation and infection than the general population.
- The risk to human health from different levels (dose response) of MRSA during carriage in animals (and in the environment) is not known.

**ToR 2. To assess the importance of food, food-producing animals, and companion animals in the risk of human infection and/or food-borne disease caused by MRSA in both the community and hospital settings.**

- Food may be contaminated by MRSA (including CC398): eating and handling contaminated food is a potential vehicle for transmission. There is currently no evidence

for increased risk of human colonisation or infection following contact or consumption of food contaminated by CC398 both in the community and in hospital.

- MRSA (including CC398) can enter the slaughterhouse in or on animals and occurs on raw meat. Although it may become part of the endemic flora of the slaughterhouse, the risk of infection to slaughterhouse workers and persons handling meat appears to be low, based on current data.
- Where CC398 prevalence is high in food-producing animals, people in direct contact with these live animals (especially farmers and veterinarians, and their families) are at risk of colonisation and subsequent infection.
- The potential for CC398-colonised humans to contribute to the spread of MRSA in hospitals currently seem to be less than for hospital associated MRSA strains.
- MRSA infections in companion animals are increasingly reported and in almost all cases, the strains causing infection in animals are the same as those commonly occurring in hospitals in the same geographical region. Humans are likely to spread MRSA to companion animals, and these can then be a reservoir for humans both in the community and in health care facilities.
- Horses can become colonised and/or infected with MRSA from humans or from other animal sources in their environment. There are sporadic reports of human disease, usually minor skin infections, attributable to an equine source.

**ToR 3. To determine which animal species (and if appropriate, foods derived there from) represent the greatest risk to humans.**

- The primary reservoirs of CC398 in affected countries are pigs, veal calves, and broilers. CC398 has also been found in companion animals and horses on farms with colonised livestock.
- MRSA has now been reported from dogs, cats and horses with sporadic reports of isolation from wide range of other companion animals. There are no specific studies which examined the relative risk of different small animals and horses as sources of infection or colonisation in humans.

**ToR 4. To identify which methods are best suited for the isolation and molecular typing of MRSA of animal origin**

- There is a wide variety of methods available for the isolation of MRSA.
- MRSA can be identified using phenotypic (antimicrobial susceptibility testing) or genotypic methods.
- For diagnosis of infection, samples taken directly from a lesion, biopsy specimens or blood cultures are cultured onto non-selective and selective media.
- For detection of carriage or contamination, swabbing of noses (for individuals), dust (for herds or focks), and sampling of food are used. Increased sensitivity is obtained when using selective liquid enrichment methods.
- *spa* typing is applicable for lineage detection in first line typing because of wide congruence with results of MLST and other typing methods.
- There are insufficient data to identify the optimal sampling and isolation methods to identify herd/flock prevalence.

**ToR 5. To indicate what control options (pre- and post-harvest) can be considered to minimize the risk of transfer of food-associated and animal-associated MRSA to humans.**

- Monitoring and surveillance are not control options as such, however these processes are essential for determining control strategies and for the evaluation of their effectiveness.
    - Surveillance of MRSA in humans, including *spa* typing of a representative number of isolates is necessary in order to monitor the occurrence of different strains of MRSA including CC398 in people.
- Animal movement and contact between animals is likely to be an important factor for transmission of MRSA. In the absence of specific studies on the spread and persistence of MRSA, general control options on farms, in slaughterhouses and in food production areas are likely to be the same for MSSA as well as MRSA, and include good husbandry practices, HACCP, GHP and GMP. Monitoring and subsequent restrictions on movement may reduce transmission.
- Since the most important routes of transmission to humans are through direct contact with live animals and their environments, the most effective control options will be at pre-harvest.
- LA-MRSA carriers in hospital and other healthcare settings can be managed in the same way as HA- and CA- MRSA carriers in both staff and patients by screening and infection control measures. Strategies for screening (together with actions taken following their results) vary considerably between different MS's. Search and destroy policy seems to be the most effective, however its implementation is difficult when MRSA is already prevalent.
- Transfer of MRSA to humans from companion animals and horses is difficult to control. Basic hygiene measures are key, especially hand washing before and after contact, and if possible, avoiding direct contact with nasal secretions, saliva and wounds. Decolonisation of these animals is a potential control option but controlled studies are lacking.

**RECOMMENDATIONS**

- It is recommended that periodic monitoring of intensively reared animals is carried out. This would provide trends in the development of this epidemic in all Member States. Data that would be comparable with the ongoing on-farm base-line study in breeding pigs would be useful in countries where the problem already exists, and may be extended to fattening pigs, veal calves and poultry. The preferred sampling method would be the collection of dust samples. In countries with a low or zero prevalence, studies at the abattoir level may be sufficient to detect the emergence of LA-MRSA. Although the preferred sampling method at the abattoir level has not yet been established, nasal swabs of pigs and cattle should be considered.
- In order to identify trends in the spread and evolution of zoonotically acquired MRSA, systematic surveillance and monitoring of MRSA in humans and food producing animals is recommended in all Member States. Harmonised data, including information on risk factors, as well as analysis of a representative sample of isolates for susceptibility to multiple antimicrobial agents, virulence associated traits, and lineage determination, should be available from a single location.

- These data should be directly integrated with data on antibiotic usage in human and veterinary medicine with respect to different animal species and production systems.
- In order to evaluate the effectiveness of control measures to reduce the carriage of CC398 in livestock, intervention studies should be carried out. Such studies should be longitudinal over consecutive production cycles.
- Further work should be performed on harmonising methods for sampling, detection and quantification of MRSA during carriage in both humans and animals, as well as for detection of MRSA as a contaminant of food, and in the environment including from dust both in air and on surfaces.
- The factors responsible for host specificity, persistence in different environments, transmission routes (including airborne transmission) and vectors, should be investigated.
- In order to evaluate the effectiveness of control measures to reduce the carriage of MRSA in companion animals and horses and their human contacts, intervention studies should be carried out.
- On the base of already existing recommendations for prevention of MRSA infections in some MSs, protocols for screening at admission to hospitals should be expanded to include humans exposed to intensively reared livestock.

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## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| **AIAO** | all in – all out |
| **AP-RAPD** | arbitrary single primer – random amplification of polymorphic DNA |
| **AR** | antimicrobial resistance |
| **AST** | antimicrobial susceptibility testing |
| **BA** | blood agar |
| **BSI** | blood stream infections |
| **Carriage/Colonization**: | occurs in both humans and animal where *S. aureus* (including MRSA) multiplies in the nares, throat or other superficial sites but without causing disease. |
| **CA-MRSA**: | MRSA infection/colonization acquired outside the hospital and health care settings and without risk factors for HA-MRSA and HCA-MRSA. |
| **CC** | clonal complex |
| **CLSI** | Clinical Laboratory Standards Institute |
| **CMRS** | Canadian (epidemic) MRSA lineage |
| **Contamination:** | occurs in humans, animals, food, the environment etc where *S. aureus* (including MRSA) is present due to exposure from another site (i.e and infected or colonised host or the environment such as dust). Animals or humans can be contaminated at external surfaces, and there is no multiplication of *S. aureus* and no clinical symptoms. |
| **CVMP** | Committee for Medicinal Products for Veterinary Use |
| **C+G** | guanine-cytosine content |
| **Decolonisation** | the process of disruption of carriage/colonisation by the eradication of *S. aureus* from specific anatomical sites |
| **EARSS** | European Antimicrobial Resistance Surveillance System |
| **ECDC** | European Centre for Disease Prevention and Control |
| **EMEA** | European Medicines & Evaluation Agency |
| **ET** | enterotoxigen(ic) |
| **EUCAST** | European Committee on Antimicrobial Susceptibility Testing |
| **GHP** | good hygienic practices |
| **GMP** | good manufacturing practices |
| **GP** | general practitioner |
| **HACCP** | hazard analysis and critical control points |
| **HA-MRSA** | MRSA infection/colonization acquired in health care settings and which emerges at least 48 hours after admission. |

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| **HCA-MRSA** | MRSA infection/colonization of hospital origin is acquired in the community but in association with previous hospitalization, residence in a nursing home, attending centres for dialysis and/or centers for diabetes. |
| **ICU** | intensive care unit |
| **Infection** | in both animals and humans, where growth of the bacterium occurs together overt or covert pathological changes indicating the presence of disease. |
| **IV** | intravenously |
| **LA-MRSA** | livestock associated MRSA; unless otherwise specified, LA-MRSA will be the preferred term used as synonym for UT-MRSA, NT-MRSA, MRSA CC398, and MRSA CC398. |
| **MIC** | minimum inhibitory concentration |
| **MLST** | multilocus sequence typing |
| **Monitoring** | Means the continuous investigation of a given population or subpopulation, and its environment, to detect changes in the prevalence or incidence of a disease or characteristics of a pathogenic agent |
| **MRSA** | meticillin resistant *Staphylococcus aureus* |
| **MSM** | men that have sex with men |
| **MSSA** | meticillin susceptible *Staphylococcus aureus* |
| **NT** | non-typeable |
| **PBP** | penicillin-binding protein |
| **PFGE** | pulsed field gel electrophoresis (macrorestriction analysis) |
| **PVL** | Panton-Valentine leucocidin |
| **RIVM** | Rijks Instituut voor Volksgezondheid en Milieu (NL) |
| **SAGAM** | Scientific Advisory Group on Antimicrobials |
| **SA** | *Staphylococcus aureus* |
| **SE** | staphylococcal enterotoxin |
| **Spa** | staphylococcal protein A |
| **SSC** | staphylococcal chromosome cassette |
| **SSTI** | skin and soft tissue infections |
| **ST** | (multilocus) sequence type |
| **Surveillance** | Represents an extension of monitoring and consists of the close and continuous observation of the occurrence of infection for the purpose of active control |
| **TSB** | trypticase soy broth |
| **Vectors** | Vectors are inanimate objects or living organisms responsible for the transmission of infectious agents. |