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## MRSA Epidemiology in Animals

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### 1. Introduction

Until the 1990s, methicillin-resistant *Staphylococcus aureus* (MRSA) was traditionally considered a pathogen causing nosocomial infections, being the so-called HA-MRSA (healthcare-associated methicillin-resistant *Staphylococcus aureus*). However, over time, cases of MRSA-positive individuals were observed who never had contact with hospital services, and strains from these individuals were identified and named CA-MRSA (community-associated methicillin-resistant *Staphylococcus aureus*). In 2003 in the Netherlands, a new MRSA strain arose in patients that could not be typed through PFGE (pulsed field gel electrophoresis) with *Sma*I, with resistance to digestion by this enzyme (Bens et al., 2006), being called since then NT-MRSA (non typeable methicillin-resistant *Staphylococcus aureus*). Investigations of this NT-MRSA intensified, and it was observed that these patients carrying this strain had previous contact with pigs and the geographic distribution of cases showed clusters near pig farms (van Loo et al., 2007). With more advanced studies, it was possible to determine strains strictly related to animals, such as those found in pigs, which were named LA-MRSA (livestock-associated methicillin-resistant *Staphylococcus aureus*) in 2010 (Vanderhaeghen et al., 2010).

The resistance to methicillin in staphylococci is mediated by the *mecA* gene that encodes a modified penicillin-binding protein (PBP), the PBP2a or 2', which shows reduced affinity to the resistant penicillins to penicillinase, such as methicillin and oxacillin and for all other beta-lactam antibiotics (van Duijkeren et al., 2004). Due to the need for better characterizing these isolates, they have been classified in a more detailed manner, beginning with the SCC*mec* types and patterns identified by PFGE, and are currently based on sequence type and *spa* typing. With the use of techniques such as MLST (multi locus sequence typing) and *spa* typing, characteristic clones from animals have been observed, and it is suspected that some have tropism by determined host species. An example of this is ST398, which in addition to being strictly linked to pigs, has carried novel types of SCC*mec*. Li et al. (2011) analyzed the SCC*mec* element structure carried by 31 CC398 MRSA strains isolated from participants of a conference. The strains were classified into novel types, IX and X, type V (5C2&5) subtype c and type IVa, all carriers of genes conferring resistance to metals. The SCC*mec* structures from CC398 strains were distinct from those usually found in humans, complementing evidence that humans are not the original host for CC398. With the absence of a complete evaluation of risk factors

for carrying this strain as much in animals as in humans, Graveland et al. (2010) carried out the first study that showed direct association between animal and human carriage of ST398. This association, in addition to the association between MRSA and the antimicrobial use in calves, highlights the need for prudent use of antibiotics in farm animals.

One clone of special interest in ruminants is the CC133. The great majority of isolates from small ruminants is represented by this clonal complex of *S. aureus*, but its evolutionary origin and molecular basis for its host tropism remain unknown. Guinane et al. (2010), attempting to determine whether the CC133 developed as result of a transmission from human host to ruminant followed by an adaptative diversification of the genome, carried out a comparative sequencing of the complete genome. Several novel mobile genetic elements were observed in the CC133 isolates encoding virulence proteins with attenuated or enhanced activity and they were widely distributed, suggesting a key role in their host-specific interaction. These data provide broad and new insights into the origin and basis molecular of *S. aureus* ruminant host specificity. The MRSA evolution and epidemiology in animals are discussed in this chapter.

## 2. History of MRSA in animals

The first report of MRSA in animals was in milk from Belgium cows with mastitis (Morgan, 2008). Until 2000, MRSA had been isolated sporadically from animals, in particular cows, small companion animals, and horses. With exception of some equine isolates, the nature of these cases suggested a human origin and no epidemics have been reported. In this respect, until the end of 20<sup>th</sup> century, both the scientific community and policy makers were convinced that animal husbandry was of little relevance for MRSA causing diseases in humans, but was particularly a problem based on antimicrobial use in human medicine. The situation has changed with a growing number of reports of MRSA in livestock, especially pigs and veal calves. MRSA has also been reported in companion animals and horses, as well as transmission between humans and animals (Catry et al., 2010). Calling attention to this dramatic increase of MRSA in animals, van Duijkeren et al. (2010) at the Veterinary Microbiological Diagnostic Center, in the Netherlands, reported 0% MRSA in isolates from equine clinical samples in 2002 and then 37% in 2008.

## 3. The importance of the different animal species

Various species have been identified as host and carriers of MRSA in different countries and settings, including dogs, cats, sheep, chickens, horses, pigs, rabbits, seals, psittacine birds, and one turtle, bat, guinea pig and chinchilla (Morgan, 2008). With differences between strains isolated from pets, wild animals and cattle, it is important to evaluate each species individually, because they present peculiar characteristics, including the series of resistance genes. Thus, the concern for MRSA types in animals has grown considering their role as a potential reservoir or vector for human infection by MRSA in the community. However the data available on MRSA transmission between humans and companion animals are limited, and further epidemiological studies are needed on this transmission and its impact on public health (Loeffler & Lloyd, 2010).

### 3.1 Companion animals

Pets have been shown to act as reservoirs of bacteria resistant to antimicrobials, and MRSA transmission between humans and animals has been described. For strains of MRSA with a low specificity to the host, the transference is likely to occur in both directions between humans and pets living at the same household (Nienhoff et al., 2009). The infections by MRSA in companion animals are predominantly of skin and soft tissues, especially during post-surgical (Morgan, 2008).

Nienhoff et al. (2009) reported two cases of transmission of MRSA strains between humans and dogs. Three positive dogs to MRSA were identified in a survey carried out in 803 dogs and 117 cats admitted to the Small Animal Clinic of the University of Veterinary Medicine Hannover, Germany. The first case was a 6-month-old female admitted to the clinic for teeth extraction. The owner, MRSA-positive, was a specialist veterinarian in swine diseases, working in pig barns 4-5 days per week and having access to MRSA ST398-positive farms. The dog and owner strains were identical through molecular typing, belonging to ST398 and *spa* type t034. The second case was an 11-year-old male admitted to the clinic because of a cardiac problem. The likely origin of the strain was the mother-in-law of the dog's owner, who is diabetic, having received nursing care at home and presenting an infected wound on the foot and an ulcer in the right eye. The MRSA isolates found in these lesions and in the dog belonged to ST225, which is frequently found in humans.

### 3.2 Cattle

Cows with mastitis have been the most likely to harbor MRSA, and they may be related to horizontal transfer via wet hands of colonized or infected dairy farm workers, and selection by the use of antibiotics to treat mastitis (Morgan, 2008). The first known case of MRSA transmission between cows and a person was reported by Juhász-Kaszanyitzky et al. (2007). MRSA strains isolated from cows with subclinical mastitis were phenotypically and genotypically indistinguishable from the strain from the person who worked with these animals. These strains were determined as ST1, *spa* type t127, SCC*mec*IVa. The authors considered these strains epidemiologically related, indicating transmission from cow to human or from human to cow.

Feßler et al. (2010) studied 25 MRSA ST398 isolates from cases of bovine clinical mastitis and two isolates from farm workers originating from 17 dairy farms in Germany, evaluating the genetic relatedness, antimicrobial resistance and virulence properties. Nine major ApaI PFGE patterns were found, three *spa* types (t011, t034 and t2576) and two types of SCC*mec* (IV and V) were identified. As described previously for ST398 from pigs, isolates from this sequence type originating in cases of bovine mastitis have also shown a high degree of variability when the ApaI PFGE profile and other genotypic and phenotypic characteristics were compared. A uniform pattern of virulence genes appeared to be conserved between ST398 isolated from both animal species.

Türkyılmaz et al. (2010) detected 14 of 16 strains from bovine milk of the lineage MRSA ST-239-III in which one was related to hospital-associated clones, and two strains were ST8/IV, which correspond to USA300, which causes severe community-acquired infections. The presence of MRSA ST239-III lineage can indicate a transmission from humans to animals, and the presence of ST8-IV can show emergence of strains from the community in the Aydın

region in Turkey. This study underscores the necessity to take measures to avoid MRSA transmission between humans and animals.

### 3.3 Horses

In horses, MRSA have been reported in infections of skin and soft tissues, bacteraemia, septic arthritis, osteomyelitis, implant-related infections, metritis, omphalitis, catheter-related infections and pneumonia. The first MRSA outbreak in horses was observed in 1993, with 11 infected horses in the post-surgical in a veterinary teaching hospital in Michigan. Subsequent outbreaks have occurred on Japan, Austria, the UK, Ireland, Canada, and other areas of the USA (Morgan, 2008).

In 2009, Loeffler et al. reported the first isolation of MRSA ST398 *spa* type t011 in animals from the UK. They were two horses in southeastern England, with isolates with identical phenotypic and genotypic characteristics as reported in horses in Belgium, Austria and Germany, which also carried the SCC*mec* type IVa. They vary from those commonly found in pigs (*spa* type t108, t034 or t571) and frequently carry SCC*mec* V, possibly indicating host-specific variation within this lineage or independent evolution. One interesting fact is that isolates from pigs and horses commonly show resistance to tetracycline and/or gentamicin, both agents frequently used in pigs and horses, respectively. These findings demonstrate the introduction of ST398 in England and provide more evidence of successful dissemination of this zoonotic pathogen in the animal reservoir. The authors recommended vigilance for MRSA ST398 in both animals and humans.

Outbreaks of MRSA were observed in horses and horse personnel in the Netherlands in the period of 2006-2008. The isolates belonged to ST8, *spa* type t064, and to ST398, *spa* types t011 and t2123, predominantly. During the outbreak of post-surgical infections by MRSA in horses in a veterinary teaching hospital, isolates from *spa* type t2123 were isolated from 7 horses, and 4 of 61 personnel indicating a zoonotic transmission; after intervention, the outbreak stopped. In another outbreak that occurred in 2008, 17 horses with MRSA were detected, with 12 *spa* type t011, 4 *spa* type t2123 and 1 *spa* type t064. From 170 personnel, 16 were positive for MRSA, with 11 *spa* type t011 and 5 *spa* type t2123. From 106 personnel who maintained close contact with horses, 15 were MRSA-positive compared with 1 MRSA-positive of 64 personnel who had no close contact with the animals. Furthermore, screening carried out on the horses on admission showed that 9.3% were MRSA-positive, predominantly *spa*-type t011. Weekly cross-sectional sampling from all horses hospitalized for 5 weeks demonstrated that 42% were MRSA-positive at least once, again predominantly with *spa* type t011, which suggests that a nosocomial transmission appeared. The research of environmental samples from veterinary hospital revealed the presence of 53% of MRSA, including samples from students and staff member rooms, all *spa* type t011, indicating that humans contribute to the microorganism dissemination. The samples cultured employing pre-enrichment with high-salt concentration presented better results than the method without pre-enrichment. These results demonstrate that the nosocomial transmission in equine clinics occurs and suggest that personnel play a role in the transmission (van Duijkeren et al., 2010).

### 3.4 Poultry

The first reports of MRSA in chicken meat occurred in Korea in 2003 (Lee, 2003) and Japan in 2005 (Kitai et al., 2005), but it was not determined as livestock-associated, raising the hypothesis of meat contamination with human strains through handlers.

LA-MRSA was reported primarily in health poultry in 2008 in Belgium, with ten recent isolates classified as ST398 *spa* types t011 and t567. In this study, strains isolated in 1970s and strains isolated recently in 2006 were evaluated. It was observed that from 12 antimicrobial agents tested, eight presented percentage of resistance significantly higher in the recent isolates (Nemati et al., 2008).

Persoons et al. (2009) evaluated samples from 50 laying hens and 75 broiler chickens in Belgium. MRSA was found in 8 broiler chickens from 2 of 14 farms sampled, belonging to ST398 *spa* type t1456. According to the author, it still remains unclear as to whether this strain is associated with poultry.

### 3.5 Pigs

Mounting evidence suggests that livestock, particularly pigs, can represent an important reservoir of CA-MRSA (community-associated - CA) strains that can colonize and infect humans in close contact with them. ST398 is the most commonly reported MRSA sequence type among large livestock in Europe. These strains frequently carry genes encoding for non-beta-lactam antimicrobial resistance, including a plasmid-borne gene with resistance to trimetoprim, *dfpK*, identified in an isolate from a pig from Germany. Furthermore, these isolates are referred to often as nontypeable by PFGE because their genome is not digested by *SmaI* enzymes, several common *spa* types have been associated with them, carrying SCCmec types IV and V and they typically lack PVL genes (David & Daum, 2010).

In 2008, with the aim of evaluating whether other professionals in contact with pigs, in addition to farmers and veterinarians, have a higher risk of carrying MRSA than the population in general, a study was carried out with 272 participants of a conference on swine health in Denmark. In total, 34 (12.5%) participants from 9 countries carried MRSA, being that 31 isolates were not typeable by PFGE with *SmaI*. They belonged to ST398, *spa* types t011, t034, t108, t571, t567 and t899. The MRSA transmission from pigs to staff demonstrated to be an international problem, creating a new reservoir for a strain that was still considered CA-MRSA (Wulf et al., 2008).

Horgan et al. (2010) evaluated the prevalence of MRSA in the swine population in Ireland. A total of 440 pigs were evaluated from 41 geographically distributed farms and 100 individuals involved in the pig industry. No MRSA isolate was recovered from pigs but two humans tested were identified as MRSA carriers. These individuals were working in the wider pig industry. These isolates belonged to ST22 and ST1307, indicating that during the period of study the porcine colonization by MRSA, in particular the animal-related strain ST398, was not common in Ireland.

Wagenaar et al. (2009) published the first report of MRSA on pig farms in China, and it was the first time that MRSA ST9 in 4 pig farms and one *single locus variant* of MLST ST9 (ST1376) were detected in a pig farm. This study shows that LA-MRSA is not restricted to the clonal lineage ST398 found in Europe and North America in commercial pigs, but that other MRSA lineages are able to spread in the livestock in the same manner, also confirming that livestock can act as a reservoir of MRSA.

Despite that LA-MRSA appears to be the predominant MRSA strain in pigs, some studies mention the detection of non-LA-MRSA strains in these animals, possibly by transmission of

human strains to the animals. In Singapore, ST22-MRSA-IV was isolated from pigs and this strain was previously found increasingly important in the hospital population there. Notably the ST22-MRSA-IV is also a major hospital clone, as is the UK-EMRSA-15 found in the UK, with both strains indicating a contamination of human origin. In Canada, 14% of MRSA isolated from pigs appeared to belong to the human epidemic clone CMRSA-2 (Canadian epidemic MRSA-2, known as USA100), while 74.4% of isolates were LA-MRSA. The remaining strains belonged to rare clones, not related to LA-MRSA or CMRSA-2. Most reports on LA-MRSA in pigs originate from the Netherlands. In Europe, LA-MRSA has also been found in pigs in Germany, Denmark and Belgium, and beyond Europe, in Canada, Singapore and the USA (Vanderhaeghen et al., 2010).

### 3.6 Products of animal origin

Besides the importance of living animals as a source of MRSA, animal origin products also play a role in disseminating these strains to the humans. Lozano et al. (2009) detected MRSA ST398 in food samples in Spain. A total of 318 samples of raw food were evaluated from food-producing animals (148 from chicken, 55 from pork, 46 from veal, 19 from lamb, 10 from turkey, 8 from rabbit and 12 minced-meat samples) and of wild animals (8 *game birds*, 4 wild boar, 4 deer and 4 hare samples). MRSA was detected in 5 of 318 (1.6%) food samples (pork, chicken, rabbit, veal and wild boar). The two strains from pork and veal corresponded to ST398-SCC*mecV* clone (*spa* types t011 and t1197, respectively), the two strains from chicken and rabbit were typed as ST125-SCC*mecIVa*-t067, and the strain from one wild boar was ST217-SCC*mecIVa*-t032, with all the MRSA being PVL negative. The characteristics of these strains suggest that they can be of both animal and human origin, and although the presence of MRSA in food is low, it must be monitored, because it can contribute to its dissemination.

Recently, Weese et al. (2011) evaluated the presence of MRSA in feedlot cattle, close to the time of slaughter, in nasal and rectal fecal samples. It was not possible to detect MRSA in these animals, in contrast to recent studies on retail beef (Weese et al., 2010), demonstrating the need for more studies of livestock, as well as farms, processing and retail environments to elucidate the epidemiology of contamination with MRSA in meat.

## 4. MRSA as zoonosis or humanosis

Several studies have been done to determine the degree to which MRSA plays a role in zoonosis or humanosis. It has been observed that usually the strains originating from companion animals are originally human strains, and that the infection with this MRSA type is considered humanosis. On the other hand, the strains originating from livestock (livestock-associated - LA) are often divergent from human strains and the infection with this type of LA-MRSA could be considered zoonosis, and in this case MRSA would be an emergent zoonotic agent. Within this context, veterinarians, cattle farmers and pet owners are considered risk groups for acquiring MRSA (Morgan, 2008).

In 2011, a case of empyema was reported in a 79-year-old man in Spain with isolation of MRSA ST398 in purulent exudates from the thorax and trachea and nasal swabs. The ineffective initial therapy with levofloxacin was modified to intravenous linezolid, but the clinical situation of the patient rapidly deteriorated, and he died of multiorgan failure. The

three MRSA strains were typed as ST398, *spa*-type t011, *SCCmec V* and *agrI* and presented the same phenotypic resistance, including  $\beta$ -lactam, tetracycline, clindamycin (but not erythromycin), ciprofloxacin and levofloxacin. It is important to point out that the patient lived with his wife and two sons near a pig farm. Both sons worked on the farm. The patient, but not his wife, sporadically helped on the farm. Nasal samples from three family members indicated MRSA carriage in one son. The characteristics of this isolate were identical to the isolate from the patient. Furthermore, nasal swabs of 18 pigs from the farm were randomly collected, and MRSA isolates were detected in 9 (50%) pigs. One MRSA isolated in each animal was minutely characterized. Eight isolates were typed as ST 398/t011/*SCCmec V*/*agrI* and one remaining isolate as ST398/t1451/*SCCmec V*/*agrI*. All MRSA isolated from the animals had the same phenotypic and genotypic resistance comparing MRSA isolated from the patient and son. These findings indicate potential pig-human zoonosis transmission of MRSA ST398 and that this clone can be associated with severe respiratory pathology in immunocompromised patients, and this microorganism can also be resistant to other first-line antimicrobial agents, such as fluoroquinolones, used to treat these infections. Furthermore, the unusual clindamycin-resistance/erythromycin-susceptibility phenotype can be a key marker (in addition to tetracycline resistance) of the possible presence of livestock-associated MRSA (Lozano et al., 2011).

As seen in the reports, animal reservoirs for MRSA are becoming recognized worldwide with increasing awareness of MRSA ST398 colonizing pig and veal farmers, and attending veterinarians, at high rates. In The Netherlands, these groups are now considered high risk, and if admitted to a hospital, they are immediately conducted to isolation, screening for MRSA and decolonization (Loeffler et al., 2009).

MRSA isolates originating from animals have been shown to hold important genes of resistance which could be transferred to less pathogenic human strains, but well adapted, in a nasal co-colonization and resulting in new human lineages, for example (Springer et al., 2009). The gene *czrC*, which confers resistance to cadmium and zinc, was determined in isolates of MRSA CC398 of *SCCmec* type V originating from 23 (74%) pigs and 24 (48%) humans from Denmark. It is suggested that resistance to heavy metals can play a role in coselection of MRSA, because it was strongly related to the clone CC398 (Cavaco et al., 2010).

In lineages MRSA ST398 and MSSA ST9 isolated from pigs in Germany, the major reservoir of these lineages, the gene *cfr* was found, a gene of multi-resistance to the drugs phenicol/lincosamine/oxazolidinone/pleuromutilin/streptogramin A. The risk of its transference to humans with exposure to pig farms is of concern since these lineages can colonize and cause infections in humans (Kehrenberg et al., 2009).

Genes encoding virulence factors can also be carried by animal strains. An example is the PVL gene, encoding an important virulence factor related to MRSA, rarely reported in animals, but can be found in companion animals. PVL-positive CA-MRSA has been reported in cats, dogs, rabbits, birds, bats, turtles, pigs and cattle. The strains associated with pigs that have been rapidly disseminating are currently PVL-negative (Morgan, 2008).

As for animal origin food, meats of several animal species have been evaluated for detection of MRSA. The contamination has been reported in meats of turkey, chicken, veal, pork, beef and lamb. The majority of the isolates were non typeable MRSA. Considering the low

number of non typeable MRSA in patients, the role of food products in disseminating MRSA seems to have been overlooked (Morgan, 2008).

## 5. MRSA detection in animals

Animal studies of MRSA are limited by certain research bottlenecks compared to human studies, including a lack of standardization regarding culture methodology, susceptibility testing, definition of genetic profile and sampling methods, which ultimately renders comparison difficult. A mass animal screening is logistically difficult, expensive, and impractical in many situations. Moreover, physical challenges exist: for example, carrying out a nasal swab in cats is only possible with appropriate animal restraints (Morgan, 2008).

Despite these drawbacks, several studies have been carried out, as more detailed studies of epidemiological aspects of animal MRSA are indispensable, primarily in food-producing animals of particular concern. These animals are not only reported as the primary source of a recently emergent new type of MRSA, LA-MRSA, but studies also suggest that they are involved in transmission of other strains of MRSA between animals and humans (Vanderhaeghen et al., 2010). MRSA screening must be performed in all diagnostic laboratories, even if done through disk diffusion with oxacillin, which is better than methicillin related to the resistance to degradation and the detection of heteroresistant strains (Weese et al., 2004).

MRSA detection in animals has been performed, generally, with isolation from samples from nasal and oral mucosae and perineum in small animals (Loeffler et al., 2005; Nienhoff et al., 2009), samples from milk in cows (Kwon et al., 2005), samples from nasal, oral and/or perineal swab, in pigs and horses (Baptiste et al., 2005; Voss et al., 2005; Huijsdens et al., 2006), samples from nose and cloacae in poultries (Nemati et al., 2008) and samples from meats (Lozano et al., 2009).

The samples were pre-enriched in different selective mediums with the aim of increasing the sensitivity of the culture technique. Lozano et al. (2009), to detect MRSA in meat, pre-enriched the samples in BHI broth (brain heart infusion) containing 6.5% of sodium chloride at 35°C for 24 h. An aliquot of each growth was seeded on ORSAB plates (oxacillin resistance screening agar base) with oxacillin (2mg/L) and incubated at 35°C for 36 h. Van Duijkeren et al. (2010) compared two methods of culture technique; the first was Müeller Hinton Broth containing 6.5% sodium chloride, which after overnight incubation at 37°C, was transferred to phenol red mannitol broth with 5µg/ml of ceftizoxime and 75µg/ml of aztreonam and after overnight incubation at 37°C was plated onto sheep blood agar and brilliance MRSA agar. In the second method, Tryptone Soy Broth was used containing 4% sodium chloride, 1% mannitol, 16µg/ml of phenol red, 50µg/ml of aztreonam and 5µg/ml ceftizoxime, plating onto sheep blood agar and MRSA brilliance agar after 48 hours of incubation at 37°C. The first method, with the pre-enrichment containing higher salt concentration, presented better results. Weese et al. (2011) used as pre-enrichment a broth containing tryptone, sodium chloride, mannitol, yeast extract and incubated at 35°C for 24 h to evaluate MRSA presence in feedlot cattle, as well the nose samples as feces. An aliquot of the growth was inoculated onto MRSA Chromogenic agar and incubated at 35°C for 48 hours.

Many methodologies have been employed to identify and characterize strains, ranging from phenotypic to genotypic. Both present advantages and disadvantages and must be

performed in accordance with the need of the study and the material and personnel available in each laboratory. Relative speed and the reliability are the desirable characteristics in both methods, because the choice of treatment and infection control measures are determined by the results of such testing (Kaya et al., 2009). Phenotypic methods at first are more accessible and nearly always cheaper; however they depend on the characteristic expression and visualization that cannot occur or be reduced, as for example, by environmental influences and/or regulatory genes (Berger-Bächli, 2002; Mohanasoundaram & Lalitha, 2008).

Methicillin-resistant *S. aureus* can be identified through different genotypic methods, such as species-specific primers for detection of DNA fragment of *S. aureus*-specific (van Duijkeren et al., 2010, as cited in Martineau et al., 1998) and with gene *mecA* (Murakami et al., 1991) by PCR (polymerase chain reaction), or the detection of DNA fragment of *S. aureus*-specific and gene *mecA* by multiplex PCR (Huijsdens et al., 2006), for example.

To determine the MRSA clones involved, in the beginning of 1990s the pulsed-field gel electrophoresis (PFGE) of genomic *Sma*I macrorestriction fragments were introduced and still represents the gold standard with respect to discriminatory power. The clonal groups determined by cluster analysis through PFGE are largely congruent with those defined by MLST. However, with the presence of some lineages of special interest (for example ST398) that are non typeable by the standard restriction enzyme *Sma*I, other methods have been used (Cuny et al., 2010).

As observed in the majority of studies discussed in this chapter, from genotypic methods for classification of predominant strains and determination of evolutionary pathways, MLST and *spa* typing have been the most widely employed: the first because it is an unambiguous discriminatory method for studying MRSA epidemiology and evolution, with results that can be truly portable between laboratories (Enright, 2003) and the second method to indicate genetic microvariation permitting investigation of outbreaks or accomplishment of phylogenetic analysis (Koreen et al., 2004). MLST characterize bacteria isolates unambiguously using the sequences of internal fragments of seven “housekeeping” genes, being a discriminatory method which permits that related strains recovered from different countries be quickly identified (Enright et al., 2002). The *spa* type identified by DNA sequence analysis of the X region of the protein A gene (*spa*) is less expensive, time-consuming, and error prone than multilocus techniques, such as MLST (Shopsin et al., 1999; Koreen et al., 2004).

The classification by MLST permits that the genomes of strains deposited in the GenBank database to be compared to establish their evolution and characteristic features. Comparing the genome of CA-MRSA and HA-MRSA from the same clonal lineage as well as their most probable MSSA ancestor, about 78% of the genes are conserved, and the remaining 22% comprise an “accessory genome” including genomic islands, pathogenicity islands, prophages, integrated plasmids, and transposons. However, comparing the *S. aureus* genome from cattle mastitis (ST151) with human *S. aureus*, it has been demonstrated that this bovine clone probably evolved from a common ancestor by acquiring foreign DNA. Subsequent microarray studies on recent epidemic strains of bovine origin (such as ST97) also revealed the presence of mobile genetic elements absent from *S. aureus* of human origin. Even with these impressive insights into the evolution of *S. aureus*, research has not yet provided many clues on the adaptation of the pathogen to the host, since only limited data

are available (Cuny et al., 2010). In this respect, further research is needed to address these gaps, as well as to better understand the evolution of these strains in humans and animals.

## **6. MRSA prevention and control in animals**

All studies on animal MRSA have helped establish critical measures for its control. Numerous reports on MRSA control in humans have been published and many of the principles may also be applied to control in animals. However, caution is necessary for extrapolating these human guidelines to animals, as disease epidemiology can differ significantly (Leonard & Markey, 2008).

It has been observed that exposure to antimicrobials is a risk factor for the acquisition and dissemination of MRSA in humans and also most probably in animals. In this respect, strategies for prevention and management of MRSA in animals should be, as much as possible, related to the use of antimicrobials. If the antimicrobial treatment is necessary in individual cases for the sake of animal welfare, the risk of the emergence of wider resistance in MRSA strains colonizing animals needs to be managed, especially considering zoonotic aspects. Options to manage this risk include the non-use of antimicrobials except as a last resort strategy, decolonization in humans, isolation of animals during treatment, and monitoring the effects of treatment in strain resistance through selective culture and susceptibility tests (CATRY et al., 2010).

### **6.1 General preventive and control measures**

Good hygiene is an important general preventive and control measure, both in homes and human and animal healthcare environments, because environmental contamination with MRSA acts as a reservoir for infection. Known MRSA-positive animals should be nursed apart from other animals, with strict washing of the hands, gloves and gowns if in close contact. Recording the history of contact with human or animal MRSA, as well as an early culture of a wound non-responsive to first-line therapy allows for earlier recognition of MRSA and its appropriate management. Furthermore, when faced with repeated and inexplicable failure of human decolonization, clinicians can investigate nearby exposure to animals and birds that could be the reservoirs (Morgan, 2008).

Below, are some precolonized specific measures cited from (Catry et al., 2010).

#### **6.1.1 Specific measures for livestock animals**

- Reduction of antimicrobial selective pressure in livestock by avoiding routine mass medication
- Prevention of transmission of MRSA between and within the farms with sanitary measures of control between herds and during transportation
- Identification and isolation of animals to minimize the risk for zoonotic infection
- Use of contact precautions such as protective outerwear, overalls, aprons or coats and boots or overshoes that are not worn elsewhere
- Protective outerwear and all the items handled during the treatment of MRSA-positive animals should be considered potentially contaminated
- Hands can be hygienically cleaned with alcohol gel pouches, which are essential but need to be used correctly

- Proper cleaning and disinfection of contaminated environments, including transport vehicles. Special attention should be paid to dust in stables
- Animal owners should be informed about the risks and necessary precautions.

#### **6.1.1.1 Reducing carriers on MRSA-positive livestock farms**

Control and/or treatment of colonized and infected animals with or without antimicrobials is necessary for the reduction of carriers.

The affected animals need to be immediately separated from healthy animals. In extreme cases culling of infected animals is a further option. Milk of animals with mastitis by MRSA must be destroyed, and in some cases the infected quarter must be prematurely dried-off.

If the antimicrobial treatment is chosen, it is necessary to evaluate its risk-benefit compared with other alternatives. The choice of antimicrobials should always be based on a susceptibility test, and all precautions should be taken that the drug reaches the infected site with appropriate concentrations.

#### **6.1.2 Specific measures for horses**

The options of control for colonized horses, as well as livestock animals, include the non-antimicrobial management and the antimicrobial treatment of colonized or infected horses. In a Canadian study, two farms with horses colonized by MRSA drastically reduced the number of affected animals with active screening and strict implementation of control protocols of infection, without the use of antimicrobial therapy. Antimicrobial treatment must be applied only if the colonization is persistent or in cases in which control measures are impossible.

Preventive measures for the infected animals are the same for the previously mentioned livestock animals. In equine hospitals, MRSA management for veterinary practices, guidelines stipulated by the British Small Animal Veterinary Association (BSAVA), for example, can also be applicable. In the confirmed or suspected cases of infection by MRSA, horses must be isolated and treated as if disseminating a nosocomial and zoonotic agent. It is necessary to take precautions with staff hygiene as well, using protective barriers, such as gloves, aprons, and boots. Moreover, the entire animal must be evaluated before being admitted to the hospital to ensure prevention of MRSA dissemination.

#### **6.1.3 Specific measures for companion animals**

In colonized animals, it is not recommended to decolonize animals having mucosae colonization with MRSA. And it is observed that apparently some pets eliminate the carriage of MRSA spontaneously if the re-colonization is prevented. Antimicrobial therapy is only indicated in exceptionally persistent cases or when control measures are impossible. If the infection is local in infected animals, meticulous management of the wound can avoid the necessity of antimicrobial use. The risk of resistance development, the susceptibility profile of the isolate, the severity of the infection and the presence of systemic disease (fever, leukocytosis), and the patient's underlying disease or any comorbidity must be taken into account when choosing the antimicrobial treatment. In deciding to treat or eventually euthanize, veterinarians must consider the national veterinary guidelines available.

Preventive measures include the strict control practices of the infection in homes, particularly frequent hand washing hygiene, avoiding high-risk contact to minimize the chance of becoming colonized and isolating the animal and other pets. Gloves, masks and eye protection must be used to attend patients, as well as planning surgeries to avoid risk for infection, removing permanent urinary catheters as soon as possible and preventing complications from intravenous and urinary catheters with appropriate asepsis.

## 7. Conclusion

This paper summarizes a wide range of information and findings from the literature on MRSA in animals and humans in contact with them. Notwithstanding, there is enormous potential for new research aiming to conclusively address certain unknown questions such as, at which point does an infection play the role of a zoonosis or humanosis? This question has yet to be answered because several animal species are involved, with distinct characteristics of transmission and isolated clones, raising appropriate concern for MRSA in animals.

Steadfast vigilance of MRSA in samples of animal origin in laboratory diagnoses is essential for: consistent and thorough monitoring of the evolution and dissemination of these strains; elucidating characteristics that determine a predilection for a determined host; determining transmission routes; identifying resistance and virulence genes received by these new lineages; and distinguishing molecular markers that allow for discriminating between CA-MRSA, HA-MRSA and LA-MRSA.

Appropriate and effective measures of control and prevention must be better determined and applied to each situation and country, according to previously reported guidelines, aiming to minimize risks to humans, since these strains have housed new virulence and resistance genes which can be transferred to human strains. Veterinarians play an important role in public health, in controlling this pathogen through measures appropriately applied in veterinary medicine, namely, the rational use of antimicrobials and appropriate management of infected animals, together with other health professionals, for prevention of MRSA dissemination.

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This book represents an overview on the diverse threads of epidemiological research, brings together the expertise and enthusiasm of an international panel of leading researchers to provide a state-of-the-art overview of the field. Topics include the epidemiology of dermatomycoses and *Candida* spp. infections, the epidemiology molecular of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from humans and animals, the epidemiology of varied manifestations neuro-psychiatric, virology and epidemiology, epidemiology of wildlife tuberculosis, epidemiologic approaches to the study of microbial quality of milk and milk products, Cox proportional hazards model, epidemiology of lymphoid malignancy, epidemiology of primary immunodeficiency diseases and genetic epidemiology family-based. Written by experts from around the globe, this book is reading for clinicians, researchers and students, who intend to address these issues.

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