

Review on Livestock Associated Methicillin Resistant *Staphylococcus aureus* and its Zoonotic Importance

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA), first was appeared as hospital associated (HA)-MRSA in 1961, then as community associated (CA)-MRSA and recently as livestock associated (LA)-MRSA. LA-MRSA was first isolated from a Belgian cow in 1972 though Significant concerns about MRSA and association with food animals were emerged after an alarming report about infections and high rates of colonization among Dutch pig farmers in 2005. The emergence and dissemination of MRSA in livestock is highly associated with usage of antibiotics in animal feed as growth promoter and as prophylaxis. Resistance is documented to a wide variety of antibiotics in addition to β -lactams. LA-MRSA isolates usually belong to clonal complex CC-398 and to the sequence type ST-398. Characteristically, LA-MRSA ST-398 strains carry SCC *mec*-type, type IV or, more frequently, type V which are also present in CA- MRSA. However, ST-398 generally does not possess the Panton-Valentine leukocidin (PVL) –genes. LA-MRSA is usually associated with suppurative conditions of the skin, wound infections and mastitis. Vets, farmers, abattoir workers and people living near high density pig farms are high risk groups and zoonotic transmission is both through direct and indirect method. Therefore measures like screening of animals and their products for MRSA during trade and in farm, wise use of antibiotics and strict implementation of hygiene should be taken to minimize the spread and control of the diseases as a result of MRSA.

Key words: Drug Resistance • Livestock • MRSA • Zoonosis

INTRODUCTION

Antimicrobial resistance is resistance of a microorganism to an antimicrobial medicine of which it was originally sensitive. Understanding the mechanism of resistance is important for developing novel drugs that target these resistant microorganisms. The development of antibiotic resistance genes can occur in the form of spontaneous or induced mutation or by the acquisition of resistance genes from other bacterial species by horizontal gene transfer via conjugation, transduction, or transformation. Acquisition of these genes results in selective survival expansion in the presence of the corresponding drugs. In this way, a gene for antibiotic resistance may readily spread through an ecosystem of bacteria genes [1].

Antibiotic resistant strains of bacteria have become newsworthy, in recent times. Ever since the outset, the use of antibiotics has met the generation of antibiotic

resistance in certain bacteria. The most capable types of bacteria in this regard, appear to be *Escherichia coli* (*E. coli*), *Salmonella* and *Staphylococcus aureus* (*S. aureus*). Due to the unwise use of antibiotics in humans and animals (Especially wide spread feeding of antibiotics to farm animals), the situation is becoming more serious, with same microorganisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA) which is becoming a serious problem in human and animal health [2].

Methicillin, a penicillin derivative, interferes with cell wall synthesis by binding to particular enzymes known as penicillin-binding proteins, which are essential for the synthesis of peptidoglycan (PGN). All members of the penicillin family of drugs, including penicillin, ampicillin, amoxicillin and methicillin, have a common basic structure comprising a β -lactam ring, which confers antibacterial activity. However, several organisms produce the enzyme β -lactamase, which degrades the β -lactam ring.

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The resistance shown by *S. aureus* to methicillin is attributable to the acquisition of the *mecA* gene, which encodes a specific penicillin-binding protein, PBP2a, with a low affinity for β -lactam antibiotics [1].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen occurring in human and veterinary medicine. It was first described as hospital-acquired MRSA in 1961 as nosocomial infections. Later, the pathogen was also observed in healthy humans without hospitalization and the term community-acquired MRSA was developed [3]. MRSA in animals was first isolated from a Belgian cow, in 1972 [4] and since 2003, MRSA belonging to Clonal Complex (CC) 398 (CC-398) has emerged in livestock and this CC is by far the most prevalent livestock-associated MRSA (LA-MRSA). The emergence in livestock caused a strong increase in MRSA occurrence in humans between 2001 and 2006 in The Netherlands. CC-398 is now being reported from different countries around the world [5] and LA-MRSA strains have been found mainly in pigs and veal calves, but they have the capacity to colonize a wide spectrum of hosts including sheep and poultry [6].

LA-MRSA was emerged as a public health concern in 2005 with reports of a specific multi-locus sequence type (ST-398) being found in higher than expected numbers in swine workers in France and Netherlands [7].

The Organism: Methicillin-resistant *Staphylococcus Aureus*: Staphylococci are spherical Gram positive bacteria that divide in several planes to form grape-like or irregular cluster. They are present in the upper respiratory tract and on other epithelial surfaces of all warm blooded animals. The most intensively studied species of Staphylococcus; *Staphylococcus aureus* is a common pyogenic agent in humans and several animal species [8].

Methicillin-resistant *Staphylococcus aureus* (MRSA), first identified in 1961, is a major cause of healthcare-related infections, responsible for a significant proportion of nosocomial infections worldwide. Frequently, the MRSA strains isolated from animals resemble human strains and presumably were transferred from their caretakers. Recently however, a new lineage has been found in livestock which was first identified in pigs in The Netherlands in 2003. These livestock-associated MRSA (LA-MRSA) isolates are genetically distinct from human isolates. LA-MRSA strains, largely comprising MLST type ST-398, currently represent the largest reservoir of MRSA outside of a hospital setting [9].

Naming Conventions for Methicillin-Resistant *Staphylococcus aureus* Strains: There are at least three different genetic techniques currently used for the classification of *S. aureus* strains, including pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and DNA sequencing of the X- region of the protein A-gene (Spa typing). Consequently a single *S. aureus* isolate can have more than one valid name, depending on the test which is used for typing. Naming conventions are complex and strains given a single name in one system are sometimes separated into more than one strain in another system although names such as ST-9 or CC-398 are used for both methicillin-resistant and methicillin-susceptible *S. aureus* of that genetic type [10].

Why the name Methicillin-resistant Staphylococcus aureus?

Cloxacillin, Flucloxacillin and Oxacillin are semi-synthetic antibiotics closely related to Methicillin. MRSA could equally be called CRSA (Cloxacillin-resistant *S. aureus*), FRSA (Flucloxacillin-resistant *S. aureus*) or ORSA (Oxacillin-resistant *S. aureus*) since any *S. aureus* highly resistant to Cloxacillin, Flucloxacillin or Oxacillin will also be highly resistant to Methicillin and vice-versa. However, although Methicillin is no longer widely used, the resistant bacteria are still referred to as MRSA because it was the first of these antibiotics to be marketed [11].

Epidemiological Definitions of Methicillin-resistant *Staphylococcus aureus* Groups: Different types of MRSA may be distinguished based on epidemiological groups. This can be a simplistic approach since in some cases strains of MRSA have spread between the groups [12].

Hospital Associated MRSA (HA-MRSA): HA-MRSA is known as nosocomial pathogens for decades. MRSA are regarded as HA-MRSA when infections caused by them are likely to be acquired in health care settings when they emerge at least 48 hours after admission in patients having particular risk factors such as prolonged hospital stay, care in intensive care units (ICUs), prolonged antibiotic treatment, surgical interventions and/or close contact with MRSA-positive individuals [13].

Health Care Associated Community MRSA (HCA-MRSA): HCA-MRSA is associated with outpatients with MRSA infection/colonization and previous hospitalization, such as residence in a nursing home,

receiving of home nursing, attending centers for dialysis and/or centers for diabetes were MRSA of hospital origin has been introduced [14].

Community Associated MRSA (CA-MRSA): CA-MRSA emerges in the community and patients that lack hospitalization as a risk factor. Close contact in sport settings, schools, day care centers, military settings and prisons are among the risk factor [12].

Livestock Associated MRSA (LA-MRSA): LA-MRSA refers mainly to the clonal spread of certain MRSA strain (ST-398) that colonizes different food animal species (Including horses) and may cause infections in humans [12].

Resistance to β -Lactam Antibiotics: Within a very short time *S. aureus* was showing signs of its remarkable ability to evolve and grow stronger when under attack by antibiotics. By 1942, even before Penicillin was available for all doctors to prescribe, penicillin-resistant strains were being found [11].

Treatment of *S. aureus* infections before the 1950s involved the administration of benzyl penicillin (Penicillin G), a β -lactam antibiotic, but by the late 1950s *S. aureus* strains resistant to benzyl penicillin were causing increasing concern. Resistant strains typically produced an enzyme, called a β -lactamase, which inactivated the β -lactam. Efforts were made to synthesize penicillin derivatives that were resistant to β -lactamase hydrolysis. This was achieved in 1959 with the synthesis of Methicillin. Unfortunately, as soon as Methicillin was used clinically, Methicillin-resistant *S. aureus* (MRSA) strains were isolated that have resistance which was not due to β -lactamase production [15].

Resistance to Methicillin: The main mechanism of Methicillin resistance in *S. aureus* is through the expression of a foreign Penicillin binding protein (PBP), PBP2a. MRSA differ genetically from Methicillin-sensitive *S. aureus* isolates by the presence, in the chromosome, of a large stretch of foreign DNA (40-60 Kb), referred to as the *mec* element and the presence of the *mecA* gene that encodes the 76 KDa penicillin-binding protein PBP2a (Also referred as PBP2). PBP2a is not able to completely compensate for the other PBPs since cells grown in the presence of methicillin exhibit a marked reduction in the degree of cross-linking. However, the limited degree of cross-linking is enough to ensure survival of the cell [15].

Identification of Methicillin-Resistant *Staphylococcus aureus*: Methicillin-resistant *Staphylococcus aureus* (MRSA) can colonize more than one site, but nasal and rectal sampling should both be done whenever possible. Infection with MRSA, including colonization, can be diagnosed by culture and identification of the organism [10].

These methods require isolation and identification of *S. aureus* as Gram positive, catalase positive and coagulase positive cocci showing beta hemolysis on blood agar. After isolation of *S. aureus*, following tests could be employed for identifying the MRSA [16].

Disc Diffusion Test: Disc diffusion test is employed by culturing *S. aureus* on Muller Hilton agar (MHA) impregnated with Oxacillin or Methicillin (1 or 5 μ g) and Cefoxitin (30 μ g) discs. MRSA is identified by assessing zone of inhibitions with Oxacillin \leq 14 mm and/or Cefoxitin \leq 21 mm. Cefoxitin disc diffusion test is considered superior to Oxacillin disc diffusion test due to its ease of reading and higher sensitivity. Cefoxitin induces *mecA* gene of MRSA and its results have been found in concordance to PCR. Thus, Cefoxitin disc diffusion test can be alternative to PCR for the detection of MRSA in resource constraint settings.

Oxacillin MIC Tests: Gradient plates of MHA containing 2% NaCl with doubling dilutions from 0.25 μ g/ml to 256 μ g/ml of Oxacillin are prepared. *S. aureus* inoculum is prepared by diluting 0.5 McFarland equivalent suspension of a strain with sterile normal saline to the concentration of 10⁴ CFU/ml. The plates are spot inoculated and incubated at 35°C for 24 h. An Oxacillin MIC of less than or equal to 2 μ g/ml is indicative of susceptible and that of > 2 μ g/ml resistant.

Chromogenic Media: These are selective and differential media used for direct detection of MRSA. This type of media contains specific Chromogenic substrate and antibiotics like Cefoxitin. MRSA will grow in the presence of antibiotics producing colored colonies due to hydrolysis of Chromogenic substances.

Polymerase Chain Reaction (PCR): Is used for detection of *mecA* gene of *S. aureus* using *mecA* gene specific primers. But, the use of PCR method is limited only for sophisticated laboratories.

The Creation of New Challenge: The latest phase in the evolution of MRSA has been its appearance in animals.

The British scientist Andrew Waller has called MRSA in farm animals and pets “the creation of a new monster”. At first, infections were reported in pets, particularly dogs and cats. The strains involved were usually similar or identical to those infecting humans and the most obvious explanation for this new veterinary problem was and still is, that the pets acquired the resistant bacteria from humans [11].

The emergence and dissemination of MRSA in food animals, livestock-associated MRSA, (LA-MRSA) appear to have taken a different course from that in companion animals. Rather than emerging from human sources, LA-MRSA seems to have evolved independently in one or more food animal species, with subsequent dissemination and interspecies transmission. Following the initial report of LA-MRSA in a dairy cow in 1972, sporadic cases of LA-MRSA mastitis in dairy cattle were described, typically at a low prevalence, among *S. aureus* isolates from clinical or subclinical mastitis. The incidence of LA-MRSA mastitis and the prevalence of methicillin resistance among bovine *S. aureus* isolates appear to be quite low, so LA-MRSA does not appear to be a common or important bovine mastitis pathogen at that time [4, 11].

The first ever report of MRSA in pigs came from the Netherlands as recently as December 2005 [11]. Significant concerns about MRSA and food animals emerged after an alarming report about infections and high rates of colonization among Dutch pig farmers in the mentioned year. Because ST-398 strains have predominated in reports of MRSA in pigs internationally, it is plausible that this strain emerged in pigs and was subsequently disseminated to other species. Nowadays LA-MRSA is present in a wide range of animal species, including dogs, cats, rabbits, horses, cattle, pigs, poultry and exotic species, both as a cause of infection as well as in healthy carriers [4].

Origin and molecular evolution of livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA)

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) seems to be primarily associated with pigs. Currently, Methicillin-susceptible *Staphylococcus aureus* (MSSA) strain ST-398, has only been described in humans and in pigs, suggesting that one of these species is the original host of MRSA ST-398. The results of an early French study support the view that MSSA ST-398 is primarily pig-associated. In that study ST-398 was found in certain MSSA clones, that were prevalent in healthy pig farmers but not in healthy non-farmer controls [4, 17].

Since nearly all the MRSA found in Dutch pigs is unclassifiable using the PFGE method, the logical next step was to look more closely at all human isolates of MRSA which were also non-typeable by PFGE, to see whether there appeared to be any link with farming. Humans infected by, or carriers of non-typeable MRSA (NT-MRSA) were found to be about 12 times more likely to be pig farmers and 18–20 times more likely to be cattle farmers, than those who were infected by, or carriers of, other MRSA strains. The scientists concluded: ‘NT-MRSA is associated with pig and cattle farming. It causes serious disease and is also transmitted between humans’. Nonetheless, it seems that more data are necessary to reliably conclude that MSSA ST-398 is essentially pig specific. Unfortunately, conversely to humans, very few databases of *S. aureus* strains isolated from pigs are available, making it difficult to assess the prevalence of MSSA ST-398 in pigs to date [4, 11, 17].

It is generally assumed that the acquisition of SCCmec in certain MRSA clone was by horizontal transfer of SCCmec from another source. This source might be another MRSA strain carrying the specific SCCmec element often suggested, a Methicillin-resistant non-*S. aureus* Staphylococcus (MRNaS), in which various SCCmec elements are known to be present and which thus could function as a reservoir for SCCmec. The role of MRNaS in the horizontal transfer of SCCmec to MSSA and the frequency of SCCmec transmission events between staphylococcal strains remains mere speculation. However the acquisition of SCCmec by MSSA ST-398 did not occur in pigs but in humans. This would be in accord with the earlier detection of MRSA ST-398 in humans than in pigs. Indeed, before the first report on LA-MRSA in pigs, MRSA ST-398 was detected in a Dutch woman and in a French pig farmer. A possible course of events could thus have been that after transfer to one or more humans, probably farmers, an MSSA ST-398 strain from pigs acquired SCCmec from a CA-MRSA strain and after re-colonization of one or more pigs, such MRSA ST-398 strain started to spread among other pigs [17].

Microbiological, Molecular and Virulence Characteristics of LA-MRSA ST-398 Strain: LA-MRSA strains were initially distinguished by their peculiar resistance to digestion by *SmaI*, the restriction enzyme most frequently used for PFGE typing of *S. aureus* due to the presence of a new methylation enzyme protecting restriction sites. Otherwise, LA-MRSA were perfectly typeable by current molecular methods: they were associated with a specific group of *spa* types with related

repeat sequences, including t-011, t-034, t-108 and t-899 and belonged to ST-398, a clone historically quite rare in humans that did not derive from the most common MRSA lineages [17, 18].

Characteristically, LA-MRSAST398 strains carry SCC mec type, type IV or, more frequently, type V. The presence of these SCC mec types in LA-MRSA is shared with CA-MRSA and suggests a transmission of genetic elements between these two MRSA groups. However, differently from CA-MRSA, ST-398 generally does not possess the phage-encoded PVL-genes, that contribute to the virulence of the former. Exceptions involve strains of human infections that may have acquired PVL-genes from human MRSA: one patient from the Netherlands and two patients from Sweden had infections associated with PVL-positive ST-398. Also sporadic ST-398 MRSA from China has been found to be PVL- positive. No specific virulence factor characteristic of ST-398 has been identified so far [18].

LA-MRSA ST-398 is generally susceptible to antibiotics other than beta-lactams, but it is characteristically resistant to tetracycline, which suggests that heavy tetracycline use in the pig industry may have favored the emergence of this clone. A recent study showed that the addition of tetracycline or zinc in animal feed increased the number of ST-398 bacterial cells in pigs' nostrils although it had no effect on MRSA transmission [11, 18].

LA-MRSA ST-398 strains have been found to carry previously unidentified resistance genes, such as a novel trimethoprim resistance gene, *dfrK* and a novel gene, *vga* (C), encoding an ABC efflux pump and conferring resistance to streptogramins and lincosamides. In addition, an isolate from the nose of a pig was found to carry the multi-drug resistance gene *cfm* that is able to confer resistance to five different antibiotic classes, including linezolid, a "last-resource" antibiotic for serious infections due to multidrug-resistant Gram-positive bacteria. Due to their characteristic multi-host specificity, ST-398 strains can represent an efficient vehicle of these resistant determinants that are plasmid-encoded, favoring their transmission and spread [18].

Livestock Associated Versus Community Associated Methicillin-Resistant *Staphylococcus aureus*: When MRSA first appeared in the community, it was unclear whether it was an overspill from hospitals, or whether Methicillin-sensitive *S. aureus* had acquired resistance in the community resulting in new strains of MRSA. Scientific studies have since shown that most community strains share few of the characteristics of most

hospital-acquired MRSA, whereas they do share important features with methicillin-sensitive strains in the community. This has led some scientists to argue that the new resistant strains may have acquired their resistance in the community, not in hospitals [19].

Toxins in Livestock Associated MRSA: While one of the most striking characteristics of CA-MRSA is the high percentage of strains which produce the Pantone-Valentine leukocidin (PVL) toxin, there is at present only limited evidence of the presence of PVL MRSA in farm animals? The ST-398 MRSA found in Dutch pigs has been shown not to produce the PVL toxin, but 9% of the ST-398 MRSA bacteria in humans in the Netherlands were found to have the PVL-genes. This suggests that ST-398 MRSA in pigs could still acquire this trait, or it may even suggest that some of the ST-398 MRSA bacteria in cattle or chickens are already PVL MRSA. In Korea, however, 14 MRSA samples collected from cattle over a number of years and from different regions all had the PVL gene. This particular PVL strain is believed to have developed in cattle rather than to have been acquired from humans [20].

Another condition sometimes associated with CA-MRSA is toxic-shock syndrome [21] a rare but potentially fatal illness. Until recently, toxic-shock syndrome in humans caused by MRSA had been found extensively in Japan, rarely in the US and not at all in Europe. In 2005, scientists in Belgium reported a case of toxic-shock syndrome caused by MRSA and Belgian scientists warned of the increasing risk of MRSA outside Japan causing the syndrome [11]. In 2006, toxic-shock syndrome caused by MRSA was also reported in Russia and the US [22]. The rise in the incidence of toxic-shock syndrome associated with MRSA may indicate a farm-animal link, as the gene which enables MRSA to produce the toxin which causes toxic-shock syndrome has been found to be particularly common in *S. aureus* from farm animals [23].

A Dutch study published in 2005 compared the genetics and virulence traits of *S. aureus* from humans with those of *S. aureus* from animals, including farm animals. In addition to finding that many of the bacteria fell within the same 'genomic classes', the scientists discovered that the distribution of eight of the ten genes was similar for both human and animal isolates across each class. Two virulence genes were more prevalent in veterinary isolates, including the gene encoding the toxic-shock syndrome toxin, which was found to be much more common in farm-animal mastitis infections than in human infections [20, 23].

Antibiotic Resistance Elements of CA-MRSA and Farm-Animal MRSA: Most CA-MRSA and most MRSA from animals share one significant characteristic which distinguishes them from most hospital-acquired MRSA: the type of antibiotic-resistance element which they carry, the gene which makes *S.aureus* bacteria resistant to methicillin in all true MRSA is called *mecA*. This gene is part of a larger genetic grouping, or 'cassette', which is found in the *S. aureus* chromosome. This cassette is called a staphylococcal chromosomal cassette *mec* (*SCCmec*) and can vary in size and in the range of genes it contains. Until a few years ago, only three different kinds of *SCCmec*, referred as *SCCmec* I, II and III, were known in hospital-acquired MRSA. However, two new cassettes, *SCCmec* IV and *SCCmec* V have been found in community-acquired MRSA in recent years [24]. Two studies have also confirmed that most community-acquired MRSA have these new *SCCmec* IV and V [25].

Similarly, although the type of *SCCmec* has not always been determined for MRSA from farm animals, when it has, the *SCCmec* identified has usually been type IV or V. The *SCCmec* types IV and V are significantly smaller than types I, II and III and unlike types II and III, they do not usually carry any antibiotic resistance genes other than the *mecA* gene [24]. One consequence of this is that CA-MRSA appears to be less resistant to other antibiotics than many hospital-acquired MRSA and this may also turn out to be the case for farm-animal MRSA [11].

Other Phenotypic and Genomic Distinguishing Features: Recently, key phenotypic and genomic distinguishing features have been identified in human MRSA and LA-MRSA isolates. For example, transfer of LA-MRSA isolates beyond the immediate animal-exposed human contacts has rarely been observed and persistent nasal colonization is infrequently detected in individuals without direct animal exposure. Consistent with this, LA-ST398 MRSA isolates have been reported to be less transmissible among humans than HA-MRSA isolates. Using *in vitro* binding assays, ST-398 MRSA isolates were reported to bind significantly less to human skin keratinocytes and keratin compared to human MSSA isolates [9].

Epidemiology and Ecology of LA-MRSA: Methicillin-resistant *Staphylococcus aureus* (MRSA) can be found

worldwide, although the prevalence varies between regions. CC-398 is the predominant MRSA among pigs in Europe, but it has also been recognized in North America and Singapore. ST-9 appears to be the prevalent MRSA strain among pigs in China, Hong Kong and Malaysia but CC-398 may be uncommon or absent in these regions [10].

Europe: The first report of MRSA in livestock was a case of bovine mastitis in Belgium in 1972 [4]. Although, molecular typing methods were not available, bio-typing strongly suggested that a human origin of the isolate. Two decades later, investigations have found cattle also to be colonized by LA-MRSA, with 88% positive farms among Dutch veal calves rearing units studied [5]. Recently, bovine MRSA has been reported in many European countries with varying rate of prevalence. Huber *et al.* [26] (2010) reported a low prevalence of MRSA in bovine milk (2 out of 142 *S. aureus* isolates) in Switzerland. Similarly, prevalence rate is 16.7% in Germany and 0.4% in Hungary [16]. In general, the occurrence of MRSA among bovine mastitis isolates is well studied and its prevalence seems to be very low [27].

In 2005, a high prevalence of LA-MRSA, ST-398 was found in Dutch pigs in slaughterhouses. In other European countries this MRSA is the same ST-398 strain as found in Dutch pigs. Huber *et al.* [26] reported, presence of MRSA, particularly ST-398, in Swiss livestock in low numbers (2.9%) compared to the herd level prevalence of 81% in Dutch pigs. Among the cited porcine studies, the highest percentage of positive farms (Living animals, not slaughterhouse) was found in Belgium with 34 out of 50 fattening pig farms studied being positive, 68% [28]. A large multinational study conducted by the European Food Safety Authority (EFSA) found the prevalence of MRSA ST-398 in swine farms to be 25.5% but varied from 0% to 50.2% among European Union Member States [7].

USA: Bovine MRSA has also been reported in different states of USA. Zero prevalence of bovine MRSA has been reported from Virginia and North Carolina however prevalence rate of 0.6% in Michigan, 1.8% in Wisconsin and 4% in Minnesota has been reported [16]. Another study in the U. S. examined 299 animals from two swine production systems in Iowa and Illinois and 45% were found to carry MRSA. All isolates typed were ST-398 [7].

Asia: The first reports of MRSA in chicken meat occurred in Korea in 2003 and Japan in 2005, but it was not determined as livestock-associated, raising the hypothesis of meat contamination with human strains through handler [29]. Some of the Asian countries have also reported the occurrence of bovine MRSA, 47.6% prevalence, 6.3% in Korea, 13.1% in India and four MRSA isolates were obtained from 263 *S.aureus* collected from 260 dairy farms of Japan [16].

Africa: MRSA in cattle has also been reported in some of the African countries like Egypt [30] and Nigeria [31]. In Ethiopia, a MRSA prevalence of 60% was reported in a total of 78 *S. aureus* isolate from cows' milk [32]. In South Africa a MRSA prevalence of 6% was reported in cows' milk produced in two commercial farms [33].

Risk Factors for Colonization and Spread of La-Mrsa

Clinical Antibiotic Usage: A causal relationship between the use of antibacterial drugs and MRSA has been demonstrated in human medicine for different antimicrobial compounds, e.g. quinolones, glycopeptides and b-lactams, in a recent meta-analysis [34].

More generally, though, where MRSA has developed resistance to a particular antibiotic class then the use of that antibiotic is likely to promote the spread of MRSA because by definition these antibiotics do not kill MRSA, but on the other hand can kill many other bacteria with which MRSA would normally have to compete. An example of this, is the case of the fluoroquinolones which can favor MRSA colonization in two principal ways: the first one, is that they can be excreted in sweat, thus killing sensitive bacteria on the skin and allowing the MRSA to fill the vacated space and the second is, that they have an effect on the MRSA bacteria themselves, inducing them to produce greater quantities of proteins which enhance their ability to attach to their host. This increased adherence makes it easier for the bacteria to survive on skin and spread [11].

Usage of Antibiotics as Growth Promoters: Animal growth promoters (AGPs) may play a role in maximizing profit, by potentially shortening the time that an animal achieves market weight i.e. ready for slaughter, thereby potentially conferring an economic advantage with their use. The constant exposure to non-therapeutic low-dose antibiotics routinely given in feed or water exerts a selection pressure for the survival of antibiotic resistant pathogens and genes. MRSA in livestock developed as a direct result of the routine and widespread use of

antibiotics, including Animal Growth Promoters (AGPs) in livestock production. It is thought to have adapted to intensively raised pigs and acquired resistance genes due to the AGPs routinely fed to them [35].

Conditions of Intensive Farming: Whereas in hospitals efforts are made to minimize the spread of MRSA from person to person, through hand-washing, cleaning, or the use of isolation wards, the high density in which animals are kept on an intensive pig or poultry farm ensures that once MRSA appears it can quickly spread to a large number of animals. The Dutch microbiologist Jan Kluytmans has said: 'If it can spread so easily in hospitals where containing measures are taken, you can imagine the effect in a pig sty where there is complete body contact – that is an MRSA paradise!' [11]. In addition to the above factors animal trading appears to be an important factor for introduction of MRSA on MRSA-negative herd [36].

Disease Conditions in Animals: Animals can be colonized for prolonged periods without developing clinical signs. Infection with MRSA results in the same syndromes as *S. aureus*, which can cause a wide variety of suppurative infections. MRSA has been specifically isolated from various skins and wound infections including abscesses, dermatitis including severe pyoderma, postoperative wound infections, fistulas and intravenous catheter or surgical implant infections. MRSA has also been found in other conditions including pneumonia, rhinitis, sinusitis, otitis, bacteremia, septic arthritis, osteomyelitis, omphalo-phlebitis, metritis, mastitis (Including gangrenous mastitis) and urinary tract infections [10].

Transmission by direct contact is probably the main route for MRSA transmission between pigs. Additionally, some studies suggested MRSA transmission between pigs in slaughterhouses due to the high density of the animals during the housing in the abattoir. MRSA can also be transmitted from sows to their offspring, vertical perinatal transmission [36].

Humans have also been shown to be susceptible to colonization therefore, it is likely that persons in contact with pigs act as vectors, transmitting MRSA while handling the animals (Within-herd dynamics) or introducing MRSA in negative farms in the case of veterinarians (Between-herd dynamics). Poultry and cattle appear to carry MRSA, though with a lower prevalence compared to pigs residing on the farm and might therefore also play a role in the dissemination of MRSA on the farm.

Furthermore, Rodents are recognized for their role in transmission and persistence of MRSA on livestock farms [10, 36].

Antibiotic therapy should be based on susceptibility testing, however certain antimicrobials, such as Vancomycin and Tigecycline, are critically important for treating human illnesses caused by MRSA. In some cases, they may be the drugs of last resort. The use of these drugs in animals may place selection pressure on isolates that can infect humans. Thus, they are controversial for treating MRSA-infected animals and should be avoided if at all possible. Local treatment with antiseptic compounds such as Chlorhexidine or Povidone iodine may be helpful in some types of infections [10].

Zoonotic Importance of La-Mrsa:

Risk Groups and Mode of Transmissions: Persons in direct contact with MRSA-colonized livestock, such as farmers, veterinarians, workers at slaughterhouses and transporters of livestock are at high risk of becoming colonized with MRSA. In turn, they may become a source of transmission to animals and other humans. Subsequent contact with household members may transfer the bacteria [37]. Animal to human transmission occurs through direct contact, environmental contamination and through handling of infected animal's product [11].

Direct Contact: Equal to other HA-MRSA and CA-MRSA, the most obvious risk factor and route of transmission of LA-MRSA is direct contact with colonized patients, i.e. animals. As a result, those who have direct contact with farm animals which carry MRSA have the highest risk of acquiring farm animal MRSA [17].

Through Environmental Contamination: There are two main ways by which antibiotic-resistant bacteria from farm animals can escape into the environment: through manure and by being carried in the air. Since manure from farm animals gets spread on the land, there is real danger of MRSA being spread with it, contaminating the water supply and crops [11]. A recent American study suggests that people living near MRSA-positive intensive pig farm may also be exposed to high concentrations of MRSA in the air [17].

Through Contaminated Meat: A Dutch study proved relatively early that LA-MRSA could be present on pork. A very recent and much larger Dutch study confirmed this and showed in addition a very wide spread of LA-MRSA

on many different meat products. Despite this relatively high number of meat contaminated with LA-MRSA, so far there are no signs that this has contributed significantly to the dissemination of LA-MRSA to humans [4, 17].

Handling raw meat with bare hands potentially allows MRSA to bypass cooking; the phenomenon is well-attested with salmonella and other food-poisoning bacteria. However, in the case of MRSA it is additionally significant since MRSA lives very easily on skin and can be readily transferred by direct contact [11].

Clinical Signs, Diagnosis and Treatment in Humans:

Zoonotic MRSA can presumably cause the same types of infections as human-associated MRSA strains i.e. skin and soft tissue infections like boils, abscess and cellulitis and MRSA infections in humans are diagnosed by culture and identification of the organism, as in animals [10].

Antimicrobials such as Vancomycin, Linezolid and Teicoplanin are regarded as critically important antimicrobials (CIAs) for the treatment of MRSA infections in human medicine [38].

Prevention and Control Measures of Mrsa in Farm Animals: an Option for Controlling the Zoonosis

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, 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 [12].

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, 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, proper cleaning and disinfection of contaminated environments, including transport vehicles [38].

Control and/or treatment of colonized and infected animals with or without antimicrobials are necessary for the reduction of carriers. The affected animals need to be immediately separated. In extreme cases culling of infected animals is a further option. Milk of animals with mastitis by MRSA must be destroyed and the choice of antimicrobials should always be based on a susceptibility test [29].

CONCLUSION

Currently antibiotic resistant microorganisms pose a great problem both in human and veterinary medicine. Penicillin resistant *S. aureus* occurred before the clinical use of penicillin, but soon after their introduction, penicillin resistant *S. aureus* dominates. This was due to the enzyme beta lactamase produced by the bacteria. This problem leads to the introduction of a new beta lactamase resistant penicillin, methicillin. But this solution was immediately followed by a new problem i.e. emergence of MRSA. This problem was due to the fact that these antibiotics kill the sensitive bacteria, rendering the resistant one without natural competition leading to their dominance. The recently discovered MRSA strain, CC-398, has emerged in the livestock production chain pre-harvest, mainly in intensified production systems like fattening pigs, veal calves and broilers. Many factors seem to be involved behind its emergence in livestock. But antimicrobial consumption is considered to be the most driving force in the emergence and spread of LA-MRSA. Despite this, many countries are still using antibiotics as growth promoters in their farm. The animal's defense against MRSA also plays an important role in the spread of MRSA. This may be in part due to the fact that they produce high level of chemicals, cathelicidines which have antibacterial properties, this means that carrier animals will not be diagnosed and treated. This will inevitably increase the opportunities for MRSA to spread in the environment and on their meat, ultimately leading to greater danger to human health. Another important factor for spreading of MRSA is trading without screening. LA-MRSA is prevalent worldwide, but still sufficient research is not done in this area. Therefore:

- Antimicrobial therapy should be based on antimicrobial susceptibility test
- The use of antimicrobials as growth promoters should be considered from both resistance and public health perspective

- People in close contact with animals should take care during handling animals and their products
- Regular screening of livestock animals is recommended to detect and isolate MRSA positive animals and to take measure before its spread.
- Trading of live animals and their products should be from MRSA negative livestock
- There are many unsolved problems regarding the zoonotic aspects of LA-MRSA, indicating the necessity of further research.

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