



## METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN ANIMALS

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### ABSTRACT

In recent years, Methicillin-resistant *Staphylococcus aureus* (MRSA) has been increasingly reported as emerging problem in veterinary medicine, particularly in small animals. Strains isolated from pet animal cases were usually indistinguishable from those isolated from human. Pets become infected through contact with infected people, and those pets in turn pass MRSA back to humans indicating its zoonotic importance. MRSA are not only carried by pet animals but can also cause clinical disease in animals. MRSA have been found in number of animals including Dogs, Cats, Horses, Sheep, Pigs and Chickens. Methicillin was first introduced in human medicine in 1959, when it was used for treating penicillin-resistant staphylococcal infections. Methicillin is classified under narrow spectrum  $\beta$ -lactamase resistant penicillins. It acts by interfering primarily with the synthesis of bacterial cell wall by binding to penicillin binding proteins. Methicillin resistance is mediated by the *mecA* gene encoding for penicillin-binding protein 2a (PBP-2a). *mecA* gene is located on a mobile genetic element called the staphylococcal cassette chromosome (SCC*mec*). Expression of PBP-2a is controlled by *mecR1* and *mecI* regulator genes located on *mecA* gene. Mutations in the *mec* regulators may result in expression of PBP2a and the strain becomes highly resistant to Methicillin. MRSA typing systems are important for investigation of outbreaks to aid the clinical treatment of patient, discrimination between successive and recurrent infections and understanding epidemiology of the infections. Molecular typing methods are the most important which mainly includes, Chromosomal DNA analysis after REA, Pulsed field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), SCC*mec* typing and *spa* typing. Regarding public health significance, several reports are suggesting that animals may serve as reservoirs for MRSA infection to humans. Control of MRSA in animals includes, preventing introduction of infection at veterinary hospitals, preventing transmission from animal to human and human to animal by taking hand hygiene and related measures, preventing transmission from animal to animal by isolating all suspect cases and preventing indirect transmission following high standards of cleaning and disinfection.

**KEYWORDS:** MRSA, animals, zoonotic.

### 1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is emerging as a zoonotic and veterinary bacterial pathogen of public health importance. It causes nosocomial and community onset infections. MRSA is considered being resistant to virtually all available beta-lactam antibiotics which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin and cephalosporins). In last four decades, it has shown increasing endemic and epidemic spread (Kuehnert *et al.*, 2005). MRSA associated infections in human patients impose serious burden in terms of treatment costs and cause significant morbidity and mortality (Bratu *et al.*, 2005). MRSA patients stay in hospital longer with higher cost of treatment than do patients with non resistant staphylococcal infections (Cosgrove, 2006). MRSA infections accounted for an estimated 19,000 human deaths in U.S. in 2005, more than AIDS. From 1999 to 2005, Hospital treated MRSA infections have more than doubled i.e. from approximately 127,000 in 1999 to 278,000 in 2005 (Klein *et al.*, 2007).

#### Veterinary significance

In recent years, MRSA has been increasingly reported as emerging problem in veterinary medicine, particularly in small animal and equine practices. Strains isolated from pet animal cases usually indistinguishable from those

isolated from human. Pets become infected through contact with infected people, and those pets in turn pass MRSA back to humans. MRSA are not only carried by pet animals but can also cause clinical disease in animals. Since then, MRSA has been found in a variety of other domestic species including Dogs (Van Duijkeren *et al.*, 2004; Walther *et al.*, 2008), Cats (Bender *et al.*, 2005), cows (Moon *et al.*, 2007), Horses (Hartmann *et al.*, 1997; Van den Eede *et al.*, 2009), Sheep (Goni *et al.*, 2004), Pigs (Voss *et al.*, 2005; Meemken *et al.*, 2008), Guinea pig, Rabbit, Turtle, Bat, Parrot (Walther *et al.*, 2008) and Chickens (Lee, 2003; Kwon *et al.*, 2006).

### 2. EPIDEMIOLOGY OF MRSA INFECTION IN ANIMALS

Given the importance of *S. aureus* as cause of mastitis in cattle and the widespread usage of intramammary antibiotics in that species, perhaps it is not surprising that the first isolations of MRSA from animals were in milk from mastitic cows (Devriese *et al.*, 1972; Devriese and Homme, 1975). About 12% nasal carriage and 3% skin carriage of MRSA in 67 horses in an equine hospital (Baptiste *et al.*, 2005) was found. In Ireland, 13% isolation rate was observed in clinically affected dogs (Abbott *et al.*, 2006). In Germany, 7.5% and 10% isolation rates were

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found in clinically affected dogs and cats respectively (Walther *et al.*, 2008). 16% Pigs 16% positive on nasal swabs, 7.4% positive on both nasal and rectal swabs and 1.4% positive on rectal swabs only (Khanna *et al.*, 2008).

Twelve of 487 dogs (2.5%), six of 48 cats (12.5%), 5 of 12 horses (42%), and 1 of 2 pigs samples were detected positive for MRSA (Lin *et al.*, 2010). (Table 1)

**TABLE 1.** Summarized chronology of publications reporting MRSA infections in animals

Sr.no	Authors	Comments
1	Devriese <i>et al.</i> (1972)	Isolation of MRSA from cows with mastitis
2	Devriese and Hommeez, (1975)	Isolations of MRSA from dairy cows, suggested to be of human origin
3	Scott <i>et al.</i> (1988)	Cat suspected to be a source of MRSA outbreak in geriatric ward
4	Smith <i>et al.</i> (1989)	Isolation of coagulase-positive <i>Staphylococcus</i> sp. (12% of isolates were oxacillin resistant) from orthopedic implant sites in dogs
5	Cefai <i>et al.</i> (1994)	Repeated nasal carriage of MRSA in two nurses linked to pet dog
6	Anzai <i>et al.</i> (1996)	Isolation of MRSA from 13 mares with metritis and a stallion with a skin lesion
7	Hartmann <i>et al.</i> (1997).	MRSA isolated from leg wound of horse
8	Shimizu <i>et al.</i> (1997)	PGFE typing of equine MRSA isolates revealed pattern distinct from human isolates
9	Lilenbaum <i>et al.</i> (1998)	Isolation of MRSA from skin scrapings from clinically normal cats
10	Seguin <i>et al.</i> (1999)	MRSA outbreak in equine patients attending a veterinary hospital. Closely related MRSA isolates also obtained from hospital personnel
11	Pak <i>et al.</i> (1999)	Isolation of MRSA from 12 dogs hospitalized due to a variety of clinical conditions
12	Tomlin <i>et al.</i> (1999)	MRSA infection in 11 dogs associated with surgery, traumatic wounds or recurrent pyoderma
13	Gortel <i>et al.</i> (1999)	MRSA isolates from wounds and skin lesions in dogs
14	Lee (2003).	MRSA isolated from dairy cows (milk samples) and chickens (muscle or joint samples)
15	Manian (2003)	Identical PGFE type of MRSA isolated from pet dog of owners with recurrent MRSA infection
16	Goni <i>et al.</i> (2004).	MRSA isolated from case of ovine mastitis
17	Van Duijkeren <i>et al.</i> (2004)	MRSA isolated from two dogs with wounds
18	Rich and Roberts (2004)	MRSA isolates (n=95) reported from dogs (69), cats (24), a horse and a rabbit. Most isolates obtained from wound infections, postoperative infections or from the skin
19	Boag <i>et al.</i> (2004)	MRSA isolates (n=12) from dogs (7) and cats (5)
20	Voss <i>et al.</i> (2005)	Association between pig farming and high MRSA carriage rates
21	Bender <i>et al.</i> (2005)	Isolation of MRSA from non-healing abscess in a cat
22	O'Mahony <i>et al.</i> (2005)	MRSA isolated from dogs (14), horses (8), a cat, a rabbit and a seal. Isolates were also obtained from attendant veterinary personnel. Non-equine MRSA isolates had PGFE pattern indistinguishable from most prevalent MRSA strain in human population. Distinct PGFE pattern for equine MRSA isolates
23	Kwon <i>et al.</i> (2005)	SCC mec characterization of MRSA from bovine milk
24	Loeffler <i>et al.</i> (2005)	MRSA isolated from staff, dogs and environment of a small animal referral hospital
25	Middleton <i>et al.</i> (2005)	14% of patients (65) with <i>S. aureus</i> infection at 7 veterinary teaching hospitals infected with MRSA
26	Hanselmann <i>et al.</i> (2005)	1% nasal MRSA carriage rate in dogs in referral hospitals
27	Weese <i>et al.</i> (2005)	MRSA isolates from horses and horse personnel typed as SCCmecIV and distinct from epidemic MRSA types predominant in human population
28	Weese <i>et al.</i> (2005)	4.7% isolation rate of MRSA in screened horses rising to 12% using targeted surveillance
29	Baptiste <i>et al.</i> (2005)	MRSA isolates from dogs and staff at veterinary hospital identical by PFGE analysis to epidemic MRSA type prevalent in human population. Five distinct MRSA strains isolated from horses
30	Abbott <i>et al.</i> (2006)	0.6% MRSA carriage in dogs rising to 8% in dogs clinically assessed as suspect cases
31	Leonard and Markey (2006)	MRSA isolates from postoperative infection sites in five dogs and from nares of veterinary surgeon indistinguishable by PFGE.
32	Cuny <i>et al.</i> (2006)	Approx. 0.48% of equine cases presented at veterinary teaching hospital infected with MRSA. Long term nasal carriage of MRSA in two attending veterinarians
33	Strommenger <i>et al.</i> (2006)	MRSA isolates from dogs and cats closely resemble hospital-derived isolates in local human population
34	Weese <i>et al.</i> (2006)	Animal to human and human to animal transmission of MRSA suspected following

35	Malik <i>et al.</i> (2006)	<i>mecA</i> gene in MRSA isolates from dogs and cats shown to be identical to that found in human strains
36	Rich and Roberts 2006)	Culture of 561 isolates of MRSA from clinical infections in animals between January 2003 and August 2006. One of 255 healthy dogs sampled found to carry MRSA
37	Juhasz- Kaszanyitzky <i>et al.</i> (2007)	27/595 mastitis samples of cows positive for MRSA in Hungary
38	de Boer <i>et al.</i> (2008)	85% isolates of animal origin and 15% possibly human origin in raw meat of different animals at a retail shop
39	Pu <i>et al.</i> (2009)	5/90 of pork (5.5%) and 1/30 beef (3.3%) carry MRSA in retail shop (pork and beef)
40	Vanderhaeghen <i>et al.</i> (2010)	The <i>mecA</i> gene was detected in 11 (9.3%) of the 118 <i>S. aureus</i> isolates, indicating that nearly 10% of the Belgian farms suffering from <i>S. aureus</i> mastitis have an MRSA problem

### 3. IDENTIFICATION OF MRSA

*S. aureus* (including MRSA strains) are clusterforming, facultative aerobic, Gram-positive cocci with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media. They also ferment mannitol turning mannitol salt agar yellow. The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins used for their identification (Bannerman, 2004).

#### Disc diffusion test

Using Mueller Hinton agar (MHA) plates, MRSA strains exhibit resistance to oxacillin or methicillin (1 or 5 µg/disc: zone of inhibition < 14mm in diameter used as marker for all β-lactams).

#### Oxacillin MIC test

Gradient plates of MHA containing 2% NaCl with doubling dilutions from 0.25 µg/ml to 256 µg/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 µg/ml is indicative of susceptible and that of > 2 µg/ml resistant (NCCLS, 2003).

#### Detection of *mecA* gene by PCR

Using *mecA* gene specific primers, MRSA can be detected by PCR methods (Geha *et al.*, 1994).

### 4. MECHANISM OF ACTION AND RESISTANCE

#### Mechanism of action

Methicillin is classified under narrow spectrum beta – lactamase resistance penicillins. It acts by interfering primarily with the synthesis of bacterial cell wall and produce effect by binding to penicillin binding proteins (PBPs). PBPs essentially involved in the maintenance of normal cell morphology and viability of bacteria. PBPs are grouped in to six types according to their molecular weight. Drugs occupy the active site of transpeptidase enzyme (PBP) and inactivate it. Inactivation of transpeptidation in cell wall synthesis leads to blockage of cell wall synthesis (Walther *et al.*, 2008).

#### Mechanism of resistance

Mutation on *mecA* gene which results in modification to PBP - 2a, results in drugs not binding to target site and organism becomes resistant to β-lactams and other antibiotics with the same target site. MRSA uses efflux phenomenon resulting in continuous pumping of

antimicrobial drugs out of bacterial cell. Alteration in outer-membrane proteins which limit the access of drugs to cell (Stevens, 2004). PBP - mediated resistance in MRSA suggested to take various forms and may arise from overproduction of PBP, acquisition of a foreign PBP with low affinity, recombination of susceptible PBP with more resistant varieties, point mutations within PBPs that consequently lower their affinity for β lactams (Deurenberg *et al.*, 2007).

Methicillin resistance requires the presence of *mecA* gene. This *mecA* gene is chromosomally located on a mobile genetic element called the staphylococcal cassette chromosome (SCC). Further *mecA* gene encodes for penicillin-binding protein 2a (PBP-2a) and responsible for synthesis of penicillin-binding protein 2a (PBP2a; also called PBP2) a 78-kDa protein. Expression of PBP-2a is controlled by *mecR1* and *mecI* regulator genes located upstream of *mecA* gene. A mutation in the *mec* regulators leads to expression of *mecA* gene (PBP-2a). A PBP2a substitute for the other PBPs and, because of its low affinity for all β-lactam antibiotics, enables staphylococci to survive exposure to high concentrations of these agents (Figure 1). Thus, resistance to methicillin confers resistance to all β-lactam agents, including cephalosporins (Deurenberg *et al.*, 2007).

### 5. BASED ON SCC*mec* TYPE, MRSA CLASSIFIED IN FIVE GROUPS

The 2.1-kb *mecA* gene is located on SCC*mec*. Currently, five main types of SCC*mec* (types I–V) have been distinguished, ranging in size from 20.9 to 66.9 kb. SCC*mec* types I (34.3 kb), IV (20.9–24.3 kb) and V (28 kb) encode exclusively for resistance to β-lactam antibiotics. In contrast, SCC*mec* types II (53.0 kb) and III (66.9 kb) determine multi resistance, as these cassettes contain additional drug resistance genes on integrated plasmids (pUB110, pI258 and pT181) and a transposon (Tn554) (Deurenberg *et al.*, 2007).

Classification of MRSA strains based on SCC*mec* gives epidemiological features of strains. That is, possible sources of acquisition and dissemination (nosocomial versus community), nature of drug-resistance (single versus multiple), genome characteristics viz: size of genome. Type of ribosome found in individual strain type. Type IV MRSA strains originating from community (Ca-MRSA) found to possess higher prevalence of certain virulence factors as compared with non methicillin -

resistant *S. aureus* (NMRSA) and health care associated MRSA (HA - MRSA) (Akande, 2010). CA- MRSA believed to have inherent potential for severe disease than NMRSA, and broader antibiotic resistance than typical

HA - MRSA (Said - Salim *et al.*, 2003). MSSA became methicillin resistant through acquisition of the *SCCmec* element, probably from other strains, and this occurred on multiple occasions (Robinson and Enright, 2004).

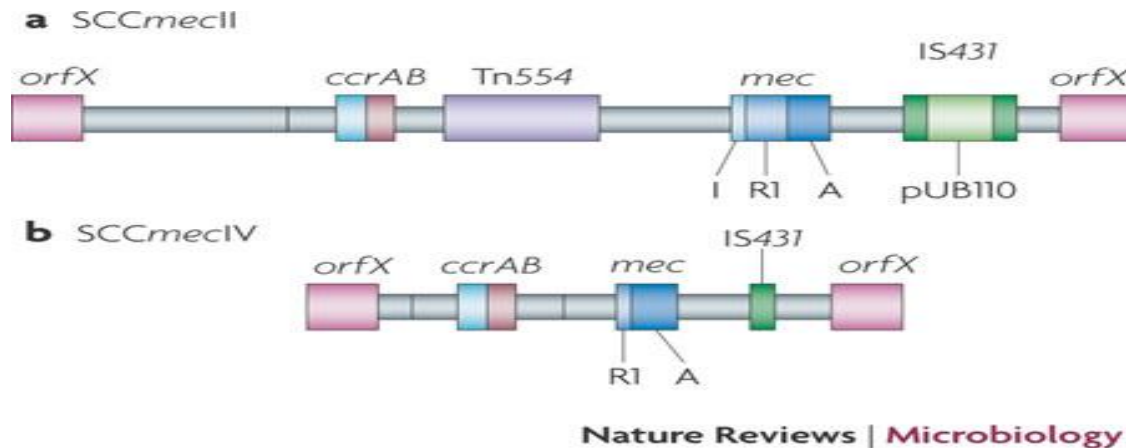


Figure 1 *mecA* gene located *SCCmec* which encodes for penicillin-binding protein 2a (PBP-2a) and allows *S. aureus* to be methicillin / oxacillin resistant. Expression of PBP-2a controlled by *mecRI* & *mecI* regulator genes located upstream of *mecA* gene. Mutations in the *mec* regulators may phenotypically be highly resistant to methicillin.

### TYPING OF MRSA STRAINS

MRSA typing systems are important for investigation of outbreaks of infection, aid the clinical treatment of patient, to discriminate between successive and recurrent infections, understanding epidemiology of infections. MRSA strains can be identified/typed using phenotypic and molecular methods.

#### Phenotypic typing methods

Using of colonial characteristics, biochemical reactions, antibiotic susceptibility pattern and susceptibility to various phages.

#### Molecular typing methods

Chromosomal DNA analysis after REA, Pulsed field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), *SCCmec* typing and *spa* typing.

#### a) Chromosomal DNA analysis after REA

Chromosomal DNA is subjected to digestion by restriction endonucleases and the resulting fragments are separated by conventional agarose gel electrophoresis. *Bgl*II and *Eco*RI used for MRSA, as they act on frequently encountered sites along the chromosome, producing short and multiple fragments (Jordens and Hall, 1988). After electrophoresis, set of profiles are compared to those of other isolates. All MRSA isolates are typeable by this method. Restriction profile consists of numerous overlapping fragments, which makes consistent analysis of results difficult (Weller, 2000).

#### b) Pulsed field gel electrophoresis (PFGE)

PFGE used in conjunction with restriction enzymes to provide a DNA fingerprint of the bacterial genome.

#### Advantage

Provides great discrimination between strains and useful in the investigation of outbreaks by allowing differentiation of unrelated strains.

#### Disadvantage

Difficulties with inter-laboratory comparison of results. Reliable comparison of strains between regions and internationally is not always possible.

#### c) MLST and *SCCmec* typing

Investigation for genetic relatedness of MRSA isolates in international epidemiological studies carried out using results of MLST and *SCCmec* typing.

#### MLST typing

MLST is an excellent tool for investigating the clonal evolution of MRSA. MLST is based on sequence analysis of 0.5-kb fragments from seven *S. aureus* housekeeping genes, i.e., *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL* (Maiden *et al.*, 1998). Sequences of the genes compared to known alleles via the MLST website (<http://www.mlst.net>). Every isolate can be described with seven integer profile. MLST scheme for *S. aureus* was developed in 2000 and details of more than 1500 isolates are available at the *S. aureus* MLST website <http://saureus.mlst.net> (Enright, 2006).

#### *SCCmec* typing

*mecA* gene is part of larger genetic element known as *SCCmec*. Element contains the *mecA* gene, chromosomal cassette recombinase gene, *mec* regulatory gene and junkyard region which contains non-essential components of *SCCmec*. Five *SCCmec* types currently described which have been characterized using PCR-based techniques. Each type is differentiated by the class of the *mec* gene and the type of recombinase genes (Ito *et al.*, 2001, 2004).

#### d) *spa* typing

Polymorphic DNA sequence analysis of the variation in X region of the protein A gene. This region consists of a number of mainly 24-bp repeats, with its diversity being

attributed mainly to deletions and duplications of the repeats and, more rarely, to point mutations. *spa* typing of limited number of animal isolates has been carried out. (Voss *et al.*, 2005; Moodley *et al.*, 2006; Strommenger *et al.*, 2006) *spa* typing differentiates strains that are indistinguishable using PFGE (Moodley *et al.*, 2006).

## 6. PUBLIC HEALTH SIGNIFICANCE

Several reports suggest that animals may serve as reservoirs for MRSA infection of humans. A cat was implicated as the source of MRSA for nurses in a geriatric nursing facility (Scott *et al.*, 1988). Dog was implicated as a reservoir for the reinfection of two nurses, after their treatment to eliminate carriage of MRSA (Cefai *et al.*, 1994).

First case of human to dog transmission was recorded in Netherlands. A nurse was identified as carrier, then treated and tested negative, few week later when she again colonized, family members, environment and pet dog were tested. Pet dog and her daughter were found positive with identical strain (Van Duijkeren *et al.*, 2004).

Recurrence of MRSA infection and nasal colonization in the MRSA infected patients was only halted following successful eradication of MRSA from their family dogs which acted as source of infection for them (Manian, 2003).

MRSA isolates from pets in the UK, Ireland and Germany were similar to human isolates. (Baptiste *et al.*, 2005; Loeffler *et al.*, 2005; O'Mahony *et al.*, 2005; Strommenger *et al.*, 2006) In USA, survey of MRSA carriage in small animal veterinary practitioners found similar strain detected in small animals (Hanselman *et al.*, 2006). Malik *et al.* (2006) compared MRSA human isolates and isolates from dogs and cats and suggested that transmission of MRSA occurs between humans and companion animals and vice versa. Epidemiological typing of equine isolates of MRSA and MRSA strains from equine-associated personnel shows that these isolates were distinct from MRSA strains which commonly cause infections in humans (Baptiste *et al.*, 2005; O'Mahony *et al.*, 2005; Weese *et al.*, 2005; Cuny *et al.*, 2006). Typing data on isolates from pigs and associated human infections and on isolates from cattle suggest MRSA from these species may represent different lineages from those present in humans (Voss *et al.*, 2005; Kwon *et al.*, 2005; Van Dijke *et al.*, 2006). The first case direct transmission of MRSA between cows to humans was documented in Hungary from milk samples (Juhasz-jaszanyitzky *et al.*, 2007).

## 7. CONTROL OF MRSA IN ANIMALS

Numerous documents on the control of MRSA in people have been published (e.g. CDC MRSA guidelines, available at [www.cdc.gov/ncidod/dhqp/ar\\_mrsa.html](http://www.cdc.gov/ncidod/dhqp/ar_mrsa.html)).

### Summary of control measures for MRSA infection and colonization in animals

#### I) Prevent introduction of infection

Screen all incoming cases for infection and/or nasal carriage at admission to veterinary hospital and isolate until negative status established. It may be possible in referral practices but only screening of suspect cases is practical in first opinion clinics.

#### II) Prevent transmission from animal-to-human and human-to-animal

Hand hygiene and related measures like Correct hand washing, Use alcohol-based hand sanitizers, Cover wounds and skin lesions, Use gloves, masks, eye protection, disposable aprons for contact with wounds, body fluids or other contaminated materials, Strict asepsis during surgery and Screening staff for MRSA colonization. Based on findings in human medicine, human hands are highly significant for MRSA transmission.

#### III) Prevent transmission from animal to animal

Isolation of all suspect cases of MRSA infection, No entry to waiting room and Hospitalize in isolation area as far as possible.

#### IV) Prevent indirect transmission

High standards of cleaning of Hand touch surfaces including doors, pens, stethoscopes, keyboards and High standards of disinfection of consulting rooms/isolation kennels/stables areas following boarding of known animals. In indirect transmission, environmental transmission may be of greater importance than in human medicine.

## 8. CONCLUSIONS

MRSA first emerged as serious pathogen in human medicine during the late 1970s and has been increasingly reported in animals during the past 10 years. MRSA can be identified using phenotypic (antimicrobial susceptibility testing) or genotypic methods. Epidemiological evidence, including phenotypic and molecular typing data, suggests that MRSA isolates from dogs and cats are indistinguishable from human isolates. Strains of MRSA isolated from horses and associated personnel are found different so far. Transfer of MRSA strains can occur between animals and humans and vice versa. Guidelines for the control of MRSA in animals have been drawn up by individual institutions based on those available for human MRSA infection. Risk factors for MRSA infection in animals are currently under investigation and such data are essential for the preparation of specific guidelines for control of MRSA in veterinary practice.

## 9. FUTURE PROSPECTS

There is a need for adequate policy framework on infection control that will reflect the current realities on the epidemiologic characters of MRSA. Strict implementation of control program to checkmate the spread of MRSA infections is very much necessary. Epidemiological studies on the spread of MRSA infections at the local, national and international levels will help in designing strategies for preventing the spread.

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