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Mini Review

METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN CATTLE: EPIDEMIOLOGY AND ZOONOTIC IMPLICATIONS

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant public health problem both in human and veterinary medicine. Strains of *S. aureus* resistant to β-lactam antibiotics are known as Methicillin-resistant *Staphylococcus aureus* (MRSA). Overuse of antibiotics has been ascribed for MRSA emergence. MRSA in cattle was first reported in 1972. Since then, many literatures describing MRSA in cattle have been published. MRSA causes incurable intra-mammary infection and skin diseases in cattle. In severe cases, it causes deep-seated infections like endocarditis and osteomyelitis. MRSA got zoonotic importance when scientists suggested the possibility of cattle serving as reservoirs for human MRSA infection. In this article, we review the current knowledge of MRSA in cattle and its zoonotic implications.

Key words: Cefoxitin; Cattle; MRSA; *mec*A gene; Zoonosis

Introduction

Antibiotics are used both for therapeutic and sub-therapeutic purpose in veterinary medicine. In addition to treatment, antibiotics are used for sub-therapeutic purpose in veterinary medicine to enhance feed efficiency and promote growth of animals. There is currently increased public and scientific concern regarding extensive use of antimicrobials for therapeutic purpose or as growth promoters in food animals, due to the emergence and dissemination of multiple antibiotic resistant zoonotic bacterial pathogens (Hardy, 2002; Normanno *et al*., 2007). Such antibiotic resistant bacteria do not respond to regular antibiotic treatments and prolong the duration of illness.

The emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious public health threat. Strains of *S. aureus* resistant to β-lactam antibiotics are known as Methicillin-resistant *Staphylococcus aureus* (MRSA) (Kumar *et al*., 2011). First described as a cause of nosocomial infection in hospital settings, now MRSA has gained attention as community pathogen (Said-Salim *et al.*, 2003). In recent years, Methicillin-resistant *Staphylococcus aureus* (MRSA) has been increasingly reported as emerging problem in veterinary medicine. MRSA has been isolated from cattle, dogs, cats, pigs, horses and poultry worldwide (Leonard and Markey, 2008).

Staphylococcus aureus in Cattle

Staphylococci are gram positive, non-motile and non spore forming bacteria. Pathogenic staphylococci are identified by their ability to produce coagulase thus clot the blood (Harris *et al.*, 2002). *Staphylococcus aureus* is one of the most extensively studied bacteria of genus Staphylococci. *S. aureus* is both commensal and pathogen. It is found as a commensal associated with skin, skin glands and mucous membranes. *S. aureus* affects skin, soft tissues, bloodstream and lower respiratory tract. It also causes severe deep-seated infections like endocarditis and osteolmyelitis (Schito, 2006).

S. aureus has been reported as most commonly isolated highly contagious pathogen recovered from bovine raw milk and infected mammary glands (Tenhagen *et al*., 2006; Haveri, 2008).Traumatized sites such as abrasions on teats, legs and navel, typically infected by *S. aureus*, are regarded as secondary sources of *S. aureus* causing mastitis. The role of *S. aureus* in causing mastitis in dairy animals has been substantiated by many authors. Sudhan *et al.* (2005) studied 352 milk samples from cattle for the isolation of pathogens associated with bovine sub-clinical mastitis (SCM) in India and found 56.89% sub-clinical mastitis caused by *S. aureus.* A study by Hameed *et al.* (2008) in Pakistan for investigating microorganism associated with mastitis in cattle revealed *S. aureus* to be the major cause of mastitis (50%) followed by *Streptococcus agalactiae* (28%) and *E.coli* (16%). Rana (2009) also reported about 51% cases of

sub-clinical mastitis in Pokhra, Nepal were caused by *S. aureus.*

Emergence of Methicillin Resistant *Staphylococcus. aureus* (MRSA)

Soon after the introduction of penicillin, around 1945, the majority of the *S. aureus* population had become resistant to penicillin through the production of beta-lactamase, an enzyme that hydrolyzes penicillin. In the late 1950s, the beta-lactamase resistant methicillin was introduced in human medicine. However, soon after its introduction, methicillin-resistant isolates of *S. aureus* were reported (Robinson and Enright, 2003). Methicillin resistance is caused by the acquisition of the *mec*A gene. This gene encodes an alternative penicillin-binding protein, called PBP2A, which has a low affinity for beta-lactam antibiotics (Vanderhaeghen *et al*., 2010). The *mec*A gene is part of a large mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). MRSA are often multidrug resistant. These microorganisms have been reported to resist most of the commonly used antibiotics like aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones (Lee, 2003).

Identification of MRSA

MRSA can be detected by either conventional culture method or molecular (PCR) method. Both of 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 -

1) ***Disc diffusion test:*** Disc diffusion test is employed by incubating *S. aureus* on Muller Hilton agar (MHA) impregnated with Oxacillin or Methicillin (1 or 5µg) and Cefoxitin (30µg) discs. MRSA is identified by assessing zone of inhibitions with oxacillin ≤ 14 mm and/or cefoxitin ≤ 21 mm (CLSI, 2007). 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 (Broekema *et al*., 2009; Rao *et al.*, 2011). Thus, Cefoxitin disc duffusion test can be alternative to PCR for the detection of MRSA in resource constraint settings.
2) ***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 (CLSI, 2007).
3) ***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.
4) ***PCR:*** Polymerase chain reaction (PCR) is used for detection of *mecA* gene of *S. aureus*. This can be done by using *mecA* gene specific primers. (Bhanderi, 2011). But, use of PCR method is limited only to sophisticated laboratories. Garcia-Alvaraz *et al.* (2011) found isolates resistant to penicillin but negative for *mecA* gene which has led scientists think about possible mechanism rendering *S. aureus* resistant to beta lactamse other than presence of *mecA* gene.

Prevalence of MRSA in Cattle

First isolation of bovine MRSA was done by Devriese *et al.* in 1972 from Belgium. Devriese and Homez (1975) reported 68 MRSA isolates from 20 Belgian herds and suggested those MRSA isolates to be of human origin. Recently, bovine MRSA has been reported in many European countries with varying rate of prevalence. Huber *et al.* (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 (Spohr *et al*., 2011) and 0.4% in Hungary (Juhasz-Kaszanyitzky, 2007). In a recent study by Paterson *et al.* (2012) 7 MRSA isolates were found out of 1500 bulk milk tank samples in UK. Bovine MRSA has also been reported in different states of USA. Zero prevalence of bovine MRSA has been reported from Virginia and North Carolina (Anderson *et al*., 2006); however prevalence rate of 0.6% in Michigan (Erskine *et al.*, 2002), 1.8% in Wisconsin (Makovec and Ruegg, 2003) and 4% in Minnesota (Haran *et al*., 2012) has been reported. Some of the Asian countries have also reported the occurance of bovine MRSA. Pu *et al.* (2014) reported 47.6% prevalence in China. Similarly, prevalence rate reported is 6.3% in Korea (Lim *et al*., 2013), 13.1% in India (Kumar *et al*., 2011). Four MRSA isolates were obtained from 263 *S. aureus* collected from 260 dairy farms of Japan (Hata *et al*., 2010). MRSA in cattle has also been reported in some of the African countries like Egypt (El-Jakee *et al*., 2011) and Nigeria (Suleiman *et al*, 2012).

MRSA has been isolated from nasal swabs of cattle and calves. Spohr *et al.* (2011) found MRSA in 5 out of 7 cows and in 4 out of 7 calves from nasal swabs in Germany. Huber *et al.* (2010) reported 3 MRSA isolates from nasal

swabs of 300 calves. Graveland *et al.* (2008) reported the colonization of MRSA in veal calves in Netherland. Initially, LA-MRSA CC938 (Livestock Associated- MRSA clonal complex 938) was considered only strain responsible for animal infection. But, García-Álvarez *et al.* (2011) discovered a divergent MRSA named as *mec*A_{LGA251} with a prevalence of 2.8% in UK. Panton-Valentine leukocidin (PVL) is a cytotoxin that is associated with the increased virulence of *S. aureus*. PVL- positive MRSA in cattle has been reported from Korea (Kown, 2005).

Zoonotic Implications of Bovine MRSA

Livestock Associated Methicillin-resistant *S. aureus* (LA-MRSA) belonging to the clonal complex 398 (LA-MRSA CC 398) is considered to be zoonotically important because of its capacity to colonize a wide range of hosts (Paterson *et al*., 2012). Bovine and human MRSA strains indistinguishable by phenotyping and genotyping methods have been found providing evidence for MRSA transmission between human and cattle (Hata *et al*., 2010; Juhasz-Kaszanyitzky, 2007; Lee, 2003). MRSA infected cattle acts as a reservoir and later transmit the infections to other animals and humans (AVMA, 2014; Spoor *et al*., 2013). MRSA colonization in cattle may be an occupational risk to the people in close contact with MRSA infected cattle *viz.* veterinarians, farmers, milkers and people working at slaughterhouses (Paterson *et al*., 2012; Juhasz-Kaszanyitzky, 2007). Transmission of animal MRSA to veterinary personnel has been found and it is more common for large animal personnel than small animal personnel (Wulf *et al*., 2008; Hanselman *et al*., 2006; O'Mahony, 2005). A study reported MRSA colonization in 32% of people with veal calf contact in the Netherlands (Graveland *et al*., 2008) and in 32% of hospitalized people who had contact with pigs and veal calves (van Rijen *et al*., 2008).

Although, MRSA has been reported as transmissible diseases of zoonotic as well as humanotic importance, the direction and routes of transmission are superficially understood. Some authors have reported bidirectional transmission of MRSA (AVMA, 2014; Price *et al*., 2012; Juhasz-Kaszanyitzky, 2007) .Animal to human transmission occurs through direct contact, environmental contamination and through handling of infected animal's product (Nunang and Young, 2007) whereas human to animal transmission is still unclear (Weese, 2010).

Prevention and Control of MRSA

All the *S. aureus* infections should also be suspected for MRSA infection both in animals and humans. MRSA isolates are resistant to beta-lactam antibiotics like penicillin, cloxacillin, and amoxicillin and are often multidrug resistant (Islam *et al.*, 2008; Lee, 2003). Bovine MRSA resistant to tetracycline has tetracycline resistant gene *tet*(M) (Paterson *et al.*, 2012). MRSA isolates both from human and animals are susceptible to vancomycin. Similarly, amikacin, linezolid, teicoplanin are also sensitive against MRSA (Oberoi *et al*., 2011; Islam *et al* ., 2008; Lee, 2003).

Modern dairy production system characterized by intensive farming, densely populated herds and high antibiotic use may bolster the emergence of MRSA in cattle in future. To prevent animal to human transmission, isolation of animal until the animal is no longer colonized is likely to be effective method to limit cross species transmission. Decolonization of animal is possible but it is effective only when re-infection of MRSA is prevented (Morgan, 2008; Weese and Rousseau, 2005). Surveillance for early identification of novel antibiotic resistant clones of *S. aureus* is recommended (Paterson *et al*., 2012). Improved biosecurity and hygiene control at farm, home, human and animal health care settings are important to prevent the spread of these pathogens.

Conclusion

This paper recapitulates a wide range of information on MRSA in cattle. Cross species spilling of MRSA has rendered it as one of the important zoonotic bacteria. New research to address many poorly understood or unknown questions are required for comprehensive understanding of its zoonotic potential. Some pertinent questions like evolution and dissemination, virulence factors, transmission routes and identifying molecular markers to differentiate human and livestock MRSA need to be addressed. Finally, MRSA has augmented the role of veterinarians in safeguarding public health mainly by rational use of antimicrobials and preventing MRSA dispersal by employing veterinary public health principles.

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