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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; mecA 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 osteomyelitis (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 mastitis in cattle caused by S. aureus.
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 mecA 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 mecA gene is part of a large mobile genetic element called Staphylococcal Cassette Chromosome mec (SCCmec). 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 diffusion 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 lactamase 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 occurrence 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 mecA LoCA251 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-Kaszanyitzyk, 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-Kaszanyitzyk, 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-Kaszanyitzyk, 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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