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Livestock-associated Methicillin-resistant *Staphylococcus aureus*: A Great Threat to Public Health

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ABSTRACT

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) globally is a public health/antimicrobial resistance issue both in humans and animals. *Staphylococcus aureus* is mainly a human pathogen its animal strains originated from humans by changing in genetic variations and host specificity. It is an important cause of human infections and a highly contagious pathogen in dairy animals. MRSA became prominent almost 50 years ago as a causative agent of nosocomial infections, meanwhile, it also became a causative agent for community infections and mastitis in dairy animals. Literature review shows the importance, reports, and prevalence of livestock-associated MRSA (LA-MRSA) from mastitis milk of cattle and goat. MRSA usually multiplies and colonizes in animals, particularly in livestock. Nowadays, LA-MRSA is an emerging issue posing to great zoonotic threat to public health and increasing treatment costs in animals. Goat milk is mainly used by poor rural families and by infants who cannot feed their mother milk. Consumption of milk having MRSA will be transmitted to humans and young ones that will become resistant to all beta-lactam antibiotics leading to public health problems. Mastitis in dairy animals caused by MRSA should be properly diagnosed and treated so that the transmission of this zoonotic pathogen can be avoided.

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an important pathogen in humans and animals that can colonize various body tissues (Graveland et al. 2011). It causes a wide range of diseases starting from mild skin infections to serious problems like toxic shock syndrome, endocarditis, and pneumonia; and some strains produce enterotoxins responsible for food poisoning (Fitzgerald 2012; Ferreira et al. 2012 Lowy et al. 1998). Almost 20% of healthy humans are carriers of *S. aureus*; in humans, it mainly causes nosocomial infection (Graveland et al. 2011; Lowy 1998, Kluytmans and Struelens 2009). In animals, Staphylococcal infections may be caused either by *S. aureus*, *S. hyicus*, *S. intermedius* and *S. delphini*. (Vanderhaeghen et al. 2010b; Graveland et al. 2011). In dairy animals mastitis is an important reason for the usage of antibiotics and one of the important causes of economic losses (De Oliveira et al. 2000). It is the major pathogen of mastitis among all causative agents, contagious and economically significant cause. The antibiotic resistance of *S. aureus* especially to methicillin or β- lactam antibiotics is one of the important concerns for its treatment (Ferreira et al. 2012)

Methicillin-resistant *Staphylococcus aureus* (MRSA)

S. aureus has the ability that it can develop resistance against different antibiotics. The *S. aureus* resistance particularly against methicillin denotes resistance against all antibiotics having beta-lactam ring in their structure as first reported in 1961 (Pantosti, 2012) *S. aureus* has developed resistance by the production of beta-lactamase soon after the discovery of penicillin around 1945. In the 1950s, methicillin which was resistant to beta-lactamase was introduced in human medicine. The development of methicillin resistance in *S. aureus* is due to attaining a particular gene namely mecA, this gene ultimately

Table 1: Prevalence of MRSA in various animal species.

Country	Year of study	Diagnostic method	Cattle		Goat		Bulk tank milk (BTM)		Reference
			%	n	%	n	%	n	
Jordan	2018	PCR targeting mecA and mecC genes Disc diffusion test	-	-	-	-	31.8%	117	Obaidat et al. 2018
Greece	2018	Disc diffusion test PCR targeting mecA gene Spa-typing PFGE	-	-	-	-	13.3%	36	Papadopoulos et al. 2018
Pakistan	2017	Disc diffusion test PCR targeting mecA gene	34	900	-	-			Aqib et al. 2017
Egypt	2017	Disc diffusion test	-	-	100%	86			Ali et al. 2017
Italy	2017	Disc diffusion test PCR MLST typing Spa-typing SCCmec-typing	-	-	-	-	8.3%	40	Basanisi et al. 2017
India	2017	Disc diffusion test PCR targeting mecA gene.	16.47%	85	-	-	-	-	Shrivastava et al. 2017
Indonesia	2016	Disc diffusion test PCR for the amplification of 23S rRNA gene	12.82%	39	4.17%	24			Widianingrum et al. 2016
Italy	2016	Disc diffusion test PCR targeting mecA MLST and SCCmec typing	-	-	1.23%	162			Caruso et al. 2016
Italy	2016	Disc diffusion test mecA PCR MLST analysis Spa-typing	-	-	-	-	2.5	486	Parisi et al. 2016
Italy	2015	Disc diffusion test PCR Spa typing	9.2%	163					Luini et al. 2015
Italy	2015	Disc diffusion test mecA specific PCR MLST typing	-	-	2%	197			Cortimiglia et al. 2015
Netherlands	2014	Disc diffusion test PCR targeting mecA and mecC spa typing and MLVA typing	3.9%	411	-	-			Duijkeren et al. 2014
Indonesia	2014	Disc diffusion test PCR for detection of mecA	-	-	14.2%	7	-	-	Suwito et al. 2014
Belgium	2013	Pulsed field gel electrophoresis (PFGE) PCR-based typing techniques (MLST, spa, SCCmec, and agr typing)	4.41	430	-	-	-	-	Bardiau et al. 2013
Turkey	2012	Disc diffusion test mecA PCR	-	-	4.8%	42			Aras et al. 2012
Spain	2012	spa typing, multi-locus sequence typing (MLST) and antimicrobial susceptibility.	-	-	20%	267			Porrero et al. 2012
Iraq	2012	Disc diffusion test	39.1%	400	-	-	-	-	Abdulkadhim, 2012
Serbia	2012	Disc diffusion test PCR for mecA gene.	1.41%	212	-	-	-	-	Zutic et al. 2012
Czech and Slovak Republic	2011	Disc diffusion test mecA PCR	51%	634	-	-	-	-	Vyletelova et al. 2011
India	2011	Disc diffusion test PCR for 16S rDNA, nuc, and	13.1%	107	-	-	-	-	Kumar et al. 2011

		mecA genes							
Belgium	2010	Disc diffusion test spa-typing MLST and SCCmec-typing	9.3%	118	-	-			Vanderhaeghen et al. 2010
France	2010	Triplex PCR targeting 16SrRNA, mecA, and *S. aureus*-specifc nuc genes antibiotic susceptibility by Disc diffusion test	0.72%	139	-	-	-	-	Haenni et al. 2010
Czech Republic	2009	Specie specific PCR Disc diffusion test mecA PCR	-	-	2.87%	278			Stastkova et al. 2009
Netherlands	2008	Disc diffusion test PCR for mecA gene Spa-typing	28%	2151	-	-	-	-	Graveland et al. 2008
Switzerland	2007	Disc diffusion test PCR Expression of mecA on DNA microarray	1.56%	128	-	-	-	-	Monecke et al. 2007
Turkey	2006	Disc diffusion test	17.5%	103	-	-	-	-	Turutoglu et al. 2006
Korea	2005	Disc diffusion test Spa typing SCCmec-typing	0.18%	9055	-	-	-	-	Kwon et al. 2005
Korea	2003	Disc diffusion test	1.34%	894	-	-	-	-	Lee et al. 2003

synthesize proteins that are specific penicillin binding proteins that do not affect all those antibiotics that come under the category of beta-lactams (Vanderhaeghen et al. 2010b; Graveland et al. 2011). This mecA gene is usually present in chromosomal cassette mec(SCCmec) (Grundmann et al. 2006). During the 1970s infections of MRSA mainly were confined to the vicinity and surroundings of the hospital i.e. HA-MRSA (Wendlandt et al. 2013; Parisi et al. 2016). Later on, MRSA transmission occurred in the community and was named CA-MRSA that have no obvious determinants for associations of MRSA infections (Doyle et al. 2012; Parisi et al. 2016). Presently new isolates of MRSA have been recovered from livestock that are recognized as LA-MRSA (Witte et al. 2007; kock et al. 2013; Parisi et al. 2016) (Table 1).

The transmission of MRSA may be done in humans who are handling or using MRSA-infected animals (EFSA, 2009; Feingold et al. 2012). *S. aureus* is the primary cause of clinical and subclinical mastitis in goats; usually its prevalence in goat clinical and subclinical mastitis is about 5.6% to 37% worldwide (Aras et al. 2012; Deinhofer and Pernthaner, 1995). The presence of MRSA in goat milk is of great concern as the goats are kept by poor rural families and their milk is consumed by young ones who cannot feed their mother milk (Haenlein, 2004). The goats that are infected by MRSA isolate of *S. aureus* will be transmitted to humans ultimately those human users will become resistant to all those antibiotics that come under the category of beta-lactams, this is a great threat to public health.

Risk Factors and Transmission of MRSA

Proper prevention of MRSA needs the determination of risk factors that increase the probability of its transmission and carriage. This information is important for establishing proper biosecurity measures and policymaking (Ferreira et al. 2012, Hanselman et al. 2006). In this respect, the colonization of MRSA is important in the personnel that are in contact with infected ones. A study was conducted in the Netherlands by Van Loo et al. 2007, who concluded that the risk of MRSA was 20 times higher in those who have contact with cattle.

New evidence supports that livestock *S. aureus* isolates can colonize humans (Garcia-Alvarez et al. 2011). While humans are an important source of new isolates that affect animals (Guinane et al. 2010; Price et al. 2012). It is usually estimated that more than 60% of human emerging pathogens come from animals (Cutler et al. 2010; Pantosti, 2012). It has been found that the important vehicle of MRSA transmission in humans is the consumption/handling of infected animals (EFSA, 2009; Feingold et al. 2012; Parisi et al. 2016). Livestock-associated *S. aureus* and its ability to cause zoonotic diseases in humans has attained attention toward its investigation and treatment (Fitzgerald, 2012). MRSA transmission in animals is mainly from human origin and the infected animals. One study (Devriese, 1975) concluded that almost 68 MRSA isolates of human origin in dairy herds in Belgium. Lee 2003 suggested a high prevalence of MRSA (above 50%) in *S. aureus* isolates as compared to animal isolates in Korea. Bovine mastitis milk being a source of MRSA in humans has been reported by various studies (Turkyilmaz et al. 2010; Aqib et al. 2017, Obaidat et al. 2018). Similarly, caprine milk is also used by poor rural families and by infants who cannot feed their mother milk. Caprine mastitis milk is the emerging issue of MRSA in humans (Ali et al. 2017; Widianingrum et al. 2016; Caruso et al. 2016; Aras et al. 2012). Studies have concluded that the source and origin of LA-MRSA is human (Ferreira et al. 2012).

Livestock-associated MRSA from Goat

The presence of MRSA in goat mastitis milk was confirmed by Aras et al. (2012). As the MRSA is usually

isolated from humans but recently recovered in various animal species (Duijkerenet et al. 2004; Stastkova et al. 2009, Turutoglu et al. 2009, Van den Eede et al. 2009). MRSA strains were isolated from both goat's milk and their handlers in the current study. They collected 42 *S. aureus* strains from goat mastitis during 2008-2009. They identified two (4.8%) strains of MRSA from these 42 *S. aureus* stains by disc diffusion test and further confirmation through PCR for the presence of the mecA gene. Ali et al. (2017) studied the incidence of MRSA in sheep and goat. They investigated 95 sheep and 86 goats that were pneumonic or having enteritis by microbiological and antibacterial susceptibility tests. They isolated *S. aureus* from nasal and fecal swabs. Furthermore, they investigated MRSA by disc diffusion of various antibiotics and concluded that all nasal and fecal swabs from goats were 100% resistant to ampicillin, amoxicillin, and penicillin, while *S. aureus* recovered from sheep were 50% resistant to methicillin and oxacillin. All *S. aureus* were sensitive to ciprofloxacin and gentamycin (Caruso et al. 2016) assessed the prevalence of MRSA in bulk tank milk of sheep and goat and also from the nasal swabs of the workers. From bulk tank milk samples two MRSA stains were recovered from out of 162 samples (1.23%) while in three nasal swabs of workers at one farm. MRSA from the bulk tank and personnel was evaluated and was found to be identical with the genetic profile of spa type t1255, ST398, SCCmec V. Presence of MRSA in goat breeding farms was evaluated (Stastkova et al. 2009). They collected 278 samples of milk, environment, and farm personnel. The isolates of *S. aureus* were identified through PCR. All identified isolates were tested with oxacillin and other antibiotics and confirmed for mecA through PCR. They confirmed eight MRSA isolates, five from goat milk and three from the carrier farm personnel. The presence of MRSA in goat bulk tank milk samples in Northern Italy was confirmed (Cortimiglia et al. 2015). They demonstrated that the MRSA strains that were isolated from bulk tank milk samples were similar to those isolated from three goats. The genotypes of these isolates showed that belong to livestock-associated MRSA. The pattern of antibiotic resistance and genes leading to the development of resistance were demonstrated in Indonesia (Widianingrum et al. 2016). The resistance level of *S. aureus* that was observed was higher in bovines and humans as compared to goats.

LA-MRSA from Cattle

In cattle, MRSA is also one of the important causes of mastitis (Fessleret al. 2010; Haran et al. 2012; Parisi et al. 2016). In the case of subclinical mastitis MRSA may be shed in milk without showing any physical or sensorial changes and ultimately transmitted through the dairy chain. *S. aureus* is an important pathogen of mastitis in dairy cattle leading to economic losses in the dairy industry (Pantosti, 2012; Piepers et al. 2007; Tenhagen et al. 2006; Vanderhaeghen et al. 2010) Development of resistance against antibiotics that can interfere treatment of its infections (Lowy, 2003; Vanderhaeghen et al. 2010). β-lactam antibiotics are frequently used for the treatment of mastitis (Sawant et al. 2005). Resistance against methicillin will show resistance against all β-lactams (Vanderhaeghen et al. 2010). *S. aureus* isolate that was resistant to methicillin from livestock (LA-MRSA) was first time isolated from dairy cattle almost 40 years ago (Devriese et al. 1972), but the particular allele of mecA gene responsible for resistance has been investigated recently in bovines (Garcia-Alvarez et al. 2011). Now the *S. aureus* isolates of the same genetics have been identified in humans in Germany, Denmark, and the UK, suggesting the possibility that cows may act as reservoirs of human MRSA infections (Garcia-Alvarez et al. 2011). Proper diagnosis and identification are needed for proper control and prevention (Fitzgerald, 2012). MRSA has been usually described commonly (Lee, 2003; Kwon et al. 2005; Juha sz-Kaszanyitzky et al. 2007; Moon et al. 2007; Hendriksen et al. 2008; Vanderhaeghen et al. 2010; Duijkeren et al. 2014; Luini et al. 2015; Aqib et al. 2018)

Conclusions

MRSA isolates with mecA genes have been recovered from livestock particularly from mastitis milk of cattle and goat, with zoonotic importance in humans leading to the development of antibiotic resistance. The presence of MRSA in livestock is associated with its transfer to humans, which has an impact on antibiotics usage in animals. Prevention of MRSA spread needs proper actions for veterinary infection control. The research work and phylogenetic studies of *Staphylococci* showed that animal strains are evolved from human isolates and these days there is a risk that these can be transmitted back to humans. Various Staphylococcal species in animals and the environment provide a large reservoir of MRSA and resistance genes. It is, therefore, emphasized that food animals should be properly monitored to prevent its spread in both populations.

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