Incidence of resistant mastitis in dairy cows in Tamil Nadu, India

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Abstract: The incidence of resistant mastitis in dairy cows in Tamil Nadu, India was 56.I %. The predominant resistant causative pathogen was *Escherichia coli* (50.64 %) followed by *Staphylococcus aureus* (44.25 %) and Methicillin resistant *Staphylococcus aureus* (5.11 %). Incidence of resistant mastitis was high in Holstein Friesian cross breed followed by Jersey cross breed and non descript. Highest incidence was observed in early stage of third lactation. *In vitro* antibiotic sensitivity test revealed the *E. coli, S. aureus* and MRSA organisms showed more sensitivity to enrofloxacin, amoxicillin +sulbactam, gentamicin and ceftriaxone and had highest resistant to penicillin followed by amoxicillin, oxytetracycline and methicillin. The study highlights the need for preventing the indiscriminate use of antibiotics.

Keywords: *Escherichia coli*, Methicillin resistant *Staphylococcus aureus*

INTRODUCTION

Mastitis is considered as most important disease affecting the productive performance of cattle world-wide contributing to the economic losses (Kumar *et al.,* 2010). Due to the involvement of multiple aetiological agents it always remains as a challenge to veterinarians all over the globe. *Staphylococcus aureus* and *Escherichia coli* are the most frequent causes of contagious and environmental clinical mastitis in dairy cattle, respectively (Olde Riekerink *et al.,* 2008). Antimicrobial therapy is commonly implemented for mastitis prevention and control. Unfortunately, despite the best possible antimicrobial treatments, failures of bacteriological cure are common, especially for *S. aureus* mastitis and antimicrobial resistance (AMR) which is considered one of the reasons for low cure rates (Barkema *et al.,* 2006). Additionally, AMR in bacteria is a public health hazard, and antimicrobial use is considered a potentially important driver of AMR. Acquired antimicrobial resistance in bacteria is an increasing threat in human as well as in veterinary medicine. The emergence of antibiotic-resistance in *S. aureus* mastitic dairy animals has been shown in recent years. Strains of *S. aureus* are reported to show resistance against multiple antimicrobials. Among the various antibiotic-resistant isolates, methicillin-resistant *S. aureus* (MRSA) is a serious cause because of its public health significance (Juhasz-Kaszanyitzky *et al.,* 2007). Beta–lactam antibiotics are frequently used in mastitis

therapy and the resistance is due to the production of beta-lactamases and low affinity penicillin-binding protein, PBP2A (Olsen *et al.*, 2006). As on date β-lactamase resistant penicillins such as methicillin and oxacillin are not used in dairy cows except for cloxacillin that is used in products for intramammary administration (Turutoglu *et al.,* 2006). Therefore, the present study was conducted to study the incidence of drug resistant mastitis in dairy cows from Tamil Nadu.

MATERIALS AND METHODS

Sampling: Four hundred and one milk samples were collected from acute mastitic cows from Large Animal Clinic Medicine Unit of Madras Veterinary College Teaching Hospital and six dairy farms in Coimbatore district. Clinical examination was carried out as described by Boddie (2000).The cows selected for the present study were subjected to detailed history. The relevant information of each animal with regard to the breed, age, lactation number and stage of lactation were recorded.

Bacterial culturing: The guidelines of National Mastitis Council (NMC) were followed for sample collection, transportation, culture and isolation of bacteria. Milk samples were inoculated into nutrient broth and streaked on to blood agar and Mac Conkey's agar plates and subsequently inoculated to specific media for pure culture of bacterial isolates. The bacteria grown on the

plates were identified up to the genus level based on the culture characters, staining and biochemical reactions. The *S. aureus* isolates were also characterized by their growth on blood agar and mannitol salt agar, the positive results for catalase and coagulase while the *E. coli* isolates were identified on eosin methylene blue agar, a negative oxidase test.

Antibiotic sensitivity and resistance: The antimicrobial susceptibility of the different *E. coli* and *S. aureus* strains to the commonly used antibiotics were determined by agar disc diffusion on Muller-Hinton agar plates with 0.5 McFarland turbidity standards (CLSI, 2008). The *Staphylococci* isolates were confirmed to be MRSA by amplifying the MRSA specific genes *mec*A and *bla*Z as described by Lee (2003) and Martineau *et al.* (2000), respectively. Based on culture, isolation and sensitivity tests, cows with resistant mastitis were grouped as follows, Group I *Escherichia coli* (n=119), Group II *Staphylococcus aureus* (n=104) and Group III Methicillin resistant *Staphylococcus aureus* (n=12).

Statistical analysis: The results were statistically analyzed, utilizing SPSS – Version 14 statistical software package.

RESULTS AND DISCUSSION

Out of 401 dairy cows, resistant mastitis was detected in 235 cows accounting to 56.1 % with the predominant resistant causative pathogen was *E. coli* (50.64 %) followed by *S. aureus* (44.25 %) and Methicillin resistant *Staphylococcus aureus* (5.11 %).

The antibiotic usage has directly contributed to an increased prevalence of resistance (DANMAP, 2001). All antimicrobial use in the herd may affect the resistance of *E. coli* isolates by increasing the presence of these antimicrobial agents in the cow's environment. Findings of above said studies are in partial agreement with the present study.

Sori *et al.* (2011) reported that 52.4 % were resistant to 2 or > 2 antimicrobial agents against *S. aureus. S. aureus* has developed multidrug resistance in many regions of the world (WHO, 2000), although reported prevalence rates indicated that wide variations existed regionally and even from herd to herd (Waage *et al.,* 2002).

In the present study, prevalence of MRSA accounted for 5.11 %. However, we found nearly 10.34 % of (12 out of 116) *S. aureus* strains to be MRSA. This was in agreement with Kumar *et al.,* (2011) also reported MRSA prevalence of 13% of *S. aureus* isolates (14/107) from cows with mastitis in a herd located in northwest India.

Breed wise, lactation wise, stage of lactation wise incidence of resistant mastitis in cows and antibiogram of the isolated bacterial pathogens are given in fig. 1, and tables 1- 3, respectively.

Incidence of resistant mastitis was high in Holstein Friesian cross breed, followed by Jersey cross and non descript in *E. coli* and *S. aureus* group and Holstein Friesian cross breed and Jersey cross in MRSA group (Fig. 1).

In the present study, incidence of *E. coli*, *S. aureus* and MRSA mastitis was high in Holstein Friesian cross breed followed by Jersey cross breed and non descript. Several authors reported that higher prevalence of mastitis in Holstein Friesian (Radostits *et al.,* 2008 and Sharma and Maiti, 2010) (Fig. 1). These differences between breeds might be due to immune response to intra mammary infection between breeds (Sharma and Maiti, 2010).

Highest incidence was observed in third lactation followed by second, first, fourth, fifth lactation and least in sixth lactation in *E. coli* and *S. aureus* group and second lactation followed by third, fourth and first lactation in MRSA group (Table 1).

In the present study, it was found that the animals in the third lactation were affected more and it was highly significant when compared to other lactation period animals (Table 1). Various authors also recorded higher incidence during third lactation (Carlen *et al.,* 2005 and Sachin Kumar, 2007). Higher incidence in third lactation could be attributed to the negative energy balance with concurrent fatty liver syndrome and oxidative stress may be correlated to production performance of the animal.

Highest incidence was observed in early stage of lactation followed by mid and late lactation in *E. coli* and *S.aureus* group and early stage of lactation followed by mid lactation in MRSA group (Table 2).

In the present study the incidence of mastitis was higher in early stage of lactation when compared to mid and late lactation (Table 2). This observation was in close agreement with Fadlemula *et al.* (2009) reported that the highest incidence of mastitis (62.7 %) was in the first month of lactation. Various workers also recorded higher incidence rate in early stage of lactation (Corbett, 2009 and Mohana Sundhari, 2010). Lairintluanga *et al.* (2003) and Sharma *et al.* (2011) opined that dairy cows seemed to have more oxidative stress and low antioxidants defence during early lactation and it could be due to stress of peak milk production during the early stage or just after parturition than in advanced pregnant cows.

Gowri (2010) recorded 11.5 % of mastitis in periparturient cows with moderate and severe fatty liver and further suggested that increase in NEFA concentration might be attributed to impairment of immune system in fatty liver cows.

Antibiotic sensitivity and resistance: *E. coli* showed more sensitivity to enrofloxacin (79 %) followed by amoxicillin and sulbactam (74 %), gentamicin (73.1 %) and ceftriaxone (69 %). The isolates had highest resistance to penicillin (63 %) followed by amoxicillin (52.1 %), oxytetracycline (47.9 %) and methicillin (45.4 %) (Table 3).

Lehtolainen *et al.* (2003) recorded that 27 % of *E. coli* isolates were resistant to one or more antimicrobial agents. Among resistant isolates highest resistance was against tetracycline, Dihydrostreptomycin (DHS) and

Table 1. Lactation wise incidence of mastitis in cows.

Lactation	*E. coli* (n=119)	*S. aureus* (n=104)	MRSA (n=12)	χ^2 test
1st	20.16%	19.23%	8.33%	
2nd	31.93%	28.84%	41.66%	
3rd	35.29%	32.69%	33.33%	7.21 NS
4th	5.88%	13.46%	16.66%	
5th	5.04%	2.88%	-	
6th	1.68%	2.88%	-	

NS – Non significant (P>0.05)

Table 2. Stage of lactation wise incidence of mastitis in cows.

Stage of Lactation	*E. coli* (n=119)	*S. aureus* (n=104)	MRSA (n=12)	χ^2 test
Early 1-3 Months	63.9%	69.2%	83.3%	
Mid 4-6 Months	31.1%	25%	16.7%	
Late 6-9 Months	5%	5.8%	-	2.73 NS

NS – Non significant (P>0.05)

ampicillin and 41 % were multiresistant. Multiresistant bacteria also tend to maintain their resistance to a particular antimicrobial even when that antimicrobial is absent from the environment if the other antimicrobials to which the resistance is linked are still present (Galland *et al.*, 2001). The present observation was in agreement with Lairintluanga *et al.* (2003) and karthikeyan (2003) reported that gram negative pathogens were more sensitive to enrofloxacin and gentamicin and less sensitive to ampicillin and penicillin.

S. aureus isolates were most sensitive to enrofloxacin (79.8 %) followed by gentamicin (71.2 %), amoxicillin and sulbactam (69.2 %) and ceftriaxone (69.2 %). The isolates showed highest resistance to penicillin (63.5 %) followed by amoxicillin (61.5 %), oxytetracycline (49 %) and methicillin (30.8 %) (Table 3).

The resistance of *S. aureus* to penicillin and ampicillin may be attributed to the production of betalactamase, an enzyme that inactivates penicillin and closely related antibiotics (Green and Bradely, 2004) and wide use of intramammary preparations containing combinations and broad-spectrum antibiotics (Pitkala *et al.,* 2007). Edward *et al.* (2002) suggested a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. The present observation was in agreement with Lairintluanga *et al.* (2003) who reported that *Staphylococcus Spp.* was highly sensitive to enrofloxacin, gentamicin and least sensitive to ampicillin.

MRSA showed maximum sensitivity to enrofloxacin and amoxicillin and sulbactam (75 %) each, gentamicin (66.7 %) and ceftriaxone (58.3 %). The isolates showed highest resistance to methicillin (100 %), amoxicillin (91.7 %), followed by penicillin (83.3 %) and oxytetracycline (41.7 %) (Table 3).

Table 3. *In vitro* antibiogram of isolated pathogens in cows.

	E. coli (n=119)		*S. aureus* (n=104)		MRSA (n=12)	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Enrofloxacin	79	21	79.8	20.2	75	25
Amoxicillin + Sulbactam	74	26	69.2	30.8	75	25
Gentamicin	73.1	26.9	71.2	28.8	66.7	33.3
Ceftriaxone	69	31	69.2	30.8	58.3	41.7
Oxytetracycline	52.1	47.9	51	49	58.3	41.7
Methicillin	54.6	45.4	47.1	52.9	-	100
Amoxicillin	47.9	52.1	38.5	61.5	8.3	91.7
Penicillin G	37	63	36.5	63.5	16.7	83.3

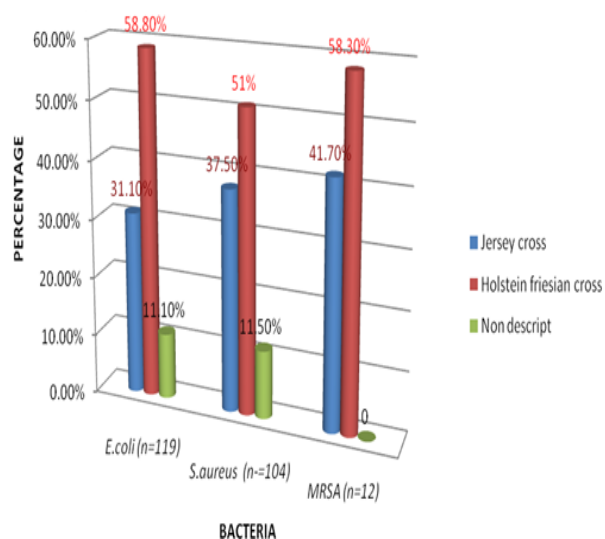MRSA- Methicillin resistant *Staphylococcus aureus*

Fig. 1. *Breed wise incidence of mastitis in cows.*

Kumar *et al.*(2011) recorded a high prevalence of MRSA (13.1 %). The isolates were also highly resistant to streptomycin, oxytetracycline, gentamicin and chloramphenicol, pristinomycin and ciprofloxacin. The present observation was in agreement with Turutoglu *et al.* (2009) and Turkyılmaz *et al.* (2010). Kumar *et al.* (2010) reported that MRSA strains were multi-drug resistant which might be due to production of betalactamase and PBP2a (penicillin binding protein).

The incidence of MRSA mastitis by targeting specific gene *mec*A and *bla*Z in the study was 10.34 per cent (12/116). Loeffler and Lloyd (2010) opined that detection of *mec*A and *bla*Z gene by PCR was gold standard test for confirmation of methicillin resistance. This high incidence in the present study might be due to indiscriminate use of antibiotics and intramammary preparations used by the owner without the prescription of the veterinarian.

Conclusion

Incidence of resistant mastitis in dairy cows of Tamil Nadu, India was high. The predominant resistant causative pathogen was *E. coli* followed by *S. aureus* and MRSA. Incidence of resistant mastitis was high in Holstein Friesian cross breed in early stage of third lactation. Antimicrobial resistance to penicillin, amoxicillin, oxytetracycline and methicillin was found in clinical mastitis cases. It highlights the need for preventing the indiscriminate use of antibiotics.

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