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Pattern of antibiotic resistant mastitis in dairy cows

D. Chandrasekaran, P. Venkatesan, K. G. Tirumurugaan, A. P. Nambi, P. S. Thirunavukkarasu, K. Kumanan, S. Vairamuthu and S. Ramesh

Department of Veterinary Clinical Medicine, Ethics and Jurisprudence,
Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India.
Corresponding author: D. Chandrasekaran, email:drchandrus@yahoo.com
PV: venkatesanperiasamy@gmail.com, KGT: tirumurugaankg@tanuvas.org.in, APN: nambi529@yahoo.com, PST: pstthiru@yahoo.com, KK: kumananrani@hotmail.com, SV: vairamuthu@tanuvas.org.in, SR: ramesh@tanuvas.org.in
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Abstract

Aim : To study the prevalence of drug resistant mastitis and their pattern of antibiotic resistance in dairy cows from Tamil Nadu.

Materials and Methods: Isolation and identification of resistant pathogens were performed from acute clinical mastitis samples. Based on culture, isolation and sensitivity tests, cows with resistant mastitis were grouped as; Group I: *Escherichia coli* (n=119), Group II: *Staphylococcus aureus* (n=104) and Group III: Methicillin-resistant *Staphylococcal aureus* (MRSA) (n=12). The isolates were tested using agar disc diffusion method for their antimicrobial susceptibility and modified resazurin assay microdilution technique for minimum inhibitory concentration (MIC) to 8 antimicrobial drugs. The organisms were also confirmed for their identity by performing PCR on the bacterial pellet targeting the specific genes such as 16s-23s rRNA, *mec*A and *bla*Z respectively for the resistant pathogens and also confirmed by sequencing.

Results: Antibiotic resistant mastitis was detected in 235 out of 401 cows accounting to 56.1%. The predominant resistant causative pathogen was *E. coli* (50.64%) followed by *S. aureus* (44.25%) and MRSA (5.11%). *In vitro* antibiotic sensitivity test and MIC breakpoints, *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. *E. coli* and *S. aureus* isolates were found to be resistant to 1 or 2 antimicrobials, whereas most of the MRSA isolates were found to be multi-drug resistant i.e resistance to 3 or more of antimicrobials. Out of 235 milk samples, the specific target gene 16s-23s rRNA (*E. coli*), 16s-23s rRNA (*S. aureus*) and MRSA (*mec*A and *bla*Z) could be amplified from 119, 104 and 12 isolates with a percentage positivity of 50.64 (119/235), 89.64 (104/116) and 10.34 (12/116) respectively.

Conclusion: Prevalence of antimicrobial resistance (AMR) in bovine mastitis pathogens was high. Most MRSA pathogens were multidrug resistant. *E. coli* and *S. aureus* isolates were resistant to few antimicrobials.

Keywords: bovine resistant mastitis, *Escherichia coli*, methicillin resistant *Staphylococcus aureus, Staphylococcus aureus.*

Introduction

Mastitis is the most common and economically significant disease affecting dairy cattle. A variety of bacteria can be isolated from bovine mastitis cases. *Staphylococcus aureus* and *Escherichia coli* are the most common causes of contagious and environmental clinical mastitis, respectively. 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 to be one of the reasons for low cure rates [1]. Additionally, AMR in bacteria is a public health hazard, and extensive use of antimicrobial is considered a potentially important driver of AMR. Several strains of *S. aureus isolated* from mastitis case have been reported to show resistance against multiple antimicrobials such as penicillin-G, gentamicin, streptomycin, ampicillin, ciprofloxacin, oxytetracycline [2].

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 [3]. 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 [4]. Methicillin-resistant *S. aureus* (MRSA) have been isolated from mastitis milk samples and have the potential to complicate treatment of bovine mastitis [5]. The presence of MRSA in bovine mastitis is a potential risk to other exposed cattle and farm workers including veterinarians [6]. In general, the emergence and transfer of AMR bacteria or genetic determinants from animals to human populations via food chain is a growing concern [7]. Comprehensive information on the prevalence of AMR in bovine mastitis pathogens in milk is lacking in India.

The objective of the present study was to study the prevalence of drug resistant mastitis and their pattern of antibiotic resistance in dairy cows from

Table-1: primers used in the study

Primer	Primer sequence	Organism targeted	Amplicon size	Reference
SU-F	5' TTC GTA CCA GCC AGA GGT GGA 3'	*S. aureus*	229 bp	[10]
SU-R	5' TCT TCA GCG CAT CAC CAA TGC C 3'			
Eco 2083	5' GCT TGA CAC TGA ACA TTG AG 3'	*E. coli*	662 bp	[11]
Eco 2745	5' GCA CTT ATC TCT TCC GCA TT 3'			
mecA	5' AAA ATC GAT GGT AAA GGT TGG C 3´	Methicillin resistant	533 bp	[12]
	5'AGT TCT GCA GTA CCG GAT TTG C 3'	S. *aureus* (MRSA)		
blaZ	5' ACT TCA ACA CCT GCT GCT TTC 3'	Methicillin resistant	173 bp	[13]
	5' TGA CCA CTT TTA TCA GCA ACC 3'	S. *aureus* (MRSA)		

Tamil Nadu, India.

Materials and Methods

Sampling and bacterial culturing: Four hundred and one milk samples were collected from acute mastitis cows from Large Animal Clinic Medicine Unit of Madras Veterinary College Teaching Hospital and six dairy farms in Coimbatore district. The guidelines of National Mastitis Council (NMC) were followed for sample collection, transportation, culture and isolation of bacteria. *S. aureus* isolates were identified as gram-positive cocci by Gram stain, growth on blood agar and mannitol salt agar and a positive test for catalase and coagulase [8]. *Escherichia coli* isolates were identified as gram negative rods by Gram stain, lactose fermenters on MacConkey agar, and a negative oxidase test. Based on incidence of common causative pathogens and sensitivity tests, isolates were categorized as resistant i.e exhibiting *in vitro* resistance to 1 or 2 antimicrobials and multidrug-resistant i.e exhibiting *in vitro* resistance to 3 or more antimicrobials. Cows with resistant mastitis were grouped as; Group I: *Escherichia coli* (n=119), Group II: *Staphylococcus aureus* (n=104) and Group III: MRSA (n=12).

Antibiotic susceptibility and resistance: Antimicrobial susceptibility testing was carried out with equivalence of 0.5 McFarland turbidity standard by agar disc diffusion method on Mueller-Hinton agar plates following the guidelines of Clinical and Laboratory Standards Institute (CLSI) [8]. All the bacteria isolated were tested *in vitro* for their sensitivity to 8 different antibiotics, that are commonly used in veterinary practice. Commercially available antibiotic discs (Himedia, Mumbai) were used in the study *viz.*, enrofloxacin (10 mcg), amoxicillin + sulbacatm (15 mcg), amoxicillin (10 mcg), gentamicin (10 mcg), ceftriaxone (30 mcg), oxytetracycline (30 mcg) and penicillin G (10 units) and oxacillin (5 mcg). The sizes of the zone of inhibition were recorded and interpreted as either susceptible or resistant to the exposed agent.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentrations of different antibiotics for *E. coli, S. aureus* and MRSA isolates were determined by modified resazurin assay microdilution technique [9]. The MIC panels consisted of dehydrated antimicrobial agents: amoxicillin, ceftriaxone, enrofloxacin, gentamicin, pencillin, oxytetracycline, amoxicillin + sulbactam and oxacillin in 96-well U bottom microtiter plates and performed in accordance with the guidelines established by the CLSI [8]. The lowest concentration of antibiotic that resulted in complete inhibition of visible growth and did not produce any turbidity was taken as the MIC end point.

Polymerase chain reaction (PCR) for identification of the mastitis causing bacteria: A single colony with typical morphology from the selective agar was suspended in nuclease free water and lysed by boiling for 10 minutes and the lysate was stored at -20°C until use. The lysate were used in a PCR reaction with primers targeting the specific gene for different strains. The PCR amplicons from some of the samples were sequenced and analysed by BLAST search to confirm their identity. The details of primers used in the study are described in Table-1.

PCR amplification was performed in a total reaction volume of 25 µl. The reaction mixture contained 12.5 µl of the master mix, 20 pmol of the forward and reverse primer and 1.5 µl of the test lysate. The amplification profile for the detection of different genes were 94°C 5 min; 35 cycles of 95°C for 45 sec, 55°C for 1 min, 72°C for 1 min; final extension of 72°C for 7 min. The PCR products were separated by gel electrophoresis in 1.5 per cent agarose gel using 1x Tris acetate EDTA buffer along with standard DNA marker (100 bp ladder, Genei, Bangalore) and visualized with ethidium bromide staining.

Results

Out of 401 clinical mastitis samples subjected to bacterial isolation, 184 (45.89%) were positive for *E coli*, 162 (40.4%) were positive for *S. aureus*, 12 (2.99 %) were positive for MRSA, 14 (3.49%) were positive for *Bacillus* spp., 13 (3.24%) were positive for *Streptococcus* spp. and 16 (3.99%) samples showed mixed infection. Mixed infections were not exhibiting resistant to antibiotics (Pansusceptible).

Antibiotic resistant mastitis was detected in 235 out of 401 cows accounting to 56.1%. The predominant resistant causative pathogen was *E. coli* (50.64%) followed by *S. aureus* (44.25%) and MRSA (5.11%).

Antibiotic susceptibility 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 (47.95) and methicillin (45.4%). Most of the *E.coli* isolates (86.55%) were found to be resistant i.e resistance to 1 or 2 antimicro-

Table-2: MIC of drugs against *E. coli* (n=119) isolated from udder of cows

Antibiotics	MIC (µg/mL) (n=119)											
	125	62.5	31.25	15.62	7.8	3.9	1.95	0.97	0.48	MIC Range	MIC_{-50}	MIC_{-90}
Gentamicin	9.2	11	2.5 |	**3.4**	11.7	5.9	16	27.7	12.6	0.48-125	1.95	62.5
Oxytetracycline	17.7	25.2	15.1	5.9 |	**16**	6.7	8.4	5	-	0.97-125	31.25	125
Ceftriaxone	4.2	9.2 |	**20.2**	3.4	12.6	1.7	13.4	20.2	15.1	0.48-125	3.9	62.5
Enrofloxacin	8.4	3.4	12.6	4.2	11.7	3.4 |	**21.8**	18.5	16	0.48-125	1.95	62.5
Amoxicillin	22.7	30.3	6.7	**23.5**	6.7	2.5	7.6	-	-	1.95-125	62.5	125
Pencillin G	19.3	41.2	8.4	**12.6**	5.9	4.2	7.6	0.8	-	0.95-125	62.5	125
Amoxicillin + Sulbactam	-	0.8	31.1	**0.8**	8.4	8.4	31.1	12.6	6.7	0.48-125	1.95	31.25
Oxacillin	6.7	33.6	16	**17.7**	5	14.3	6.7	-	-	1.95-125	31.25	62.5

Numbers indicate percentage of isolates. Numbers in bold indicate percentage of isolates with MIC values greater than the highest concentration in the dilution range and vertical line indicate clinical breakpoints, with the value to the right of the line being susceptible and those to the left being resistant. MIC_{50} and MIC_{90} values are concentrations at which ≥ 50 % and ≥ 90 % of isolates are inhibited respectively.

Table-3: MIC of drugs against *S.aureus* (n=104) isolated from udder of cows

Antibiotics	MIC (µg/mL) (n=104)											
	125	62.5	31.25	15.62	7.8	3.9	1.95	0.97	0.48	MIC Range	MIC_{-50}	MIC_{-90}
Gentamicin	7.7	13.5	3.8	**2.9** |	15.4	11.5	10.5	13.5	21.2	0.48-125	3.9	62.5
Oxytetracycline	19.2	24	12.6	**1.9** |	13.5	11.5	7.7	9.6	-	0.97-125	31.25	62.5
Ceftriaxone	11.5	13.5	6.7	2.9	11.5	**9.6** |	9.6	23.1	11.5	0.48-125	3.9	62.5
Enrofloxacin	8.6	9.6	5.8	3.8	22.1	**8.6** |	7.7	12.6	21.2	0.48-125	3.9	31.25
Amoxicillin	22.1	34.6	7.7	5.8	11.5	6.7	11.5	-	- |	1.95-125	62.5	125
Pencillin G	21.2	44.2	2.9	13.5	8.6	4.8	2.9	1.9	-	0.97-125 |	62.5	125
Amoxicillin + Sulbactam	-	4.8	32.7	2.9	**8.6** |	14.4	14.4	18.3	3.8	0.48-62.5	3.9	31.25
Oxacillin	14.4	24	14.4	14.4	11.5	**17.3** |	1.9	-	-	1.95-125	31.25	125

Numbers indicate percentage of isolates. Numbers in bold indicate percentage of isolates with MIC values greater than the highest concentration in the dilution range and vertical line indicate clinical breakpoints, with the value to the right of the line being susceptible and those to the left being resistant.MIC_{50} and MIC_{90} values are concentrations at which ≥ 50 % and ≥ 90 % of isolates are inhibited respectively.

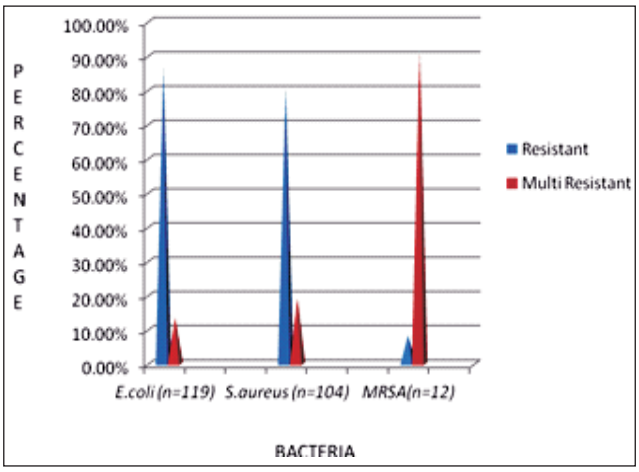

Figure-1. Incidence of resistance mastitis in cows

bials and few *E. coli* isolates (13.45%) were found to be multi-drug resistant (Figure-1) i.e resistance to 3 or more of antimicrobials.

S. aureus isolates were most sensitive to enrofloxacin (79.8%) followed by gentamicin (71.2%), amoxicillin and sulbactam (69.2%) and ceftriaxone (69.2%t). The isolates showed highest resistance to penicillin (63.5%) followed by amoxicillin (61.5%), oxytetracycline (49%) and methicillin (52.9%). Most of the *S. aure*us isolates (80.77%) were found to be resistant i.e resistance to 1 or 2 antimicrobials *and* few *S. aureus* isolates (19.23%) were found to be multi-drug resistant (Figure-1) i.e resistance to 3 or more antimicrobials.

MRSA showed maximum sensitivity to enrofloxacin (75%), amoxicillin and sulbactam (75%) followed by 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%). Few MRSA isolates (8.33%) were found to be resistant i.e resistance to 1 or 2 antimicrobials *and m*ost of the MRSA isolates (91.67%) were found to be multi-drug resistant (Figure-1) i.e resistance to 3 or more of antimicrobials.

MIC: Minimum inhibitory concentration of common antibiotics against *E. coli*, *S.aureus* and MRSA are presented in Table-2, 3 and 4.

The breakpoints for Enrofloxacin, oxytetracycline, amoxicillin, oxytetracycline, ceftriaxone, penicillin G, oxacillin, amoxicillin + sulbactam and gentamicin were ≥ 2, 8, 16, 32, 16, 16, 16 and ≥ 16 µg/mL for *E. coli* respectively.

Based on the breakpoints, the results indicated that *E. coli* was sensitive to gentamicin (56.3%), enrofloxcain (56.3%), amoxicillin + sulbactam (50.4%), ceftriaxone (86.6%) and resistant to amoxicillin (53%), oxytetracycline (58%), penicillin G (60.5%) and oxacillin (56.3%).

The breakpoints for penicillin, amoxicillin, oxacillin, ceftriaxone, enrofloxacin, amoxicillin + sulbactam, gentamicin, oxytetracycline, oxacillin and ceftriaxone were ≥ 0.25, ≥ 0.5, ≥ 4, ≥ 4, ≥ 4, ≥ 8, ≥ 16 and ≥ 16

Table-4: MIC of drugs against MRSA (n=12) isolated from udder of cows

Antibiotics	MIC (µg/mlL) (n=12)											
	125	62.5	31.25	15.62	7.8	3.9	1.95	0.97	0.48	MIC Range	MIC_{-50}	MIC_{-90}
Gentamicin	33.3	25	16.7	-	**8.3** |	8.3	8.3	-	-	1.95-125	31.25	125
Oxytetracycline	25	41.7	16.7	-	- |	8.3	8.3	-	-	1.95-125	62.25	125
Ceftriaxone	16.7	33.3	33.3	-	8.3 |	**8.3**	-	-	-	3.9-125	62.25	62.5
Enrofloxacin	-	16.7	16.7	16.7	8.3	25	8.3	-	**8.3** |	0.48-62.5	15.62	62.5
Amoxicillin	33.3	50	16.7	-	-	-	-	-	- |	31.25-125	62.5	125
Pencillin G	33.3	41.7	25	-	-	-	-	-	- |	31.25-125	62.5	125
Amoxicillin + Sulbactam	8.3	25	16.7	-	- |	**33.3**	16.7	-	-	1.95-125	31.25	62.5
Oxacillin	66.7	33.3	-	-	-	- |	-	-	-	62.5-125	125	125

Numbers indicate percentage of isolates. Numbers in bold indicate percentage of isolates with MIC values greater than the highest concentration in the dilution range and vertical line indicate clinical breakpoints, with the value to the right of the line being susceptible and those to the left being resistant. MIC_{50} and MIC_{90} values are concentrations at which $\geq$ 50 % and $\geq$ 90 % of isolates are inhibited respectively.

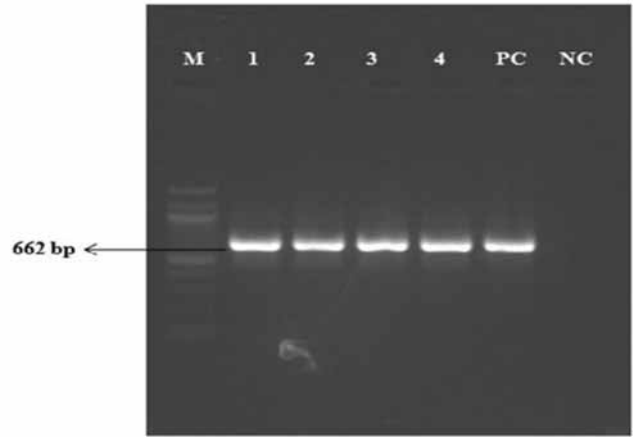

Figure-2: Amplification of 16s-23s rRNA gene of *E. coli.* M: 100bp ladder, 1-4: *E. coli* test samples, PC: Positive control, NC: Negative control.

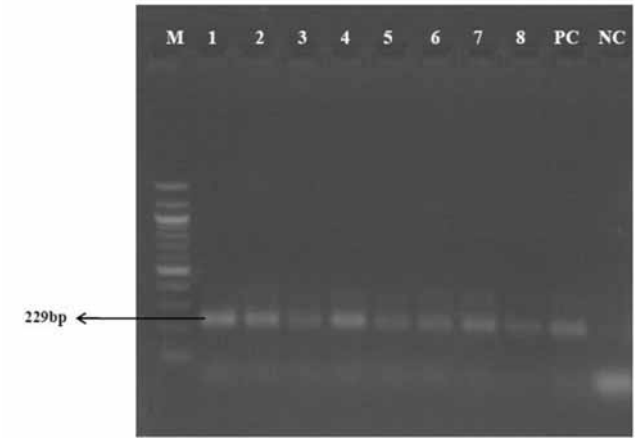

Figure-3: Amplification of 16s-23s rRNA gene of *S. aureus.* M: 100bp ladder, 1-8: *S. aureus* test samples, PC: Positive control, NC: Negative control.

µg/ml for *S. aureus* respectively.

Based on the breakpoints, the results indicated that *S. aureus* was sensitive to gentamicin (56.7%), enrofloxcain (50.1%), amoxicillin + sulbactam (50.9%), ceftriaxone (53.8%) and resistant to amoxicillin (100%), oxytetracycline (55.8%), penicillin G (100%) and oxacillin (80.8%).

The breakpoints for penicillin, amoxicillin, enrofloxacin, oxacillin, amoxicillin + sulbactam, ceftriaxone, gentamicin and oxytetracycline were 0.12, 0.25, $\geq$ 0.5, 2, 4, 4, 8 and 8 µg/ml for MRSA respectively.

Based on the breakpoints, the results indicate that MRSA was sensitive to gentamicin (24.9%), enrofloxcain (8.3%), amoxicillin + sulbactam (50%), ceftriaxone (8.3%) and resistant to amoxicillin (100%), oxytetracycline (73.4%), penicillin G (100%) and oxacillin (100%).

Confirmation of the mastitis bacteria by targeting specific genes for different strains: Out of 235 milk samples, specific target gene of 16s-23s rRNA (*E. coli*) of 662 bp (Figure-2) could be amplified from 119 isolates with a percentage of positivity as 50.64 (119/235), the 229 bp of 16s-23s rRNA (*S. aureus*) could be amplified from 104 isolates (Figure-3) with a percentage of positivity as 44.25 (104/235). Screening for the specific target gene for both *mec*A (MRSA) of 513 bp (Figure-4) and *bla*Z (MRSA) of 639 bp (Figure-5) resulted in positivity in 12 samples with a percentage of positivity as 10.34 (12/116) among the *S. aureus* isolate.

Discussion

In the present study, no resistance were observed for the *Streptococcus* spp. and *Bacillus* spp. Antimicrobial resistance has been reported to be most common among the Staphylococcal mastitis isolates with a much lower proportion of Streptococcal isolates exhibiting resistance [14]. The antibiotic usage has directly contributed to an increased prevalence of resistant *E. coli* mastitis [15]. 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. The incidence of resistant *S. aureus* mastitis was higher which might be due to indiscriminate use of antibiotics and intramammary preparations containing combinations and broad-spectrum antibiotics [16].

MRSA strains have been observed to be multi-drug resistant, such as aminoglycosides, macrolides, lincosamides, streptogramins, tetracyclines, etc., which are often used in the treatment of mastitis [17]. In the present study, multidrug resistance to methicillin, amoxicillin, penicillin and oxytetracycline was commonly observed in MRSA mastitis. MRSA strains

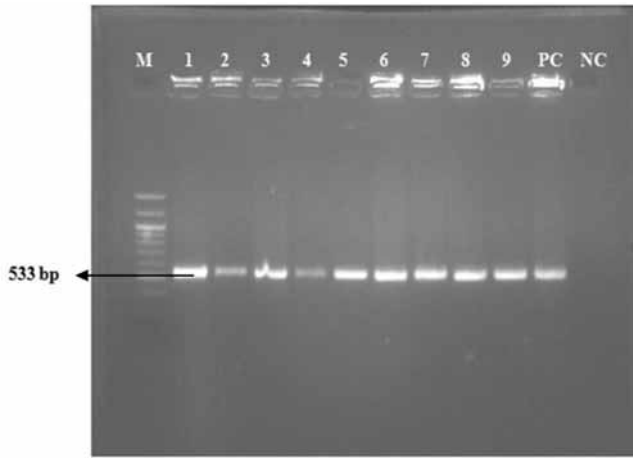

Figure-4: Amplification of *mecA* gene of MRSA. M: 100bp ladder, 1-9: MRSA test samples, PC: Positive control, NC: Negative control.

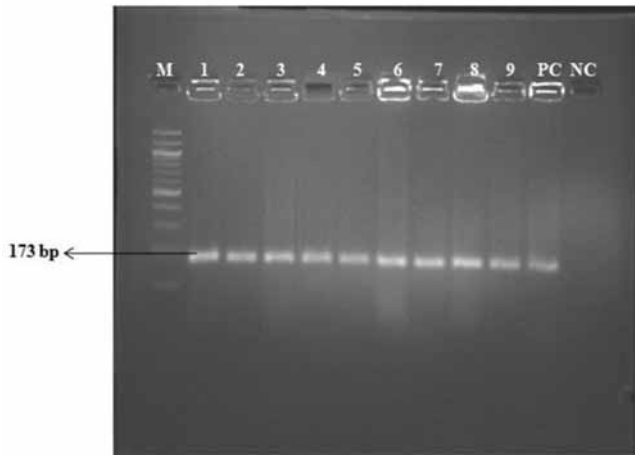

Figure-5: Amplification of *blaZ* gene of MRSA. M: 100bp ladder, 1-9: MRSA test samples, PC: Positive control, NC: Negative control.

were multi-drug resistant which might be due to production of betalactamase and PBP2a (penicillin binding protein) [17]. A previous study indicates the prevalence of 13 percent of *S. aureus* MRSA isolates from cows with mastitis in a herd located in northwest India [2]. High incidence in the present study might be due to indiscriminate use of antibiotics and consequent transfer of plasmid mediated antibiotic resistance and concurred with above authors. Indiscriminate use of antibiotics and intramammary preparations used by the owner without the prescription of the veterinarian is also attributed to be one of the reasons for increasing incidence of these strains.

Gram negative pathogens were more sensitive to enrofloxacin and gentamicin and less sensitive to ampicillin and penicillin [18]. In the present study, incidence of resistant *E. coli* mastitis was higher which could be due to the wide antibiotic usage [15].

The data for $MIC_{50,}$ clinical breakpoints of amoxicillin, penicillin G, oxacillin and amoxicillin + sulbactum against *E. coli* were not available. However, the clinical break point of ampicillin and amoxicillin-clavulanate were taken for the present study.

The breakpoints for gentamicin and enrofloxacin were $\geq$ 16 and $\geq$ 2 μg/ml for *E.coli* respectively [19]. The breakpoints for amoxicillin, oxytetracycline, ceftriaxone, penicillin G, oxacillin and amoxicillin-clavulanate were 16, 8, 32, 16, 16 and 16 μg/mL for *E. coli* respectively [20].

Based on the MIC break point for pencillin G, oxytetracycline, amoxicillin and oxacillin in *E. coli* mastitis, they were considered as resistance and could be attributed to the indiscriminate use of these drugs. It highlights the need for systematic study of resistance pattern before initiating antibiotic therapy.

The data for $MIC_{50,}$ clinical breakpoints of amoxicillin + sulbactum against *S. aureus* were not available. However, the clinical break point for amoxicillin-clavulanate were included in the study.

The breakpoints for amoxicillin, amoxicillin + clavulanate, enrofloxacin, gentamicin, oxytetracycline, penicillin and oxacillin were $\geq$ 0.5, $\geq$ 8, $\geq$ 4, $\geq$ 16, $\geq$ 16, $\geq$ 0.25 and $\geq$ 4 μg/ml for *S. aureus respectively* [19]. The breakpoints for ceftriaxone were $\geq$ 4 μg/mL for *S. aureus* [20]. The high resistance of pencillin G, oxy-tetracycline, amoxicillin and oxacillin in *S.aureus* mastitis in the present study could be attributed to the indiscriminate use of these drugs and intramammary preparations used by the owner without the prescription of the veterinarian.

The breakpoints for gentamicin, oxytetracycline, ceftriaxone, enrofloxcain, amoxicillin, penicillin G, amoxicillin + sulbactam and oxacillin were 8, 8, 4, $\geq$ 0.5, 0.25, 0.12, 4, and 2 μg/mL for MRSA respectively [9]. The high resistance of pencillin G, oxytetracycline, amoxicillin and oxacillin in *MRSA* mastitis in the present study could be attributed to the indiscriminate use of these drugs and intramammary preparations used by the owner without the prescription of the veterinarian.

Based on the antibiotic susceptibility test and MIC break points, *E. coli, S. aureus* and MRSA showed maximum sensitivity to enrofloxacin, amoxicillin and sulbactam followed by gentamicin and ceftriaxone.

These susceptible antibiotic drugs will be used as the effective drugs against *E. coli, S. aureus* and MRSA resistant isolates. The present study demonstrated that the resistant strains may be transferred to milk from infected udders, poor farm practices and due to poor handling during milking, transmission to the milk utensils, which could be the reason for infection in human beings.

The present study has demonstrated the existence of alarming levels of resistance of *E. coli, S. aureus* and MRSA to commonly used antimicrobial agents in the study farms and the results are in accordance with reports from earlier studies in other countries. Edward *et al*., [21] suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. It is therefore, very important to implement a systemic application of an *in vitro* anti-biotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections.

Only a few studies in India have been carried out to assess MRSA status among mastitis infections [2]. The presence of MRSA signifies alarming levels of resistance. Further it highlights the need for preventing the indiscriminate use of antibiotics. Avoiding routine antimicrobial use in food animals and to decrease selection pressure against anti-microbials, might decrease the prevalence of MRSA among cows.

Conclusion

AMR prevalence was more common in bovine mastitis pathogens namely *E. coli* and *S. aureus.* Overall, antimicrobial resistance to penicillin, amoxicillin, oxytetracycline and methicillin was found in clinical mastitis cases. Multidrug resistance was more commonly observed in MRSA isolates than in *E. coli* and *S. aureus* isolates. 12 out of 116 *S. aureus* isolates screened was positive for MRSA (Prevalence of 10.34%). This study result suggests that a high risk for transmission of AMR bacteria from milk or milk products to human populations.

Authors' contributions

DC planned, designed and carried out research work under his Ph.D thesis programme in collaboration with guide APN and advisory members PST, KK and SV. PV and KGT assisted in the designing and performance of PCR and SR assisted in the designing of MIC. DC and KGT revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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