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: tirumurugaank@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 Staphylococcus 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, mecA and blaZ 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 (meca and blaZ) 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 Tamil Nadu.
Table-1: primers used in the study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
<th>Organism targeted</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU-F</td>
<td>5’ TGC GTA CCA GGC AGA GTT TGA 3’</td>
<td>S. aureus</td>
<td>229 bp</td>
<td>[10]</td>
</tr>
<tr>
<td>SU-R</td>
<td>5’TCT TCA GGG CAT CAG CAA TG C C’</td>
<td>E. coli</td>
<td>662 bp</td>
<td>[11]</td>
</tr>
<tr>
<td>Eco 2083</td>
<td>5’ GCT TGA CAC TGA ACA TTG AG 3’</td>
<td>Methicillin resistant S. aureus</td>
<td>533 bp</td>
<td>[12]</td>
</tr>
<tr>
<td>Eco 2745</td>
<td>5’ GCA CTT ATC TCT TCC GCA TT 3’</td>
<td>Methicillin resistant S. aureus (MRSA)</td>
<td>173 bp</td>
<td>[13]</td>
</tr>
<tr>
<td>mecA</td>
<td>5’ AAA ATC GAT GGT AAA GGT TGG C 3’</td>
<td>S. aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>su-R</td>
<td>5’ ACT TCA ACA CCT GCT GCC TT 3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>su-F</td>
<td>5’ TCA GCA CTT TTA CTA GCA ACC 3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 + sulbactam (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, penicillin, 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-

Tamil Nadu, India.
Available at www.veterinaryworld.org/Vol.7/June-2014/5.pdf

**Figure-1.** Incidence of resistance mastitis in cows 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%). The isolates showed highest resistance to penicillin (63.5%) followed by amoxicillin (61.5%), oxytetracycline (49%) and methicillin (52.9%). Most of the *S. aureus* isolates (80.77%) were found to be resistance 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 most 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* and *S. aureus* 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%), enrofloxacin (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 µg/mL for *E. coli* respectively.

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (µg/mL) (n=119)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>9.2 62.5 31.25 0.95 62.5</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>17.7 25.2 15.1 6.7 4.2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>4.2 9.2 20.2 3.4 16 5.9</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>8.4 3.4 12.6 1.7 31.25 21.8</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>22.7 30.3 6.7 6.7 4.2 2.9</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>19.3 41.2 8.4 6.7 4.2 2.9</td>
</tr>
<tr>
<td>Amoxicillin + Sulbactam</td>
<td>- 0.8 31.1 8.4 8.4 31.1</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>6.7 33.6 16 17.7 5 14.3</td>
</tr>
</tbody>
</table>

- 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 and MIC 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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (µg/mL) (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>7.7 13.5 3.8 2.9 2.9</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>19.2 24 12.6 1.9 1.9</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>11.5 13.5 6.7 2.9 11.5 9.6</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>8.6 9.6 5.8 3.8 22.1 8.6</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>22.1 34.6 7.7 5.8 11.5 6.7</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>21.2 44.2 2.9 13.5 8.6 4.8</td>
</tr>
<tr>
<td>Amoxicillin + Sulbactam</td>
<td>- 4.8 32.7 2.9 8.6 14.4</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>14.4 24 14.4 14.4 11.5 17.3</td>
</tr>
</tbody>
</table>

- 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 and MIC values are concentrations at which ≥ 50% and ≥ 90% of isolates are inhibited respectively.

Veterinary World, EISSN: 2231-0916 391
Based on the breakpoints, the results indicated that was sensitive to gentamicin (56.7%), enrofloxacain (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, enrofloxacain, oxacillin, amoxicillin + sulbactam, ceftriaxone, gentamicin and oxytetracycline were 0.12, 0.25, 0.5, 2, 4, 4, 8 and 8 µg/ml for MRSA respectively.

The results indicate that MRSA was sensitive to gentamicin (24.9%), enrofloxacain (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 mecA (MRSA) of 513 bp (Figure-4) and blaZ (MRSA) of 639 bp (Figure-5) resulted in positivity in 12 samples with a percentage of positivity as 10.34 (12/116) among the isolate.

In the present study, no resistance were observed for the spp. and 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 mastitis [15]. All antimicrobial use in the herd may affect the resistance of isolates by increasing the presence of these antimicrobial agents in the cow’s environment. The incidence of resistant 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

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (µg/mL) (n=12)</th>
<th>MIC Range</th>
<th>MICa</th>
<th>MICa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>125</td>
<td>62.5</td>
<td>31.25</td>
<td>15.62</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>33.3</td>
<td>25</td>
<td>16.7</td>
<td>-</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>16.7</td>
<td>33.3</td>
<td>33.3</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>33.3</td>
<td>50</td>
<td>16.7</td>
<td>-</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>33.3</td>
<td>41.7</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>3.3</td>
<td>41.7</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Pencillin G</td>
<td>3.3</td>
<td>41.7</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicillin + Sulbactam</td>
<td>8.3</td>
<td>25</td>
<td>16.7</td>
<td>-</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>66.7</td>
<td>33.3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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. MICa and MICa values are concentrations at which ≥ 50 % and ≥ 90 % of isolates are inhibited respectively.
were multi-drug resistant which might be due to production of beta-lactamase 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 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 ≥ 16 and ≥ 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 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 ≥ 0.5, ≥ 8, ≥ 4, ≥ 16, ≥ 16, ≥ 0.25 and ≥ 4 μg/ml for S. aureus respectively [19]. The breakpoints for ceftriaxone were ≥ 4 μg/mL for S. aureus [20]. The high resistance of pencillin G, oxytetracycline, 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, enrofloxacin, amoxicillin, penicillin G, amoxicillin + sulbactam and oxacillin were 8, 8, 4, ≥ 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 antibiotic 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.

## References

15. DANMAP. (2001) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, Copenhagen, Denmark.