World Journal of Pharmaceutical research

Volume 3, Issue 1, 571-586. Research Article ISSN 2277 – 7105

ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* AND *COAGULASE-NEGATIVE STAPHYLOCOCCI* (CNS) ISOLATED FROM BOVINE MASTITIS IN THE REGION OF NORTH KARNATAKA, INDIA

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Article Received on
12 October 2013
Revised on 08 November 2013,
Accepted on 07 November 2013

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ABSTRACT

The present investigation was carried out to determine the species and antimicrobial susceptibilities of the *Staphylococci* isolated from clinical and subclinical Bovine mastitis milk. The samples were collected from North Karnataka, India (09 districts) from March 2012 to August 2012. A total of 392 Milk samples suffering from mastitis were screened and a total of 375 *staphylococcus* species were recovered. The molecular characterization confirmed that the isolates belong to *Staphylococcus aureus, Staphylococcus sciuri, and Staphylococcus saprophyticus.* The isolates were subjected to the antibiotic resistance screening. The overall antibiotic resistance test showed that the isolated *Staphylococcus* species were resistant to Methicillin (100%) followed by Penicillin G (95.97%), Cefpodoxime (88.85%), Oxacillin (87.61), Cefaclor (73.61%), Cefixime (72.91%), Ampicillin (69.73%), Ceftriaxone (61.94%), Streptomycin (36.77%), Amoxyclav (35.16%), Erythromycin (27.96%), Amikacin (24.99%), Norfloxacin (17.4%), Gentamicin (14.91%), Azithromycin (12.4%), Tetracycline (9.62%), Chloramphenicol (8.78%), Ciprofloxacin (5.14%), Ofloxacin (3.76%) and all the *Staphylococcus* strains were susceptible to Vancomycin. The present study demonstrated the presence of alarming level of resistance of frequently and commonly used antimicrobial agents to the isolated bacteria. Therefore, an examination of the antibiotic resistance profiles of the isolates may be recommended earlier to the use of antibiotics in both treatment and prevention of the disease.

Key words: Bovine Mastitis, *Staphylococcus aureus*, *Coagulase Negative Staphylococcus* (CNS), Antibiotics.

INTRODUCTION

Mastitis, inflammation of the mammary gland, is one of the most costly and complex diseases of the dairy industry. The economic consequences of bovine mastitis are related to treatment, production losses, culling and changes in milk quality [1]. Bacteria isolated with greatest frequency are *Staphylococcus aureus, Staphylococcus spp., Bacillus spp., Corynebacterium spp., Escherichia coli, Streptococcus spp., Pseudomonas spp.,* and *Klebsiella spp.* [2, 3]. Variation in prevalence of mastitis might be due to the different regions, breeds, therapeutic practices, management conditions and presence of microorganisms in environment [4]. Among the various causative agents, *Staphylococcus aureus* is one of the most prevalent and contagious pathogens of intra-mammary infections in dairy cattle globally [5]. Epidemiological studies revealed the transmission of *Staphylococcus aureus* from cow to cow, the primary source of which is the milk from infected glands, and also from dairy cows to humans and humans to cows [6]. *Coagulase-negative staphylococci* (CNS) have traditionally been considered as minor pathogens. Their importance has increased and they have become the predominant pathogens isolated from subclinical mastitis in several countries [7, 8, 9]. However, in recent years, the incidence of *Coagulase-negative Staphylococcus* (CNS) mastitis has increased substantially [10, 11]. *Coagulase-negative Staphylococci* (CNS) are associated with bovine intra-mammary infections (IMI) and may cause both subclinical and clinical mastitis. In most cases, the inflammatory reaction is relatively mild. The prevalence of *Coagulase-negative staphylococci (*CNS) IMI may vary significantly between regions and countries [12].

The public health significance of *Staphylococci* isolated from milk and dairy products is important. Cattle can be a source of antibiotic resistant strains for humans [13]. Antimicrobials are an important tool in mastitis control programs. Therefore, surveillance of antimicrobial resistance is important to ensure optimal results of antimicrobial use and minimize the risk for development and spread of antimicrobial resistance. The usage of antibiotics correlates with the emergence and maintenance of antibiotic-resistant traits within pathogenic strains [14]. These traits are coded for by particular genes that may be carried on the bacterial chromosome, plasmids [15], hence these are easily transferred among isolates.

The prevalence of antibiotic resistance usually varies between isolates from the different sampled stations and even between isolates from different herds on the same farm [16].

The evolution of antibiotic resistance in *Staphylococcus aureus* strains is a serious cause of concern in dairy animals [5]. Antibiotic-resistant *Staphylococcus aureus* isolates pose a severe challenge to both veterinary and health professions and dairy cattle producers because they have a negative impact on therapy [17, 18]. *Staphylococcus aureus* has been the main subject of studies on antibiotic resistance because of its importance for all forms of mastitis in dairy cows [19, 20]. Multiple antibiotic resistant *Staphylococcus aureus* strains have been isolated from milk obtained from cattle, beef and human samples in many parts of the world [14, 21]. Very few studies have investigated differences in antimicrobial resistance among CNS species identified by genotyping [22].

Identification of major mastitis pathogens and their antimicrobial susceptibility is important when selecting appropriate treatment regimen. Therefore, the present investigation was designed to determine the species and antimicrobial susceptibilities of the *Staphylococci* isolated from clinical and subclinical Bovine mastitis milk.

MATERIALS AND METHODS

Study area

The North Karnataka is located within 15°00' North (N) and 18°30' North (N) latitudes and 74° East (E) and 77°50' (E) East longitude. The border is bounded by Maharashtra and Goa States in the north and northwest and the State of Andhra Pradesh in the east. This region is mainly called as Bayaluseeme region comprising the plains of the Deccan plateau.

Source of milk samples

The samples were collected from North Karnataka, India (09 districts) from March 2012 to August 2012. The lactating cattles of the dairy farms of the North Karnataka Region has been examined from dairy herds in different smallholder farms as well as large scale farms randomly. The study includes Holstein Freshein (H.F), Jerscy, Dharwari and Murrah. A Total of 392 milk samples were collected fortnightly. Surf Field Mastitis Test (SFMT) and increased $_{P}H$ of the milk have been done to confirm the clinical and subclinical mastitis.

Surf Field Mastitis Test [23]

The samples were subjected to Surf Field Mastitis test (SFMT). The principle of the test is

that when detergent is added into milk sample, it causes rupture of somatic cell and release DNA and other cell contents. DNA is acid in nature, while detergent contains alkyl-arylsulfonate, which is basic in nature. DNA and detergents unite to form a gel; consistency of gel depends upon the number of somatic cells. More cells more thick gel and vice versa. For this purpose, 3% surf solution (pH = 10.3) was prepared by adding three grams of commonly used detergent powder (Surf Excell, Uniliver, India) in 100 mL of water. Quarter milk samples and surf solution were then mixed in equal quantities in petri-dishes separately for each quarter. The change in consistency of milk indicated mastitis, while no change in consistency of milk indicated healthy samples. The mastitis was graded into further four categories based on the severity of disease from lower to higher intensity as, + = moderate, + + = severe, + + + = more severe, + + + + = very severe.

Sampling method

Quarter foremilk samples were collected aseptically for bacteriological assay as described by Honkanen-Buzalski. Before sampling, teat ends were disinfected with cotton swabs soaked in 70% ethanol and allowed to dry and the first streams of milk were discarded. Milk samples were collected in sterile 15 ml tubes. The milk samples were transported in a cold container to the laboratory of the P. G. Department of studies in Microbiology and Biotechnology, Karnatak University, Dharwad for further analysis.

Identification and biochemical characterization

A total of 392 Milk samples suffering from mastitis were brought to the laboratory. The isolation *of Staphylococcus* stains was carried out using the standard method [24]. Briefly, 100µl of aseptically collected milk samples from each sample was spread over a Mannitol Salt Agar (MSA) plate and incubated at 37°C for 24 hrs. After incubation, the selected colonies were subjected to Gram staining, morphological characters, catalase test, Mannitol fermentation, Vogues-Proskauer and Carbohydrates fermentation and Coagulase test.

Antibacterial Resistance Test

Antibiotic resistance screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique was adapted for antibiogram. The antibiotic discs and Mueller- Hinton Agar were purchased from Hi-Media Pvt. Ltd, Mumbai. The following antibiotics are used for resistance test – Amikacin, Amoxyclav, Ampicillin, Methicillin, Oxacillin, Penicillin G, Cefaclor, Cefixime, Cefpodoxime, Ceftriaxone, Ciprofloxacin, Norfloxacin, Ofloxacin, Gentamicin,

Azithromycin, Erythromycin, Streptomycin, Vancomycin, Tetracycline and Chloramphenicol.

Molecular characterization

The strains identified were further subjected for molecular identification to confirm by analysing 16S r DNA sequence. Three strains were selected for 16S rDNA sequencing each representing from group of similar phenotypic characters.

Isolation of DNA [25].

2 ml of overnight grown Nutrient broth culture was centrifuged at 10,000 rpm at 4^0C for 10 minutes. The pellet was re suspended in 10 min 10mM Tris, 100 mM Sodium chloride solution and centrifuged at 10,000 rpm 4^0C for 10 minutes. After discarding the supernatant, the pellet was re suspended in 100 µl of $T_{50}E_{20}$ buffer containing 20µl of lysozyme (50mg/ml) and incubated at 37^0C for 20 min, in that solution 1µl of RNase (10 mg/ml) was added and incubated at room temperature for 20 minutes. To this mixture 100µl of SDS (2% in $T_{50}E_{20}$) was added and incubated at 50^0C for 45 min with proper mixing. 2µl of Proteinase K (20mg/ml) was added and incubated at 55^0C for 30 min. The sample was extracted in same volume phenol, Chloroform and Iso-amyl alcohol (25:24:1) and DNA was precipitated with one volume of isopropanol and 0.1 volume of 3M of Sodium acetate. The pellet was washed with 70% Ethanol, dried and dissolved in 100 µl of $T_{10}E_1$ buffer and stored at -20^0C for further use. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation). The DNA was stored at -20ºC for further use.

Polymerase chain reaction

PCR amplification was performed using Applied Biosystem verti thermal cycler. The primers for PCR amplification were obtained from Sigma-Aldrich.

Universal Primer [26]

27 forward – 5' AGAGTTTCCTGGCTCAG 3'

1492 reverse – 5' ACGGCTACCTTGTTACGATT 3'

The PCR was performed in 20µl reaction mixture containing 2µl of 10X assay buffer, 1µl dNTP mix of 2.5 mM, 0.5µl of mgcl2, 1µl each of forward and reverse primer (5pmol), 0.5µl of Taq polymerase, 1µl of template DNA and 13.5µl of HPLC grade water with the following amplification for 16s rDNA initial denaturation at 95ºC for 4 min followed by 38 cycles of denaturation, annealing and extension (94ºC for 1 min, 59.9ºC for 2 min and 72ºC for 2 min) and final extension at 72ºC for 20 min followed by hold for infinity at 4ºC. The presence of

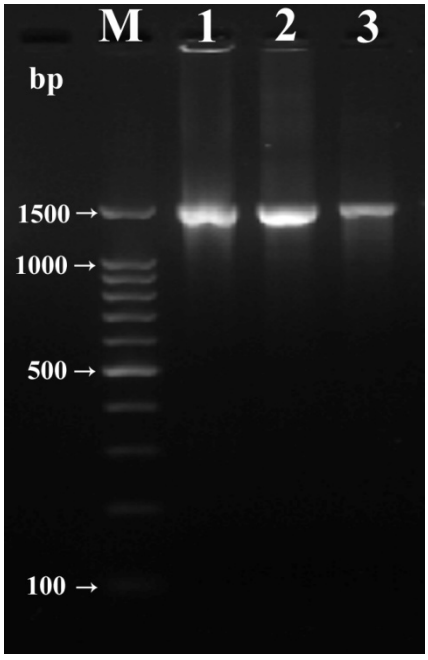
PCR products was determined by 2.5% agarose gel electrophoresis and to analyse the size of amplified PCR product DNA markers of 100bp was used which was provided by the Puregene. The amplified product was sent for sequencing to SciGenom Labs Pvt Ltd, Cochin, Kerala.

Construction of phylogenetic tree.

By using the sequence the bacteria were identified and constructed phylogenetic tree by using NCBI(http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome) and ClustalW (http://www.genome.jp/tools/clustalw/) websites.

RESULTS

A total of 375 *Staphylococcus* species were recovered from 392 milk samples based on Biochemical results. The species were Gram +ve cocci, +ve for Catalase, +ve and -ve for Mannitol, +ve for Vogues-Proskauer (VP), +ve and -ve for Carbohydrate fermentation, +ve and -ve for Coagulase test. The partial amplification of 16S rDNA (Fig 1) confirmed on the agarose gel electrophoresis that the isolates belong to *Staphylococcus aureus* (Fig 2), *Staphylococcus sciuri* (Fig 3) and *Staphylococcus saprophyticus* (Fig 4).



M- DNA Ladder 100bp, Lane 1, 2, 3 – Amplified DNA

Fig 1: Agarose gel electrophoresis to confirm the amplified DNA.

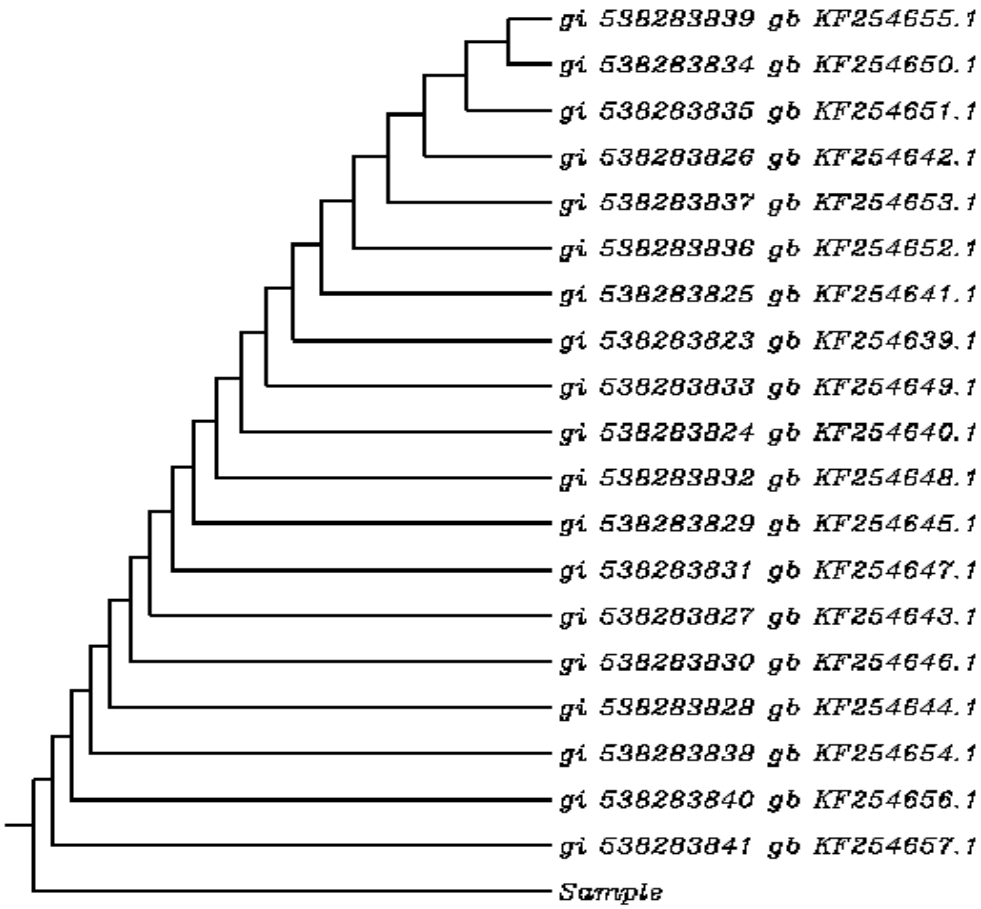


Fig 2: Phylogenetic tree of *Staphylococcus aureus*

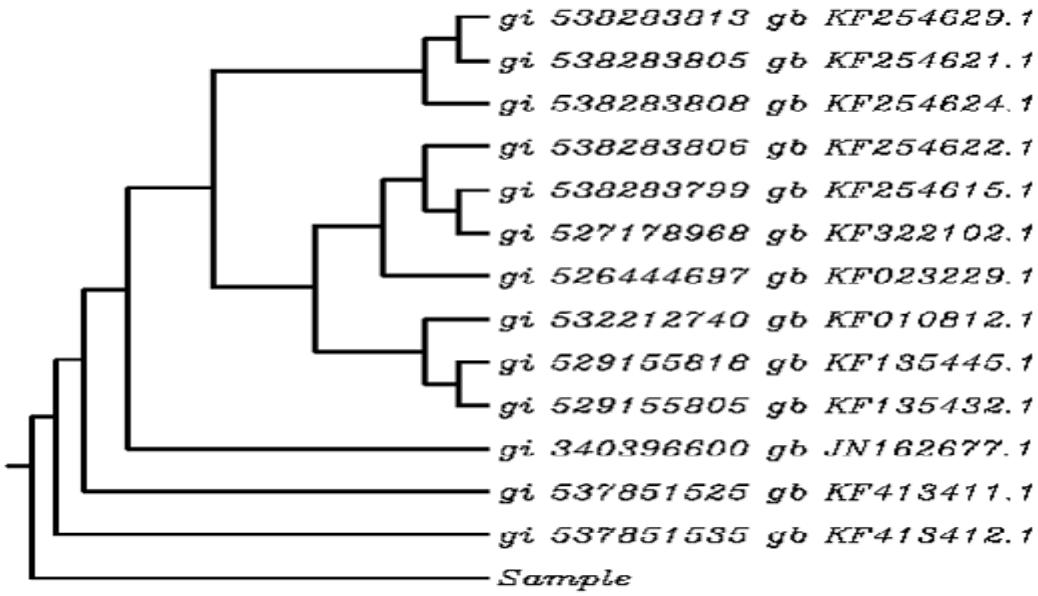


Fig 3: Phylogenetic tree of *Staphylococcus saprophyticus*

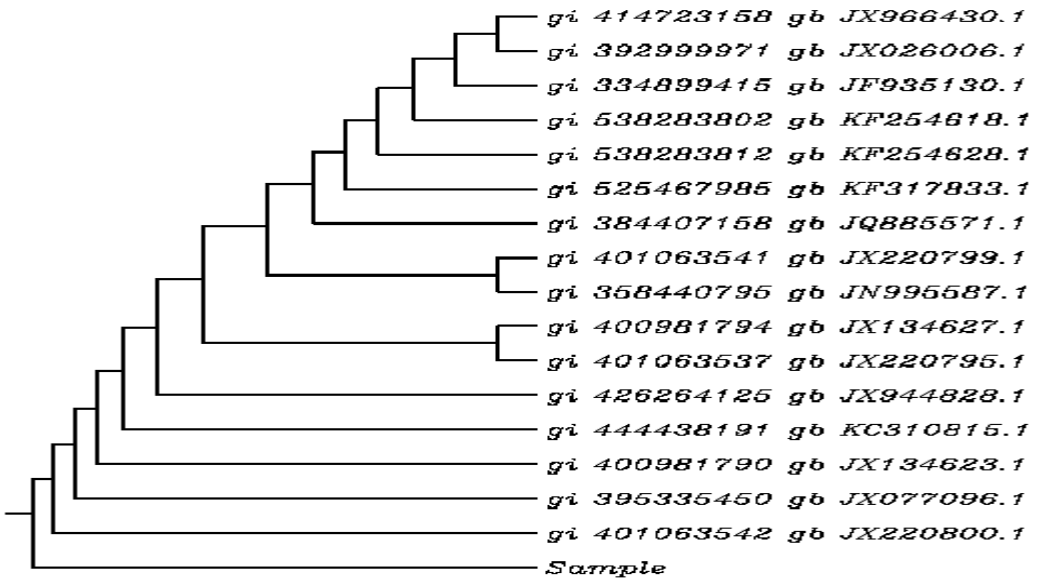


Fig 4: Phylogenetic tree of *Staphylococcus scuiri*

The antibiotic resistance test showed that the highest number of *Staphylococcus aureus* were resistant to Methicillin(100%) followed by Penicillin G (96.19%), Cefaclor (95.71%), Oxacillin (95.23%), Cefixime (91.90%), Ampicillin (88.57%), Cefpodoxime (86.19%), Streptomycin (41.42%), Ceftriaxone (36.61%), Norfloxacin (32.38%), Azithromycin (22.38%), Gentamicin (19.52%), Amikacin (16.66%), Amoxyclav (16.61%), Tetracycline (13.8%), Chloramphenicol (13.33%), Ciprofloxacin (6.66%), Ofloxacin (5.71%), Erythromycin (3.80%) and all isolated *Staphylococcus aureus* strains were susceptible to Vancomycin (Table 1). The antibiotic resistance test showed that the highest number of *Coagulase Negative Staphylococcus* were resistant to Methicillin(100%) followed by Penicillin G (95.75%), Cefpodoxime (91.51%), Ceftriaxone (87.27%), Oxacillin (80%), Cefixime (53.93%), Amoxyclav (52.72%), Erythromycin (52.12%), Cefaclor (51.51%), Ampicillin (50.9%), Amikacin (33.33%), Streptomycin (32.12%), Gentamicin (10.3%), Tetracycline (5.45%), Chloramphenicol (4.24%), Ciprofloxacin (3.63%), Norfloxacin and Azithromycin (2.42%), Ofloxacin (95.23%). All the isolated CNS strains were susceptible to Vancomycin (Table 1). The overall antibiotic susceptibility test revealed that the highest number of *Staphylococcus* species was resistant to Methicillin (100%) followed by Penicillin G (95.97%), Cefpodoxime (88.85%), Oxacillin (87.61), Cefaclor (73.61%), Cefixime (72.91%), Ampicillin (69.73%), Ceftriaxone (61.94%), Streptomycin (36.77%), Amoxyclav (35.16%), Erythromycin (27.96%), Amikacin (24.99%), Norfloxacin (17.4%), Gentamicin (14.91%), Azithromycin (12.4%), Tetracycline (9.62%), Chloramphenicol (8.78%), Ciprofloxacin (5.14%), Ofloxacin (3.76%) and all *Staphylococcus* strains were susceptible to Vancomycin (Table 1).

Table 1: Antibacterial Resistance pattern for isolated *Staphylococcus* species

Bacterial Isolates	Antibiotics Used																			
	N (%)	AK	AMC	AMP	MET	OX	P	CF	CFM	CPD	CTR	CIP	NX	OF	GEN	AZM	E	S	VA	TE	C
S.aureus	210 (56)	35 (16.66)	37 (17.61)	186 (88.57)	210 (100)	200 (95.23)	202 (96.19)	201 (95.71)	193 (91.90)	181 (86.19)	77 (36.61)	14 (6.66)	68 (32.38)	12 (5.71)	41 (19.52)	47 (22.38)	29 (3.80)	87 (41.42)	0 (0)	29 (13.80)	28 (13.33)
CNS	165 (44)	55 (33.33)	87 (52.72)	84 (50.90)	165 (100)	132 (80.00)	158 (95.75)	85 (51.51)	89 (53.93)	151 (91.51)	144 (87.27)	6 (3.63)	4 (2.42)	3 (1.81)	17 (10.30)	4 (2.42)	86 (52.12)	53 (32.12)	0 (0)	9 (5.45)	7 (4.24)
Total	375 (100)	90 (24.99)	124 (35.16)	270 (69.73)	375 (100)	332 (80.61)	360 (95.97)	286 (73.61)	276 (72.91)	332 (88.85)	221 (61.94)	20 (5.14)	72 (17.4)	15 (3.76)	58 (14.91)	51 (12.40)	115 (27.96)	140 (36.77)	0 (0)	38 (9.62)	35 (8.78)

CNS- Coagulase Negative *Staphylococcus*, N – No.of isolates, AK- Amikacin, AMC-Amoxyclav, AMP-Ampicillin, MET-Methicillin, OX-Oxacillin, P-Penicillin G, CF-Cefaclor, CFM-Cefixime, CPD-Cefpodoxime, CTR-Ceftriaxone, CIP-Ciprofloxacin, NX-Norfloxacin, OF-Ofloxacin, GEN-Gentamicin, AZM-Azithromycin, E-Erythromycin, S-Streptomycin, VA-Vancomycin, TE-Tetracycline, C-Chloramphenicol

DISCUSSION

In the Bovine mastitis the incorrect or incomplete treatment of animals also contributes significantly to the development of bacterial resistance against them. In the present study, the information on the *in vitro* activity of 20 antibacterial agents against staphylococcal strains isolated from bovine mastitis was studied. From the present study, a large number of the isolates were found to be resistant to earlier and established antibiotics compared to the newer developed antibiotics. Appearance of resistance against a particular antibiotic in a specific region may be due to its frequent and long-term use [27, 28].

The antibiotic resistant of this study revealed that the isolated *Staphylococcus aureus* showed resistant to multi drugs. These results were compared to the reports of [29], where the *Staphylococcus aureus* isolates were similar to Methicillin (100%) and higher to Penicillin G (62.1%), Ampicillin (56.3%), Amoxicillin (45.6%), Cloxacillin (17.5%), and Gentamicin (56.3%). The findings were similar to the findings of [30] were the resistance to the Penicillin (98.02%) and Ampicillin (84.62%). The results were higher to the reports of [31] where showed resistance to Penicillin(86.76%), Ampicillin (70.50%), Amoxicillin (63.23%) and Gentamycin (47.05%) and to the reports of [32], to Penicillin (41.44%), Streptomycin (25.65%), Erythromycin (13.81%), Tetracycline (11.84%), Ampicillin (3.94%) and also to the reports of [33], to Penicillin G (81.87%) and Ampicillin (81.40%).

The resistance to Penicillin G must be of concern, since this antibiotic represents the main antibiotic group recommended for S*taphylococcal* mastitis treatment and regular use of antibiotics for the treatment of cows may result in the spread of resistant strains. [29]. Antibiotic resistance is carried on plasmids and transposons which can pass from one *Staphylococcal* species to another [34].

It has been also reported that MRSA isolates that are resistant to beta-lactam antibiotics may develop induced resistance to Vancomycin [35]. But in the present study all the isolates were shown susceptible to Vancomycin but these results were contradictory to the reports of [36], in Ethiopia, where they reported the *Staphylococcus aureus* were resistance to Vancomycin (26.5%) and also to the reports of [37] in India, showed resistant to Vancomycin (1.63%) isolated from human clinical specimens. The presence of antibiotic-resistant *Staphylococcus aureus* has been reported to negatively affect the treatment of its associated infections in humans and animals [18, 38].

Coagulase Negative Staphylococcus (CNS) in cows has frequently been considered as minor udder pathogens, causing relatively less udder health problems. However, CNS infections may cause substantial herd problems due to high prevalence of subclinical and clinical mastitis [39]. The proportion of CNS among bacteria isolated from milk samples from clinical mastitis is very low in many countries [12].

The present study revealed that the antibiotic resistance to the isolated Coagulase Negative *Staphylococcus* (CNS) showed resistant to many drugs. However, these results were lower to the reports of [40] to the Erythromycin (73.2%), Oxacillin and Ampicillin (70.2%), Gentamicin (53.8%), Tetracycline (52.3%), Vancomycin (51.8%), Ciprofloxacin (26.9%), and to the reports of [41] were Ampicillin (70.59%), Gentamicin (57.06%), Amikacin (33.89%) and higher to the Penicillin (58.3%, 76.77%) respectively. This Penicillin resistance rate is also higher to the previous reports of other countries such as Korea (52.9%), Switzerland (31%), Finland (32%), and the USA (22.1%) [42, 43]. However the findings also similar to the reports of [10] were they showed 21.1% isolates were resistant to only one antibiotic (Penicillin) and 7.3% strains showed resistance to 2 or more drugs. Similarly, [44] have also reports that the percentage of antibiotic resistant in CNS was 97.7% for Ampicillin which is higher to our findings and 77.2% for Oxacillin, which is lower than our findings.

Staphylococcus infections are difficult to treat as many strains are resistant to antibiotics used in mastitis. Due to long-term and widespread use of β-lactam antibiotics for the treatment of mastitis treatment, many strains have become resistant to Penicillin, Ampicillin and Amoxicillin [45, 27]. However, the antimicrobial resistance of *Staphylococcus* species is increasing and the results of our study were in line with other reports. The present study demonstrated the presence of alarming level of resistance of frequently and commonly used antimicrobial agents to the isolated bacteria from Bovine mastitis. One of the important reasons for the failure of treatment of mastitis is the indiscriminate use of antibiotics without in vitro sensitivity of causal organisms [46]. Therefore, an examination of the antibiotic resistance profiles of the isolates may serve as a major tool in evaluating both the hygienic conditions employed during milking and the health hazards that humans may encounter when infected by antibiotic resistant strains [36] and also implementation of a systematic application of an *in vitro* antibiotic susceptibility test, earlier to the use of antibiotics in both treatment and prevention of Bovine mastitis is very important.

CONCLUSION

Staphylococcus aureus, *Staphylococcus sciuri* and *Staphylococcus saprophyticus* was isolated from the collected milk samples. Many isolates were showed multi drug resistant. Staphylococcus infections are difficult to treat as many strains are resistant to antibiotics used in mastitis. The development of antibiotic resistance in the bacteria that affects animal health is of growing concern in veterinary medicine. Therefore, the present study suggests the examination of the antibiotic resistance profiles of the isolates must be done earlier to the use of antibiotics in both treatment and prevention of Bovine mastitis.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, New Delhi, for funding the Interdisciplinary Program for Life Science Project (BT/PR/4555/INF/22/126/2010 dated 30-09-2010), Bioinformatics Infrastructure Facility Project (BT/BI/25/001/2006 VOL II dt 05-03-2012). and P. G Departments of Microbiology and Biotechnology Karnatak University, Dharwad for providing the facilities.

REFERENCES

1. Cristina Bogni1, Liliana Odierno, Claudia Raspanti, José Giraudo, Alejandro Larriestra, Elina Reinoso, Mirta Lasagno, Mirian Ferrari, Edith Ducrós, Cecilia Frigerio, Susana Bettera, Matías Pellegrino, Ignacio Frola, Silvana Dieser and Claudina Vissio., Science against microbial pathogens: communicating current research and technological advance. formatex 2011, 483-494
2. El-Khodery SA, Osman SA, Acute coliform mastitis in buffaloes (Bubalus bubalis): clinical findings and treatment outcomes. Trop Anim Health Prod 2008; 40:93–99.
3. Saini SS, Sharma JK, Kwatra MS, Prevalence and etiology of subclinical mastitis among crossbred cows and buffaloes in Punjab. Indian J Dairy Sci, 1994; 47:103–106.
4. Sadashiv SO and Kaliwal BB, Prevalence of Bovine mastitis in North Karnataka, India. Int. J. Pharm. & H. Care Res., 2013; 01(04);169 – 177.
5. Wang Y, Wu CM, Lu LM, Ren GWN, Cao XY and Shen JZ Macrolide-incosamideresistant phenotypes and genotypes of Staphylococcus aureus isolated from bovine clinical mastitis. Vet. Microbiol. 2008; 130:118–125

6. Melchior MB, Fink-Gremmels J, Gaastra W. Extended antimicrobial susceptibility assay for Staphylococcus aureus isolated from bovine mastitis growing in biofilms. Vet Microbiol. 2007; 125:141-9.
7. Kirkan S, Goksoy EO and Kaya O,. Identification and Antimicrobial Susceptibility of Staphylococcus aureus and coagulase negative staphylococci from bovine mastitis in the Aydin region of Turkey. Turk J Vet Anim Sci, 2005; 29:791-796.
8. Taponen S, Koort J, Bjorkroth J, Saloniemi H and Pyorala S, Bovine intra-mammary infections caused by coagulase-negative staphylococci may persist throughout lactation according to amplified fragment length polymorphism-based analysis. J Dairy Sci, 2007; 90: 3301-3307.
9. Waller KP, Aspa A, Nyman A, Persson Y and Andersson UG,.CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis Vet Microbiol, 2011; 152: 112-116.
10. Gentilini E, Denamiel G, Betancor A, Rebuelto M, Rodriguez Fermepin M, De Torrest RA, Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. J Dairy Sci, 2002; 85: 1913-1917.
11. Rajala-Schultz PJ., Smith KL., Hogan JS., Love BC.: Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. Vet Microbiol, 2004; 102: 33-42.
12. Pyörälä S and S Taponen, Coagulase-negative staphylococci emerging mastitis pathogens. Vet Microbiol, 2009; 134: 3-8.
13. Thatcher FS and Simon W, The resistance of staphylococci and streptococci isolated from cheese to various antibiotics. Can. J. Public Health. 1955; 46: 407-409.
14. Shitandi A, Sternesjö Á: Prevalence of multidrug resistant Staphylococcus aureus in milk from large and small-scale producers in Kenya. J Dairy Sci, 2004; 87:4145–4149.
15. Rychlik I, Gregorova D, Hradecka H: Distribution and function of plasmids in Salmonella enterica. Vet Microbiol, 2006; 112(1):1–10.
16. Waage S, Bjorland J, Caugant DA, Spread of Staphylococcus aureus resistant to penicillin and tetracycline within and between dairy herds. Epidemiol Infect 2002; 129:193–202.
17. Sears PM, McCarthy KK: Management and treatment of staphylococcal mastitis. Vet Clin North Am Food Anim Pract, 2003; 19:171–185.
18. Brouillette E, Malouin F, The pathogenesis and control of Staphylococcus aureus-induced mastitis: Study models in the mouse. Microbes Infect, 2005; 7:560–568.

19. Kaszanyitzky EJ., Janosi SZ., Egyed Z., Agost G., Semjen G.: Antibiotic resistance of staphylococci from humans, food and different animal species according to data of the Hungarian resistance monitoring system in 2001. Acta Vet Hung 2003; 51:451-464.
20. Malinowski E, Klossowska A, Kaczmarowski M, Lassa H, Kuzma K. Antimicrobial susceptibility of staphylococci isolated from affected with mastitis cows. Bull Vet Inst Pulawy, 2002; 46: 289-294.
21. Pesavento G, Ducci B, Comodo N, Lo Nostro A: Antimicrobial resistance profile of Staphylococcus aureus isolated from raw meat: A research for methicillin resistant Staphylococcus aureus (MRSA). Food Cont , 2007; 18(3):196–200.
22. Sampimon, OC., Coagulase-negative staphylococci mastitis in Dutch dairy herds. Thesis, Dutch Animal Health Service (GD), Deventer, The Netherlands, 2009.
23. Muhammad, G, Naureen A, Asi MN, Saqib M and Fazal-ur-Rehman (2010). Evaluation of a 3% surf solution (surf field mastitis test) for the diagnosis of subclinical bovine and bubaline mastitis. Trop. Ani. Health Prod., 42: 457–464.
24. Fall 2011 – Jackie Reynolds, Richland College, BIOL 2421.
25. Mary Suchita Xalxo, Characterization of crylla and crylle from native Bacillus thringiensis isolates, M.Sc Thesis University of Agricultural Sciences, Dharwad, 2006.
26. Lane DJ, 16S/23S rRNA sequencing. In Nucleic Acid Techniques in Bacterial Systematics, pp. 1991: 115–175. Edited by E. Stackebrandt & M. Goodfellow. New York: Wiley.
27. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH, Park YH, Joo YS and Koo HC Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J. Dairy Sci. 2007; 90: 1176–1185.
28. Kumar R, Yadav BR and Singh RS, Genetic Determinants of antibiotic resistance in Staphylococcus aureus isolates from milk of mastitic crossbred cattle. Curr. Microbiol. 2010; 60: 379–386.
29. Hulya turutoglu, Senay ercelik and Dilek ozturk, Antibiotic resistance of staphylococcus aureus and coagulase-negative staphylococci isolated from bovine mastitis. Bull Vet Inst Pulawy 2006: 50: 41-45
30. Mahantesh. M. Kurjogi and Basappa. B. Kaliwal, Prevalence and Antimicrobial Susceptibility of Bacteria Isolated From Bovine Mastitis , Adv. Apspl. Sci. Res, 2011; 2(6):229-235.
31. C. G. Unakal and B. B. Kaliwal, Prevalence and antibiotic susceptibility of Staphylococcus aureus from bovine mastitis. Veterinary World, 2010; 3(2):65-67

32. Muhamed Mubarack H, Doss A, Vijayasanthi M, R Venkataswamy, Antimicrobial drug susceptibility of Staphylococcus aureus from subclinical bovine mastitis in Coimbatore, Tamilnadu, South India Vet. World, 2012; 5(6): 352-355.
33. Costa GM, Paiva LV, Figueiredo HCP, Figueira AR, Pereira UP, Silva N, Population diversity of Staphylococcus aureus isolated from bovine mastitis in Brazilian dairy herds Research in Veterinary Science, 2012; 93:733–735.
34. Werckenthin C, Cardoso M, Martel JL, Schwarz S. Antimicrobial resistance in staphylococci from animals with particular reference to bovine Staphylococcus aureus, porcine Staphylococcus hyicus, and canine Staphylococcus intermedius. Vet Res, 2001; 32: 341-362.
35. Gündoğan N, Citak S, Yucel N, Devren A, A note on the incidence and antibiotic resistance of Staphylococcus aureus isolated from meat and chicken samples. Meat Sci, 2005; 69:807–810.
36. Deresse Daka, Solomon G, silassie and Dawit Yihdego, Antibiotic-resistance Staphylococcus aureus isolated from cow's milk in the Hawassa area, South Ethiopia Annals of Clinical Microbiology and Antimicrobials. 2012; 11:26.
37. Unakal CG and Kaliwal BB. "Vancomycin-Resistant Staphylococcus aureus Containing van A Gene Isolated from Clinical Samples of Community Health Care Centers of North Karnataka, India," Proceedings of International Society Biotechnology Conference (ISBT-2008), Gangtok, 28-30 December 2008; 464-468.
38. Moneoang MS, Bezuidenhout CC: Characterization of enterococci and E. coli isolated from commercial and communal pigs from Mafikeng in the North West Province, South Africa. Afr J Microbiol Res, 2009; 3(3):88–96.
39. Wilson DJ, Ruben N and Gonzalez H. Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects on somatic cell count and milk production. J Dairy Sci, 1997; 80:2592-2598.
40. Beytullah Kenar, Yahya Kuyucuoğlu and Esra Şeker, Antibiotic Susceptibility of Coagulase-Negative Staphylococci Isolated from Bovine Subclinical Mastitis in Turkey, Pak Vet J, 2012; 32(3): 390-393.
41. Kaliwal BB, Sadashiv SO, Mahantesh M Kurjogi, Rajeshwari D Sanakal, Prevalence and Antimicrobial Susceptibility of Coagulase- Negative Staphylococci isolated from Bovine Mastitis, Veterinary World, 2011; 4(4):158-161.

42. Pitkälä A, Haveri M, Pyorälä S, Myllys M and Honkanen-Buzalski T,. Bovine mastitis in Finland 2001 prevalence, distribution of bacteria, and antimicrobial resistance. Dairy Sci, 2004; 87: 2433-2441.
43. Rajala-Schultz PJ, Torres AH, Degraves FJ, Gebreyes WA and Patchanee P. Antimicrobial resistance and genotypic characterization of coagulase-negative staphylococci over the dry period. Vet Microbiol, 2009; 134: 55-64.
44. Ali A. AL-Edany , Mohammed H. Khudor , Khadeeja S.AL-Mousawi, Comparison of three indirect tests for the diagnosis of bovine subclinical mastitis caused by coagulase negative staphylococci with their susceptibility to seven antibiotics. Bas.J.Vet.Res, 2012; 11: 1.
45. Aarestrup FM, Wegener HC, Rosdahl VT, Jensen NE, Staphylococcal and other bacterial species associated with intramammary infections in Danish dairy herds. Acta Vet Scand 1995; 36: 475-487.
46. Amritha.G. Kulkarni and B. B. Kaliwal, Bovine Mastitis: A Review, Int.J.Rec.Sci.Res, 2013; 4(5):542 -548.