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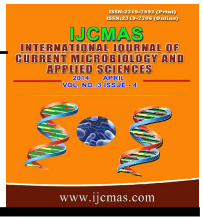

Original Research Article

Isolation, characterization and antibiotic resistance of *Bacillus sps.* from bovine mastitis in the region of north Karnataka, India

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A B S T R A C T

The present investigation was carried out to isolate the *Bacillus spps.* from clinical and subclinical Bovine mastitis milk and to determine antimicrobial susceptibility. 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 221 *Bacillus spps.* were recovered. The molecular characterization confirmed that the isolates belong to *Bacillus subtilis, Bacillus methylotrophicus, Bacillus gaemokensis, Bacillus cereus and Bacillus mycoides.* The isolates were subjected to the antibiotic resistance screening. The antibiotic resistance test showed that the isolated *Bacillus spps.* were resistant Methicillin (100%) followed by Penicillin G (91.40%), Oxacillin (80.54%), Cefixime (54.75%), Cefaclor (51.13%), Ampicillin (50.67%), Ceftriaxone (35.29%), Streptomycin (28.50%), Erythromycin (20.36%), Amikacin (17.64%), Norfloxacin (13.12%), Gentamicin (12.21%), Amoxyclav (10.40%), Cefpodoxime (8.59%), Tetracycline (7.69%), Chloramphenicol (6.33%), Azithromycin (5.42%), Ciprofloxacin (4.07%), Ofloxacin (2.26%) and all *Bacillus spps.* 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 must be done earlier to the use of antibiotics in both to choose appropriate antibiotic for treatment and prevention of Bovine mastitis.

K e y w o r d s

Bovine Mastitis, Milk, *Bacillus sps.* Antibiotics, Resistance

Introduction

Bovine Mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). It is a harmful disease affecting the dairy industry worldwide and is a matter of great concern for leading milk producing country like India because of the losses incurred due to high morbidity, discarded milk, treatment costs and reduced milk production, thus drawing in more attention

towards its treatment and control (Nihar *et al.,* 2013). Apart from the economic losses, mastitis can have serious implications on public health. Mastitis which is mostly caused by the interaction of multiple pathogenic agents (primarily bacteria), can expose human beings to various organisms through infected milk, thus serving as a media for transmission of various zoonotic diseases like T.B, brucellosis, diphtheria, scarlet fever and Q fever (Mahantesh and Kaliwal, 2011).

Mastitis is produced by a variety of pathogenic microorganisms. The majority of cases in bovines are infectious and it has been estimated that up to 200 microbial species are potential causative agents (Quinn *et al.,* 1994, Blowey and Edmondson, 1995). Cows and herds vary in susceptibility and extent, type and duration of infection, although some of mammary pathogens can be isolated from the environment of the cow, manure and bedding, water supplies, soil and inadequately cleaned milking machines (Jain, 1979 Philpot, 1979). In bovine mastitis bacteria isolated with greatest frequency are *Staphylococcus aureus, Staphylococcus spp., Bacillus spp., Corynebacterium spp., Escherichia coli, Streptococcus spp., Pseudomonas spp.,* and *Klebsiella spp*. (El-Khodery *et al.,* 2008).

Variation in prevalence of mastitis might be due to the different regions, breeds, therapeutic practices, management conditions and presence of microorganisms in environment (Sadashiv and Kaliwal, 2013). Bacteria belonging to the genus Bacillus have been associated with bovine, ovine, and porcine abortions worldwide. (Agerholm *et al.,* 1995; Kirkbride *et al.,* 1993).

The success of bovine mastitis therapy basically depends on the understanding of clinical presentation and antimicrobial susceptibility of the etiological agent, among various other factors (Miltenburg *et al.,* 1996) and the increased antimicrobial resistance of the organisms in animals treated with antibiotics and their zoonotic transmission continues to be a matter of great concern globally (Unakal and Kaliwal, 2010). The important reason for therapy failure in the management of mastitis could arise from various factors involving pathological changes in the udder, etiology, lower efficacy of antimicrobials, and improper veterinary services (Adesola, 2012)

Isolation, identification and characterization of mastitis pathogens are a fundamental aspect of milk quality and udder health control programs. There is a need to discuss public health and food safety issues associated with food borne pathogens found in the dairy environment. Because of worries about antimicrobial residues, antimicrobial resistance, milk quality and animal welfare, there is an increasing demand for development and evaluation of the milk culture method and rapid and accurate identification of bacterial species. Antibiogram studies of mastitis pathogens are important to suggest suitable antibiotic treatment to provide quality milk to the consumers and to prevent antibiotic resistance, potential health risk for humans (Nadeem *et al.,* 2013). Identification of mastitis pathogens, and their antimicrobial susceptibility is important when selecting appropriate treatment regimen (Sadashiv and Kaliwal, 2014). Therefore, the present investigation was designed to isolate, characterize the *Bacillus spps.* and their antibiotic resistance, 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 (Muhammad *et al.*, 2010)

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 *Bacillus spps.* was carried out using the standard method (Fall, 2011). Briefly, 100µl of aseptically collected milk samples from each sample was spread over a Nutrient Agar and incubated at 37°C for 24-48 hrs. After incubation, the selected colonies were subjected to Gram nature, morphological character.

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 rDNA sequence. Three strains were selected for 16S rDNA sequencing each representing from group of similar phenotypic characters.

Isolation of DNA

DNA Extraction was carried out using method modified from Mary Suchita Xalxo, 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°C 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°C 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 (Lane, 1991)

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 MEGA 5 Software.

Results and Discussion

A total of 221 *Bacillus* species were recovered from 392 milk samples based on Gram nature and morphological character. The partial amplification of 16S rDNA confirmed on the agarose gel electrophoresis. (Fig. 1).

By using NCBI and neighbour joining method in MEGA 5 the strains were identified as *Bacillus subtilis (Fig. 2), Bacillus methylotrophicus (Fig. 3), Bacillus gaemokensis (Fig. 4), Bacillus cereus (Fig. 5) and Bacillus mycoides (Fig. 6).*

The antibiotic susceptibility test to the revealed that the highest number of *Bacillus spps.* was resistant to Methicillin (100%) followed by Penicillin G (91.40%), Oxacillin (80.54%), Cefixime (54.75%), Cefaclor (51.13%), Ampicillin (50.67%), Ceftriaxone (35.29%), Streptomycin (28.50%), Erythromycin (20.36%), Amikacin (17.64%), Norfloxacin (13.12%), Gentamicin (12.21%), Amoxyclav (10.40%), Cefpodoxime (8.59%), Tetracycline (7.69%), Chloramphenicol (6.33%), Azithromycin (5.42%), Ciprofloxacin (4.07%), Ofloxacin (2.26%) and all *Bacillus spps.* were susceptible to Vancomycin (Table 1).

Bovine Mastitis is a result of interaction between three elements like bacteria, cow and environment (Mohammed *et al.,* 2012). In the present study *B.subtilis, B.methylotrophicus, B.gaemokensis, B.cereus* and *B.mycoides* have been isolated and characterized. Similarly Mohammed *et al.,* (2012) also isolated *Bacillus coagulans, B. cereus, B. subtilis, B. licheniformis, B. circulans, B. lentus, B. mycoides, B. amyloliquefaciens, B. megaterium* from Bovine mastitis. Nieminen *et al.,* (2007) also reported the presence of *B. pumilus B. licheniformis* and *B. cereus.* Parkinson *et al., (*1999) reported the Presence of Bacillus cereus from the mastitis milk. *Bacillus cereus* is recognised as being ubiquitous in the farm environment and the numbers of *Bacillus cereus* spores in soil rises throughout the winter (Davies and Wray, 1996). It is a common contaminant of milk at all stages of processing (Crielly *et al.,* 1994). The organism is not generally considered to be a primary mastitis pathogen, but causes mastitis after accidental introduction to the udder (Parkinson *et al.,* 1999). The presence of *Bacillus spps.* in the study may be due to the environmental factors like soil, water and manure, these are the main source of bacteria and when animals are exposed to soil, water and manure these bacteria infect animals via teat canals (Mohammed *et al.,* 2012). Therefore, the present study suggest that mastitis can be controlled by hygienic

conditions, cleaning manure, keeping the animals away from the stagnant water, washing udder before milking with germicidal solution.

The development of molecular biological methods, such as nucleic acid analysis, protein patterns or fatty acid profiles, has added possibilities for the rapid identification of bacteria (Busse *et al.*, 1996). Species-specific DNA sequences can be used for the identification of bacterial species. The 16s-23s rDNA has proven useful for identification of strains and species (Gurtler & Stanisich, 1996). The 16s rDNA sequencing makes it possible to identify and distinguish closely related bacterial species. In the present study the characterization of the *Bacillus spps.* have been done by using the 16s rDNA sequence. Similarly, many molecular tools like 16s-23s rRNA spacer regions used by Forsma *et al.*, (1997) PCR–DGGE method used by Ying *et al.*, (2009), 16s rDNA sequencing used by Shea Beasley (2004) for the identification of the bacterial species. The present molecular identification work suggest that, the 16s rDNA sequencing is more accurate for the species identification.

In the mastitis the improper or incomplete treatment of animals also contributes significantly to the development of bacterial resistance against them. The usage of antibiotics correlates with the emergence and maintenance of antibiotic-resistant traits within pathogenic strains (Shitandi *et al.*, 2004). These traits are coded for by particular genes that may be carried on the bacterial chromosome, plasmids (Rychlik *et al.*, 2006), hence these are easily transferred among isolates. In the present study, *In vitro* antimicrobial susceptibility test of twenty one antimicrobial agents was conducted and studied against isolated 221 *Bacillus spps.* The most commonly used antibiotics on conventional dairies were Penicillin, Cephalosporin and Tetracycline's. For mastitis, Penicillin, Ampicillin and Tetracycline's were commonly used (Mohammed *et al.*, 2012). The antibiotic resistant of the present study revealed that the isolated *Bacillus spps.* showed resistant to multi drugs. These results were in line with the reports of Mohammed *et al.*, (2012), were the resistance of Ampicillin(84%), Cefotaxime (77%), Ceftizoxime (55%), Amikacin and Ofloxacin (25%) and Tetracycline (17%). The reports were higher to the reports of Firaol *et al.*, (2013) to the Penicillin G (66.67%), lower to Chloramphenicol (88.89%) and Gentamycin (100%). From the study, a large number of isolates were found to be resistant to previous 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 (Moon *et al.*, 2007; Kumar *et al.*, 2010)

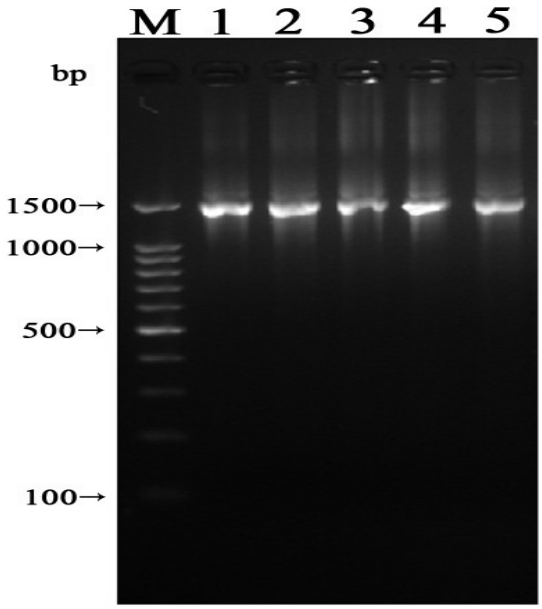
The resistance to the Penicillin G is also in line with the work of Nadeem *et al.*, (2013). The resistance to Penicillin G must be of concern, since this antibiotic represents the main antibiotic group recommended for mastitis treatment and regular use of antibiotics for the treatment of cows may result in the spread of resistant strains (Hulya *et al.*, 2006; Nadeem *et al.*, 2013). The prevalence of antibiotic resistance usually varies between isolates from the different sampled places and even between isolates from different herds on the same farm(Waage *et al.*, 2002).

Table.1 Antibacterial Resistance pattern for isolated *Bacillus Sps.*

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
Bacillus Spps.	221	39	23	112	00	178	202	113	121	19	78	09	29	5	27	12	45	63	00	17	14
%		17.64	10.40	50.67	100	80.54	91.40	51.13	54.75	8.59	35.29	4.07	13.12	2.26	12.21	5.42	20.36	28.50	00	7.69	6.33

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

Fig.1 Agarose gel electrophoresis to confirm the amplified DNA



M- DNA Ladder 100bp
Lane 1, 2, 3, 4, 5 – Amplified DNA

Fig.2 Phylogenetic tree of *Bacillus subtilis*

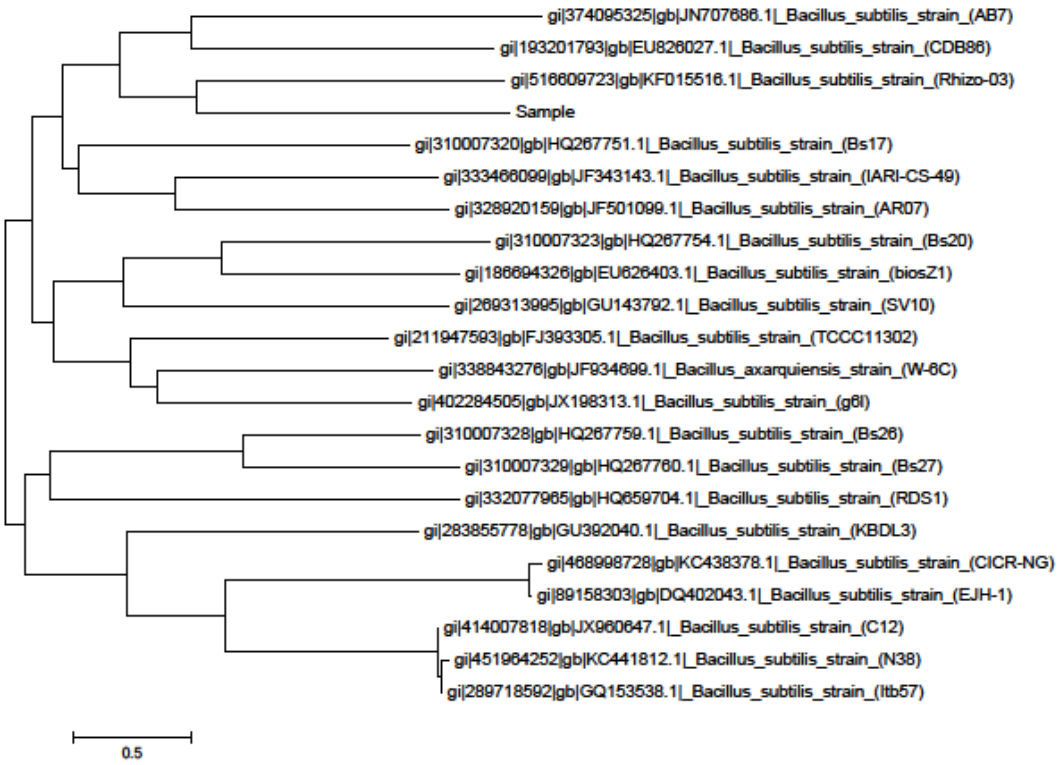


Fig.3 Phylogenetic tree of *Bacillus methylotrophicus*

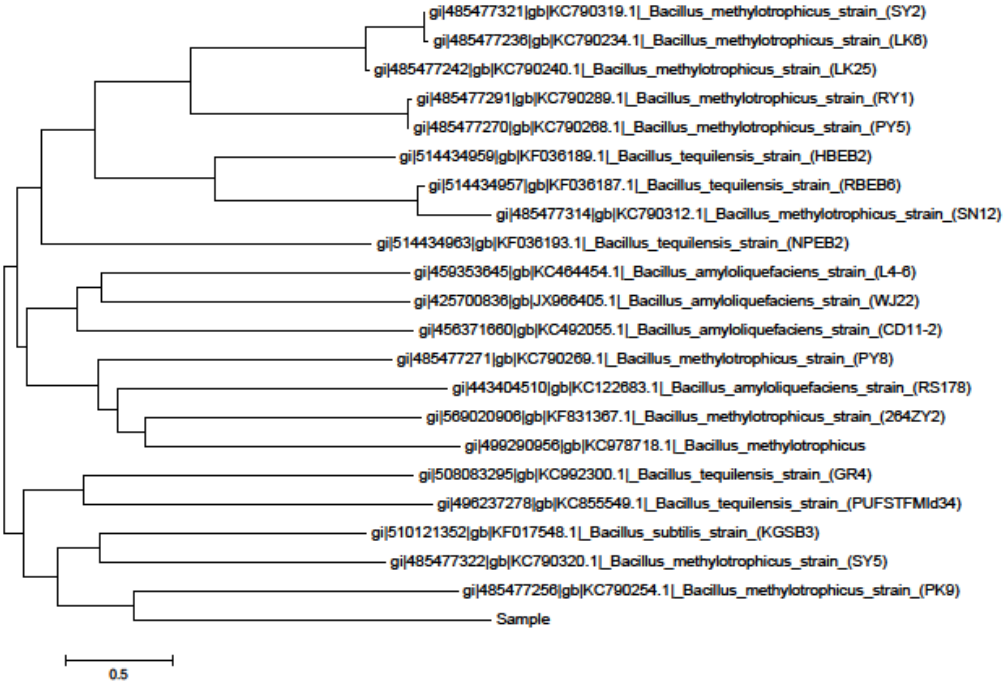


Fig.4 Phylogenetic tree of *Bacillus gaemokensis*

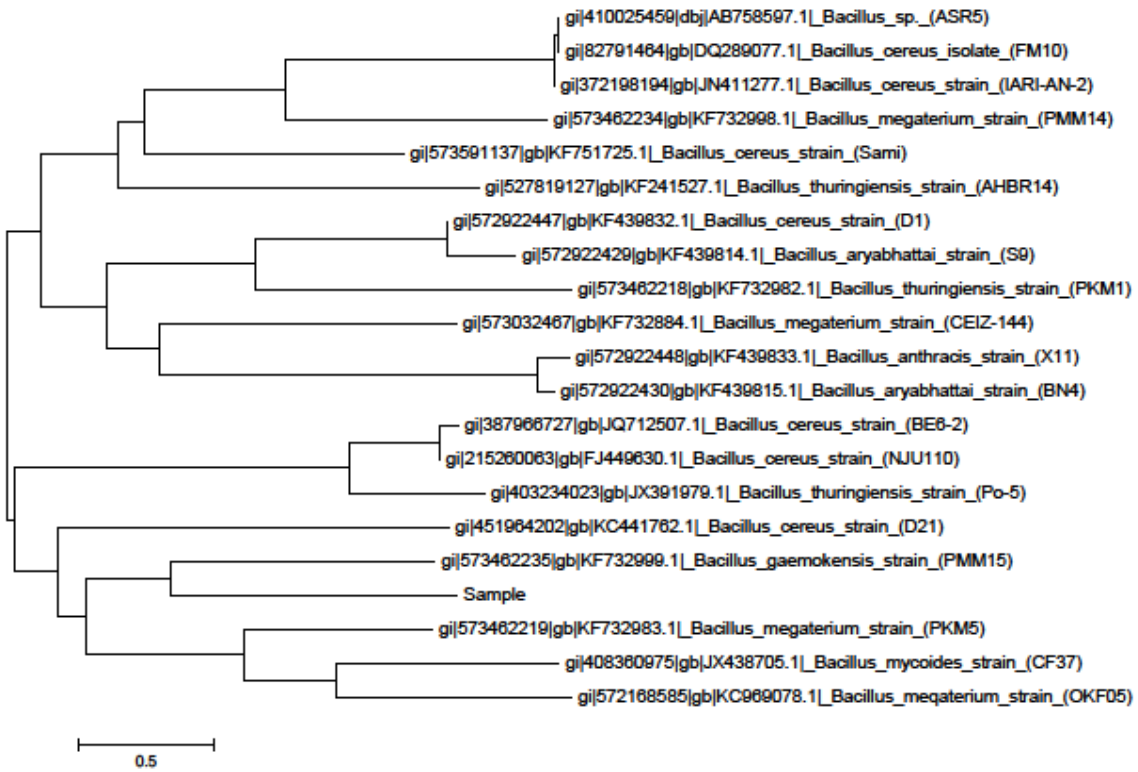


Fig.5 Phylogenetic tree of *Bacillus cereus*

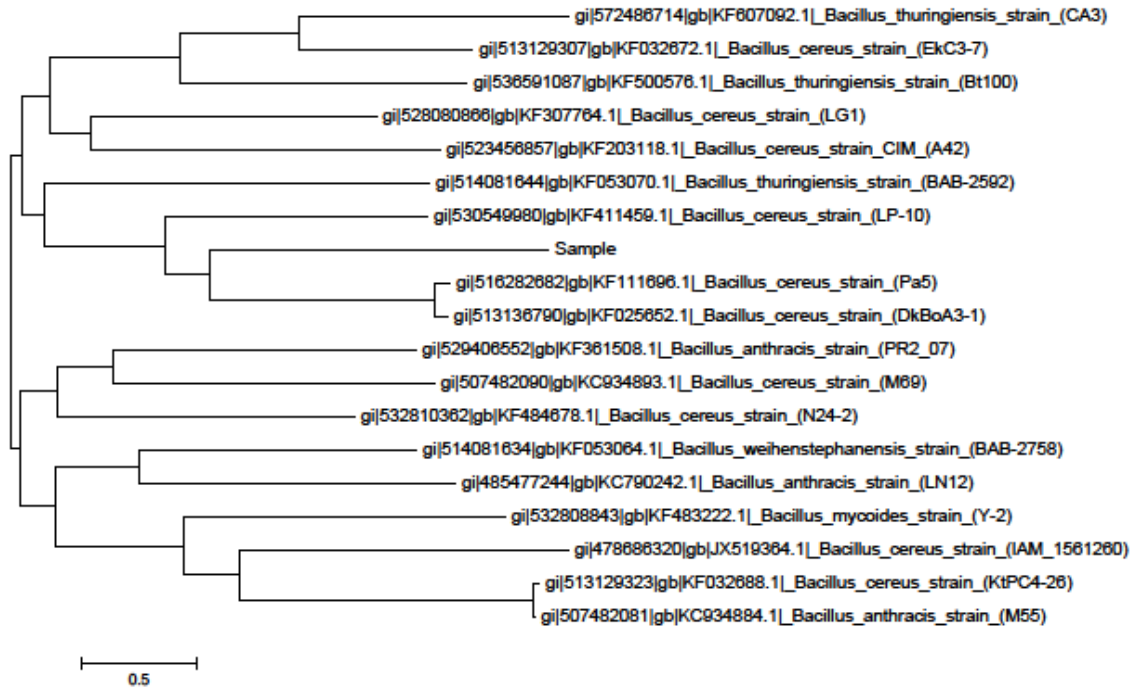
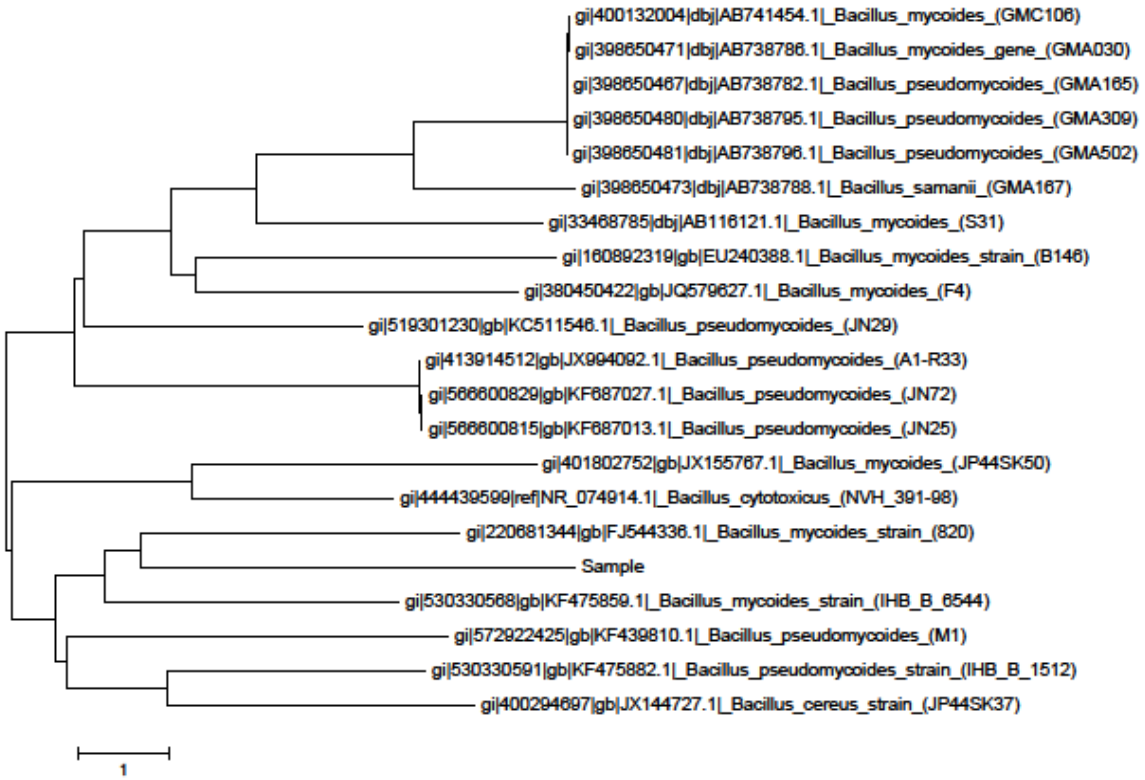


Fig.6 Phylogenetic tree of *Bacillus mycoides*



Antibiotic resistance is carried on plasmids and transposons which can pass from one species to another (Werckenthin *et al.*, 2001). The possible reason for detection of this species of resistant bacteria at high prevalence supports its contagious nature that gives the chance to circulate and infect the udder once introduced to a heard (Sharif *et al.*, 2009).

It is possible that mastitogenic bacteria can lose the sensitivity to antibiotics over the time or even acquire sometimes this feature (Edward *et al.*, 2008). It is necessary to monitor mastitis pathogens to assess any changes in their antibiotic resistance patterns. Careful use of antibiotics can avoid the increase and dissemination in antimicrobial resistance arising from the use of antimicrobial drugs in animals (Fluit *et al.*, 2006; Schwarz *et al.*, 2001). Also previous investigations of Malinowski *et al.*, (2006) showed that recovery rates from acute mastitis equals "zero" if the pathogens were resistant to antibiotics used to intrammmary treatment. Lack of stringent regulations and monitoring in the dispensing and use of antimicrobials in veterinary establishments and mass inoculation of herds of animals by some farmers has risen as a contributory factor to increase antimicrobial resistance (Alemu *et al.*, 2012).

Some natural products like Bacteriocin may be used as an alternative type of antibiotic(Kaur *et al.*, 2012). These natural agent which may inhibit growth of huge number of microbes. They exist in various ecological niches. (Suneel and Kaliwal 2013) have Reported the bacteriocins produced by the *Lactococcus garvieae* shown antibacterial effect against the *B. cereus* and *B.subtilis.*

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. Important reasons for the failure of treatment of mastitis are the indiscriminate use of antibiotics without in vitro sensitivity of causal organisms (Amritha and Kaliwal, 2013). 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 (Deresse Daka *et al.,* 2012) 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.

Bacillus spps. were isolated and characterized from the collected milk samples. Many isolates of *Bacillus spps.* were showed multi drug resistant. It is 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 to choose appropriate antibiotic for treatment and prevention of Bovine mastitis.

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References

Acar, J. and Rostel, B, 2001. Antimicrobial resistance: An overview. Rev. Sci. Technol. 20: 797-810

Adesola A. E, 2012. Antimicrobial Resistance Pattern of Streptococci and Staphylococci Isolated from Cases of Bovine Clinical Mastitis in Nigeria. Nat. Sci. 10(11):96-101.

Agerholm JS, Krogh HV, Jensen HE, 1995. A retrospective study of bovine abortions associated with *Bacillus licheniformis.* J Vet Med B 42:225–234,

Alemu, S. and B.M. Zewde, 2012. Prevalence and antimicrobial resistance profiles of Salmonella enterica serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. Trop Anim Health Prod., 44: 595-600. DOI 10.1007/s11250-011-9941-y.

Amritha.G. Kulkarni and B. B. Kaliwal, 2013. Bovine Mastitis: A Review, Int.J.Rec.Sci.Res,; 4(5):542 -548.

Blowey, R. and Edmondson, P. 1995, Mastitis Control in Dairy Herds: an Illustrated and Practical Guide. Ipswich, UK: Farming Press Books.

Busse, H.-J., Denner, E. B. M. & Lubitt, W, 1996. Classification and identification of bacteria: current approaches to an old problem. Overview of methods used in bacterial

systematics. J Biotechnol, 47, 3-38.

Crielly EM, Logan NA, Anderton A, 1994. Studies on the *Bacillus* flora of milk and milk products. Journal of Applied Bacteriology 77, 256- 63,

Davies RH, Wray C, 1996. Seasonal variation in isolation of *Salmonella typhimun'um, Salmonella enteritidis, BaCIllus cereus,* and *Closm'dium perfringens* from environmental samples. Zentralblatt fUr Veterinarmedizin 43, 119-27,

Deresse Daka, Solomon G, silassie and Dawit Yihdego, 2012. Antibiotic-resistance Staphylococcus aureus isolated from cow's milk in the Hawassa area, South Ethiopia Annals of Clinical Microbiology and Antimicrobials. 11:26.

Edward Malinowski, Henryka Lassa, Sebastian Smulski, Anna Kłossowska, And Michał Kaczmarowski, 2008. Antimicrobial susceptibility of bacteria isolated from cows with mastitis in 2006-2007, Bull Vet Inst Pulawy 52, 565-572

El-Khodery SA, Osman SA, 2008. Acute coliform mastitis in buffaloes (Bubalus bubalis): clinical findings and treatment outcomes. Trop Anim Health Prod; 40:93–99.

Fall 2011 – Jackie Reynolds, Richland College, BIOL 2421.

Firaol Tam 1 iru, 2Sefinew Alemu and 3Awoke Tsega, 2013. Aerobic Microorganisms Isolated from Mastitic Bovine Milk and Their Antimicrobial Susceptibility Profiles, Ethiopia., *Am-Euras. J. Agric. & Environ. Sci., 13 (7): 920-925*

Fluit A.C., van der Bruggen J.T., Aarestrup F.M., Verhoef J., Sen W.T.M, 2006. Priorities for antibiotic resistance surveillance in Europe. Clin Microbiol Infect, 12, 410-417.,

Forsma Pa ivi, Ti Isa la-Ti misjarvi An u and Alatossava Ta pan I, 1997. Identification of staphylococcal and streptococcal causes of bovine mastitis using 16s-23s rRNA spacer regions. Microbiology, 143; 3491-3500

from large and small-scale producers in Kenya. J Dairy Sci, 2004; 87:4145–4149.

Gurtler, V. & Stanisich, V. A, 1996. New approaches to typing and identification of bacteria using the 16s-23s rDNA spacer region. *Microbiology, 142,* 3-16**.**

Hajime Nagahatab, Yasunori Tanji a, 2009. Characterization of bacterial population of raw milk from bovine mastitis by culture-independent PCR–DGGE method. Biochemical Engineering Journal, 45; 76–81

Hulya turutoglu, Senay ercelik and Dilek ozturk, 2006. Antibiotic resistance of staphylococcus aureus and coagulase-negative staphylococci isolated from bovine mastitis. Bull Vet Inst Pulawy: 50: 41-45

Jain, N.C., 1979. Dommon pathogens and factors in infection and mastitis. J. Dairy Sci ., 62: 128-134.

Kaur B, Garg N, Sachdev A, 2013. Optimization of bacteriocin production in pediococcus acidilactici ba 28 using response surface methodolog. Asian J Pharm Clin Res, 6;192-195

Kirkbride CA, 1993. Diagnoses in 1,784 ovine abortions and stillbirths. J Vet Diagn Invest **5:**398–402

Kumar R, Yadav BR and Singh RS, 2010. Genetic Determinants of antibiotic resistance in Staphylococcus aureus isolates from milk of mastitic crossbred cattle. Curr. Microbiol. 60: 379–386.

Lane DJ, 1991. 16S/23S rRNA sequencing. In Nucleic Acid Techniques in Bacterial Systematics, pp.: 115–175. Edited by E.

Stackebrandt & M. Goodfellow. New York: Wiley

Mahantesh, M. K. and Basappa, B. K, 2011. Prevalence and antimicrobial susceptibility of bacteria isolated from bovine mastitis. Adv. Appl. Sci. Res 228(6):229-235

Malinowski E., Niewitecki W., Nadolny M., Lassa H., Smulski S, 2006. Effect of lysozyme dimer injections on results of intramammary treatment of acute mastitis in cows. Vet Med, 62, 1395-1399.).

Mary Suchita Xalxo, Characterization of crylla and crylle from native Bacillus thringiensis isolates, M.Sc Thesis University of Agricultural Sciences, Dharwad,

Miltenburg, J. D., de Lange D., Crauwels, A.P.P., Bongers, J.H., Tielen, M.J.M., Schukken, Y.H. and Elbers, A.R.W, 1996. Incidence of clinical mastitis in a random sample of dairy herds in the southern Netherlands. Vet. Rec. 139: 204- 207.

Mohammed Salih RR and Mohamed Ahmed FA, 2012. Treatment Trial of Bovine Bacterial Mastitis in Khartoum State, Sudan. World's Vet. J. 1(1): 20-24.

Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH, Park YH, Joo YS and Koo HC, 2007. Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J. Dairy Sci.; 90: 1176–1185.

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.]

Nadeem Akram, Azhar H. Chaudhary, Shabbir Ahmed, Manzoor A. Ghuman, Ghazala Nawaz, Sajjad Hussain, 2013. Isolation of bacteria from mastitis affected bovine milk and their antibiogram, *European Journal of Veterinary Medicine*, 2 (1): 38-46

Nadeem Akram, Azhar H. Chaudhary, Shabbir Ahmed, Manzoor A. Ghuman, Ghazala Nawaz, Sajjad Hussain, 2013. Isolation of bacteria from mastitis affected bovine milk and their antibiogram, *European Journal of Veterinary Medicine*, 2 (1): 38-46

Nieminen. T, Rintaluoma. N, Andersson A, Taimisto M, Ali-Vehmas T, Sepp¨al¨a A, Priha O M, Salkinoja-Salonen M, 2007. Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk doi:10.1016/j.vetmic.2007.05.015

Nihar Nalini Mohanty, Priyaranjan Das, Shaswati Subhadarsini Pany, Laxmi Narayan Sarangi, Siddharth Ranabijuli and Hemant Kumar Panda, 2013. Veterinary World, Vol -06: 739-743

Parkinson T.J., Merrall M. & Fenwick S.G, 1999. A case of bovine mastitis caused by Bacillus cereus, New Zealand Veterinary Journal, 47:4, 151-152.

Rychlik I, Gregorova D, Hradecka H, 2006. Distribution and function of plasmids in Salmonella enterica. Vet Microbiology, 112(1): 1-10.

Sadashiv S O and Kaliwal B B, 2013. Prevalence of Bovine mastitis in North Karnataka, India. Int. J. Pharm. & H. Care Res. 01(04);169 – 177.

Sadashiv S. O and B. B. Kaliwal, 2014. Antibiotic resistance of *Staphylococcus aureus* and Coagulase-Negative Staphylococci (CNS) isolated from Bovine mastitis in the region of north Karnataka, India, *World Journal of Pharmaceutical Research*., Vol.-3(01): 571-586

Samad MA, 2008. Animal Husbandry and

Veterinary Science, volume II, LEP pub no.11, Bangladesh

Schwarz S., Kehrenberg C., Walsh T.R, 2001. Use of antimicrobial agents in veterinary medicine and food animal production. Int J Antimicrob Agents, 17, 431-437.).

Sharif, A., M. Umer and G. Muhammad, 2009. Mastitis Control in Dairy Production. J. Agric. Soc. Sci., 5: 102-105.

Shea Beasley, 2004. Isolation, identification and exploitation of lactic acid bacteria from human and animal microbiota. presentation on October 29th, 2004. Forestry Sciences of the University of Helsinki for public criticism in auditorium 2041 at the University of Helsinki,

Shitandi A, Sternesjö Å: Prevalence of multidrug resistant Staphylococcus aureus in milk from large and small-scale producers in Kenya. J Dairy Sci, 2004; 87:4145–4149.

Suneel Dodamani and Basappa Kaliwal, 2013. Production of bacteriocin by lactococcus garvieae and influence of various supplements on its antimicrobial activity. American Journal Of Pharm Research, 3:(12).

Unakal, C. G. and Kaliwal, B. B, 2010. Prevalence and antibiotic susceptibility of Staphylococcus aureus from bovine mastitis. Veterinary World 3(2):65-67.

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.

Werckenthin C, Cardoso M, Martel JL, Schwarz S, 2001. Antimicrobial resistance in staphylococci from animals with particular reference to bovine Staphylococcus aureus, porcine Staphylococcus hyicus, and canine Staphylococcus intermedius. Vet Res, 32: 341-362

Ying Kuang, Kaori Tani, Aidan J. Synnott, Kazuhito Ohshima, Hidetoshi Higuchi,Hajime Nagahata, Yasunori Tanji, 2009. Characterization of bacterial population of raw milk from bovine mastitis by culture-independent PCR–DGGE method. Biochemical Engineering Journal 45 (2009) 76–81