

Research Article

EMERGENCE OF COAGULASE POSITIVE METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BUFFALO MASTITIS MILK SAMPLES

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Received 12 September 2018, revised 28 November 2018

ABSTRACT: The present study was undertaken to screen methicillin resistant *Staphylococcus aureus* (MRSA) in mastitis buffalo milk samples from northern India. A total of 120 buffalo mastitis milk samples were collected from individual quarter over a one year period mainly from animals reared by marginal farmers. The isolates were presumptively confirmed by growing the organism in selective media and biochemical tests. A total of 52 samples were presumptively confirmed as Staphylococci on the basis of growth in Mannitol Salt Agar (MSA); out of which 12 samples were coagulase positive *Staphylococcus aureus* (*S. aureus*) by growth in Baird Parker Agar (BPA) and tube coagulase test. Antibacterial profiling and growth on Methicillin resistant *Staphylococcus aureus* (MeReSa) Agar indicated five isolates as MRSA. Further PCR of specific gene segments and their subsequent characterization based on 16S ribosomal RNA, of staphylo-coagulase and *mecA* confirmed the findings. The study accentuates the importance of determining the causal agents of mastitis, antibacterial profiling so as to enable the clinicians and researchers to intervene and adopt effective control measures.

Key words: MRSA, Buffalo, Staphylo-coagulase, Antibiotic profiling.

INTRODUCTION

Buffalo (*Bubalus bubalis*), a predominantly well adapted tropical animal is the lifeline of dairy economy while providing source of livelihood and nutritional security to a vast majority of the Indian population (NDDDB 2012). India alone contributes 17% of the world milk production, of which buffalo milk share is more than 55% (FAO 2012). Mastitis is an inflammatory condition of the mammary gland primarily caused by diverse microbial pathogens. The problem is aggravated in recent years by the emergence of antimicrobial resistant pathogens and the livestock associated zoonotic pathogens (Pantosti 2012, Paterson *et al.* 2014).

Staphylococcus aureus (*S. aureus*) is recognized as the major bacterial etiological agent causing subclinical and clinical mastitis in the dairy herds worldwide (Bramley 1992). Staphylocoagulase (*coa*), encoded by virulence-associated gene cluster present in the core genome of the bacteria, is produced by some of the pathogenic strains of *S. aureus*. This polypeptide binds and activates

prothrombin, converting fibrinogen to fibrin and promoting clotting of plasma or blood which triggers abscess formation and bacterial persistence in the mammary tissues (Cheng *et al.* 2010). *S. aureus* exhibits a remarkable propensity to develop antimicrobial resistance to most of the commonly used antibiotics; the resistance mechanism of which the bacteria acquire by horizontal gene transfer Lindsay (2014) or through spontaneous mutations and positive selection (Pantosti *et al.* 2007). Methicillin resistant *S. aureus* (MRSA); which initially emerged as human nosocomial infections, has spread in the dairy herds in several countries (Kock *et al.* 2010). The resistance mechanism has been attributed to the staphylococcal chromosomal cassette (SSC) *mec* elements, a part of which (*mecA* fragment) encodes the penicillin-binding protein 2a (PBP2a) which confers resistance to most of all types of β -lactam antibiotics (Hiramatsu *et al.* 2013).

In developing countries like India, diagnosis of causal agents of mastitis relies on conventional growth of the

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organism in specific media, phenotypic characterization by different biochemical tests and antibiogram profiling by conventional assays which is time consuming and lack both sensitivity and specificity (Chambers 1997). With the advancement of rapid high throughput robust nucleic acid amplification techniques like PCR, the sensitivity and specificity of detecting the pathogen has increased manifold. Most of the conventional PCR have targeted the 16S ribosomal RNA (rRNA) gene fragment to identify the organism; while the putative virulence and antibiotic resistance genes have been characterized to trace the polymorphism within the strains (Paterson *et al.* 2014).

Though there are several reports by different researchers for identification of the causal agents of subclinical/clinical mastitis from India; few reports are there on the identification of MRSA as the cause of clinical mastitis in the buffalo population. Thus the present study was undertaken to screen the MRSA as the cause of clinical mastitis in buffaloes reared by marginal farmers in small dairy herds located in Uttar Pradesh, India.

MATERIALS AND METHODS

Microbiological and biochemical analysis

Milk samples were collected from buffaloes (n=120) of different parts of the Bareilly district of Uttar Pradesh,

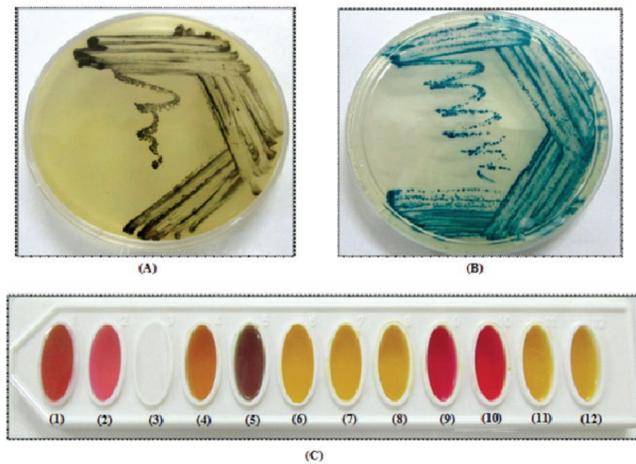


Fig. 1. (A) Growth of coagulase positive *S.aureus* in Baird Parker media with egg yolk and Sodium terrulite supplementation; (B) Growth of MRSA in Hichrome MeReSa Agar Base using MeReSa Selective Supplement; (C) Biochemical tests as performed by HiStaph™ Identification.

(Kit- Well 1: Voges Proskauer's test- (+), Well 2: Alkaline Phosphate - (+), Well 3: ONPG - (-), Well 4: Urease - (+), Well 5: Arginine utilization - (+), Well 6: Mannitol - (+), Well 7: Sucrose - (+), Well 8: Lactose - (+), Well 9: Arabinose - (-), Well 10: Raffinose - (-), Well 11: Trehalose - (+), Well 12: Maltose- (+).

India and affected udder quarter from individual animal showing clinical signs of mastitis over a one year period. The animals were mainly reared by marginal farmers having small herd size in North India (Uttar Pradesh). Initial identification of *S. aureus* was done by inoculating the milk sample in 5% sheep blood agar media and incubated at 37°C for 24 hours under aerobic condition. The presumed Staphylococcal isolates having translucent, creamy white colonies showing hemolysis were sub-cultured to Mannitol Salt Agar (HiMedia Laboratories, India) and Baird Parker Agar (HiMedia Laboratories, India) with egg yolk and potassium terrulite supplementation to confirm the presence of pathogenic *S. aureus* and coagulase positive *S. aureus* respectively. Culture smear microscopy was done by standard Gram-staining procedure for all the pure cultures. Standard biochemical tests for confirmation of *S. aureus* were done by HiStaph™ Identification Kit (HiMedia Laboratories, India) following manufacturer's recommendations. Further screening for the presence of coagulase activity by Tube coagulation test was done according to the procedure recommended by Quinn *et al.* (1994). MRSA was identified by streaking individual colonies from either Mannitol Salt Agar or Baird Parker Agar to Hichrome MeReSa Agar Base using MeReSa Selective Supplement (HiMedia Laboratories, India) with overnight incubation at 37°C.

Buffalo-1/MRSA/2014/Ind KJ509926	YVYHANGQVS YGARPTQKRP SKTNAINVTI HADGTATYGP RVTK
JKS-11 India KF923237N.QVS..A .P.Q
KVAFSU-109/11 India JX240356YK.....
Z172 Taiwan CP006838
55/2053 USA CP002388
T0131 China CP002643
CW1 Korea CP003979N.QVS..A .P.Y
Isolate 1 Poland EU490368Y.....
Rmb9393 Brazil CP005288
MW2 Japan BA000033N.QVS..A .P.Y
EGY1 Egypt KF846303R.....N.QVS..A .P.Y
ST398 Netherlands AM990992N.QVS..A .P.Y
IFH467 Bovine Japan AB373757YK.....N.QVS..A .P.Q
JKD6008 ST239 New Zealand CP002120S.....E.....Q .N.P .K.....

Fig. 2. Multiple sequence alignment report of deduced amino acid of Buffalo-1/MRSA /2014/India of staphylocoagulase gene with different *S. aureus* isolates. Sequence isolate name, country and the NCBI nucleotide Accession numbers are given in the parenthesis.

Nucleic acid amplification of the 16S rRNA, *coa* and *mecA* gene segments

Extraction of Nucleic Acid: Single colonies grown either on Mannitol Salt Agar or Baird Parker Media or MeReSa Agar were inoculated in nutrient broth and grown overnight at 37°C. Bacterial DNA was extracted from 5 ml of overnight grown cultures by HiPurA™ Bacterial Genomic DNA Purification Kit (HiMedia Laboratories, India) following the manufacturer's recommended protocol specific for Gram positive bacteria. The extracted DNA was assessed for quantity and purity using NanoDrop Spectrophotometer (ND-1000, Thermo Fisher Scientific, USA) and stored at -20°C until further use.

Amplification of Nucleic Acid: Universal primers were employed for the amplification of 16S rRNA for molecular identification of the bacterial species; while *coa* and *mecA* specific primers of *S. aureus* were used for the amplification of the respective genes. The details of the primers have been enumerated in Table 1. The primers were custom synthesized from Eurofins Genomics India Pvt. Ltd (Bengaluru, India). PCR was performed using approximately 100 ng of bacterial genomic DNA, 25µl of 2X KOD Hot Start Master Mix (Novagen®, EMD Chemicals Inc, San Diego, CA, USA), 10 µM of the different primer combination for the respective gene fragments and the volume was made up to 50 µl with Nuclease Free water (NFW). The PCR amplification was performed in a thermo-cycler

(Mastercycler® personal, Eppendorf, Hamburg, Germany) with the cycling parameters as mentioned in Table 1. A non-template control was run in all the PCR experiments to rule out the possibility of contamination in the samples. The PCR amplified products were resolved either on 1% or 1.5% agarose gel in 1X Tris acetate EDTA (TAE) buffer depending on the expected size of the amplified products. The agarose gel stained with ethidium bromide was visualized under UV light in a gel documentation system (Molecular Imager® Gel Doc™ XR+ System, BioRad, CA, USA).

Sequencing of the amplified product and Bioinformatics analysis: The PCR products were excised from the gel and extracted using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. Sequencing of the PCR purified products was carried out using the Big dye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc, CA, USA) following the manufacturer's instructions on an automated DNA sequencer (Applied Biosystems 3730xl DNA Analyzer, Applied Biosystems, CA, USA) at Eurofins Genomics India Pvt. Ltd (Bengaluru, India). The sequence chromatogram was annotated with BioEdit Sequence Alignment Editor software vs 7.0.5 (Isis Therapeutics, Carlsbad, CA, USA). The annotated sequences were identified based on being the closest match to the sequences submitted in the NCBI database on BLAST analysis. 16S rRNA, *coa* and *mecA* gene sequences from *S. aureus* strains were retrieved from

Table 1. Showing primer sequences, amplicon size, cycling parameters for amplification of 16S ribosomal RNA, staphylo-coagulase and *mecA* gene segments.

Primer Code	Gene	Primer Sequence (5'-3')	Amplicon size (bp)	Cycling parameters	Reference
16S 27F	16S rRNA	AAGGAGGTGWTCCARCC	1564	95°C-4 min	Lane 1991
16S 1525R		AGAGTTTGATCMTGGCTCAG		95°C-1 min 51°C-1 min x 35 cycles 70°C-1.5 min 70°C-5 min	
Coa-HF	Coa	ATAGAGATGCTGGTACAGG	674	95°C-4 min	Hookey <i>et al.</i> 1998
Coa-HF		GCTTCCGATTGTTTCGATGC		95°C-1 min 52°C-1 min x 35 cycles 70°C-1 min 70°C-5 min	
mecAFor	mecA	AAGCAATAGAATCATCAGAT	451	95°C-4 min	Kamal <i>et al.</i> 2013
mecARev		AGTTCTGCAGTACCGGATTTGC		95°C-1 min 52°C-1 min x 35 cycles 70°C-1 min 70°C-5 min	

Table 2. Antibacterial susceptibility of buffalo MRSA isolates (R = Resistant, S = Susceptible).

Antibiotic Groups	Antibiotic	Abbreviation	MRSA isolates (n = 5 isolates)				
			Buffalo-1/ MRSA	Buffalo-2/ MRSA	Buffalo-3/ MRSA	Buffalo-4/ MRSA	Buffalo-5/ MRSA
Penicillins	Penicillin	P	R	R	R	R	R
	Oxacillin	OX	S	S	R	S	R
	Amoxyclav	AMC	S	S	S	S	S
	Ampicillin	AMP	R	R	R	R	R
	Methicillin	MET	R	R	R	R	R
Cephalosporins	Cephalothin	CEP	R	R	S	R	S
Sulphonamides	Co-Trimoxazole	COT	S	S	S	S	S
Aminoglycosides	Gentamicin	GEN	S	S	S	S	S
	Amikacin	AK	S	S	S	S	R
Quinolones	Oflaxacin	OF	S	S	S	S	S
Macrolides	Erythromycin	E	S	S	S	S	S
	Azithromycin	AZM	S	S	S	S	S
	Clarithromycin	CLR	S	S	S	S	S
Lincosamide	Clindamycin	CD	S	S	S	S	S
Glycopeptides	Vancomycin	VA	S	S	S	S	S
	Teicoplanin	TEI	R	R	R	R	R
Tetracycline	Tetracycline	TE	S	S	S	S	S
Aminocoumarin	Novobiocin	NV	R	R	R	R	R
Chloramphenicol	Chloramphenicol	C	S	S	S	S	S
Oxazolidonones	Linezolid	LZ	S	R	R	R	S

the NCBI nucleotide database and multiple sequence alignment (MSA) for both the nucleotide and the deduced amino acid (*coa* and *mecA*) was performed using the in-built ClustalW algorithm in MEGA5.1 software (Tamura *et al.* 2011). The sequences analyzed in this study were submitted to the NCBI GenBank with the following Accession numbers KJ509925, KJ509926 and KJ509927 for *mecA*, *coa* and 16S rRNA gene segments respectively.

Antimicrobial susceptibility test: The resistance pattern of MRSA isolates was determined using an agar disk diffusion method according to standards of Clinical and Laboratory Standards Institute (CLSI, 2013). The test was performed using Icosa G-I-Plus discs (HiMedia Laboratories, India) and the antimicrobial sensitivity is depicted in Table 2.

RESULTS AND DISCUSSION

From the total of 120 milk samples screened the isolates obtained from 52 samples (43.33 %) in Mannitol Salt Agar after sub-culturing from 5% sheep Blood-Agar media revealed characteristics colonies presumptively identified as *S. aureus*. The samples were subjected to Gram-staining and biochemical analysis which further identified the presence of *S. aureus* in all the pure cultures.

The result of the biochemical tests as done by HiStaph™ Identification Kit of a representative sample is depicted in [Fig.1(C)]. Single colonies of bacteria grown on Mannitol Salt Agar were further sub-cultured to Baird Parker Agar revealed characteristics black colonies [Fig. 1 (A)] confirmative of coagulase positive *S. aureus* in 12 samples (10%). All the isolated *S. aureus* samples (n=52) were further screened by Tube Coagulation Test which revealed viscous clot of rabbit plasma in varying proportions in the same samples (n=12) which were positive in Baird Parker Agar. The remaining 40 samples were regarded as coagulase negative Staphylococci (CNS).

MRSA was confirmed in five samples (4.16%) out of the total 120 mastitis milk, which is 9.62% of the total *S. aureus* isolates. MRSA isolates showed characteristics sky blue colonies in Hichrome MeReSa Agar Base using MeReSa Selective Supplement [Fig.1(B)] on overnight incubation at 37°C. Interestingly all these five samples were also coagulase positive.

Molecular identification by amplification of the 16S ribosomal RNA gene fragment in all the samples (n=52) by PCR revealed a single band of approximately 1564 bp in agarose gel. The samples which were sequenced, annotated and on BLAST analysis had 100% sequence

similarity at the nucleotide level with other *S. aureus* strains in the NCBI database.

All the samples were amplified using primers specific for the 3' terminal of the coagulase gene (Hookey *et al.* 1998) which revealed amplified product of approximately 674 bp in the samples. The sample which was sequenced, annotated and on BLAST analysis divulged 95% sequence identity at the nucleotide level representing significant sequence heterogeneity of our buffalo isolate with other *S. aureus* isolates in the database. To confirm the sequencing result, the sequencing was repeated twice and was done in the other strand also with the reverse primer. All the sequencing results were identical. Multiple sequence alignment at the deduced amino acid level of our isolate with other isolates from India and other parts of the world revealed repeat elements [ETNAYNVT] in all the isolates (Fig. 2). A unique amino acid substitution Pro → Thr before the second repeat was observed in only our isolate which was not found in any other isolates previously reported.

The presence of the *SSCmec* cassette was confirmed with the amplification of 451 bp product in the same five samples (which were positive in HiChrome MeReSa agar) out of the total 52 samples screened. The samples which were sequenced, annotated and on BLAST analysis revealed 100% sequence identity with *SCCmec* IVa from other MRSA isolates present in the database.

The samples were further screened by agar disk diffusion for antibacterial profiling. The relative resistance and sensitivity of these five isolates to different antibacterial agents have been enumerated in Table 2. Interestingly, these MRSA isolates showed resistance to oxazolidinones and glycopeptides groups also.

Buffalo is the main milch animal contributing significantly to the milk production in India, but very few work in screening of MRSA have been undertaken in this country. Kumar *et al.* (2011) screened buffaloes for presence of *S. aureus* as the causal agent of mastitis in an institutional dairy herd from Northern India; but our study is the first of its kind undertaken where special emphasis were given to collect samples from marginal farmers maintaining few animals. More than 70% of the total buffalo population is reared by marginal farmers in rural India (NDDDB 2012). These animals are the source of their livelihood and nutritional security to their family.

The presence of MRSA in cattle has been first reported from Belgium dairy herds Devriese *et al.* (1972). The incidences have increasingly been reported from different species in several countries in recent years. MRSA have been reported from different countries of Europe (Spohr *et al.* 2011), South America (de Medeiros *et al.* 2011), Africa (Kateete *et al.* 2013) and Asia (Kumar *et al.* 2011)

with varying prevalence.

The present study indicated MRSA in five isolates (4.16%), which is 9.62% of the total *S. aureus* isolates. In a study conducted by Kumar *et al.* (2011) in organized institutional buffalo herd, MRSA was identified in 5.4% of the *S. aureus* isolates, which is similar with the study conducted in North-Eastern Brazilian buffalo herds. Further, deMedeiros *et al.* 2011, reported 5.52% prevalence of *mecA* gene in isolated *S. aureus*.

MRSA has been identified based on phenotypic detection methods like growth in specific media and antibiogram profiling; whereas molecular identification have been based on the amplification of the *mecA* gene. PCR amplification and subsequent sequence based confirmation is regarded as a reliable method to identify MRSA (Paterson *et al.* 2014) rather than phenotypic methods which inherently may be misleading and erroneous (Oliveira and Lencastre, 2002). Interestingly in our study the phenotypic detection by the growth in specific media (HiChrome MeReSa Agar) and the PCR based confirmation were identical. Five samples were found positive by the growth in the specific media and also in by sequence based confirmation.

Coagulase is considered as an important virulence determinant of *S. aureus* pathogenicity (Cheng *et al.* 2010) and has been routinely used as typing of *S. aureus* isolates (Hookey *et al.* 1998). The reason for high proportion of sequence variation in the C-terminal of the staphylocoagulase protein in some of the *S. aureus* isolates is imprecise; the probable mechanism may be due to deletion or insertion of some of the repeat regions or through spontaneous mutations and positive selection (Saei *et al.* 2009).

In our study, the *coa* gene from the *S. aureus* isolate which was sequenced revealed only 95% sequence heterogeneity at the nucleotide level with other *S. aureus* isolates present in the NCBI database. The consequence of such significant changes in the coagulase may alter the structure of the protein or create antigenic variations which causes escape from the inhibitory effects of the host anti-coagulase agents like gamma globulin present in the serum (Duthie and Lorenz 1952, Stutzenberger and San Clemente 1967, Al-Ajealy *et al.* 2017). The emergence of such *S. aureus* strains having these altered staphylocoagulase protein may increase the inflammatory response in the mammary tissue of the animals.

CONCLUSION

There has been a tremendous increase in the cattle and buffalo population in India owing to the huge demand of milk and milk products; the human-livestock interface is intricately intertwined in these regions of the world. The

indiscriminate use of the antimicrobials in treating cases of mastitis by clinicians poses a grave concern over the emergence and positive selection of the antimicrobial resistant bacterial strains. The resistant strains possible adaptation to the human population raises a public health concern. All these factors in conjugation raise a disquieting situation in India where regular intensive screening of the causal agents of mastitis and antibiogram profiling is a prerequisite for developing effective control strategies. In The present study, we have found *S. aureus* is the main organism for causing clinical mastitis in buffalo and its resistance is gaining through holding coagulase and *mecA* genes. Though the findings came out from small geographical location of the country in a miniscule level considering the huge livestock population, has been effective to address some of the concerns affecting the dairy husbandry scenario of this country.

ACKNOWLEDGEMENTS

The authors acknowledge the contribution of Director, Indian Veterinary Research Institute, Bareilly, India for providing necessary facilities to undertake this research work. The authors also acknowledge the contribution of the farmers and the field veterinarians who have provided the buffalo milk samples.

REFERENCES

Al-Ajealy BA, Al-Shukri MSM, Al-Jumaily HS (2017) Detection of newly defined superantigenic toxin genes and coagulase gene polymorphism in *Staphylococcus aureus* isolates. *Rev Med Microbiol* 28: 158-163.

Bramley AJ (1992) Mastitis. In: *Bovine medicine: diseases and husbandry of cattle*. (Andrews AH, RW Blowey, H. Boyd, RG Eddy, Eds). Blackwell Scientific Publication. Boston. 289-300.

Chamber HF (1997) Methicillin resistance in Staphylococci: molecular and biochemical basis and clinical implications. *Clin. Microbiol Rev* 10: 781-791.

Cheng AG, Mcadow M, Kim HK, Bae T, Missiakas DM, Schneewind O (2010) Contribution of coagulases towards *Staphylococcus aureus* disease and protective immunity. *PLoS Pathog* 6: e1001036.

Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement M100-23. Available from: shopping.netsuite.com/c.1253739/site/Sample_pdf/M100S24_sample.pdf (accessed on 22 December 2016).

de Medeiros ES, Franca CA, Krewer Cda C, Peixoto Rde de Souza MAF Jr., Cavalcante MB, daCosta MM, Mota RA (2011) Antimicrobial resistance of *Staphylococcus* spp. isolates from cases of mastitis in buffalo in Brazil. *J Vet Diagn Invest* 23: 793-796.

Devriese LA, VanDamme LR, Fameree L (1972) Methicillin-(cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. *Zentralbl Veterinarmed B* 19: 598-605.

Duthie ES, Lorenz LL (1952) Staphylococcal coagulase; mode of action and antigenicity. *J Gen Microbiol* 6: 95-107.

FAO (2012) Statistical year book. Available from: <http://www.fao.org/docrep/015/i2490e/i2490e00.htm> (accessed April 5, 2016).

Hiramatsu K, Ito K, Tsubakishita S, Sasaki T, Takeuchi F, Morimoto Y (2013) Genomic basis for Methicillin resistance in *Staphylococcus aureus*. *Infect Chemother* 45: 117-136.

Hookey JV, Richardson JF, Cookson BD (1998) Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. *J Clin Microbiol* 36: 1083-1089.

Kamal RM, Bayoumi MA, Abd El Aal SFA (2013) MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. *Food Control* 33: 49-53.

Kateete DP, Kabugo U, Baluku H, Nyakarahuka L, Kyobe S, Okee M, Najjuka CF, Joloba ML (2013) Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. *PLoS One* 8: e63413.

Kock R, Becker K, Cookson B, Van Gemert-Pijnen JE, Harbarth S, Kluytmans J (2010) Methicillin resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Euro Surveill* 14: 19688-19694.

Kumar R, Yadav BR, Anand SK, Singh RS (2011) Molecular surveillance of putative virulence factors and antibiotic resistance in *Staphylococcus aureus* isolates recovered from intra-mammary infections of river buffaloes. *Microb Pathog* 51: 31-38.

Lane DJ (1991) 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematic*. (Stackebrandt, E., M. Goodfellow, Eds). John Wiley and Sons. New York. 115-175.

Lindsay JA (2014) *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. *Int J Med Microbiol* 304: 103-109.

National Dairy Development Board (NDDDB) (2012) Available from: <http://www.nddb.org/English/statistics/Pages/Statistics.aspx> (accessed April 5, 2016).

Oliveira DC, Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 46: 2155 -2161.

Pantosti A (2012) Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. *Front Microbiol* 9: 127.

Pantosti, A, Sanchini A, Monaco M (2007) Mechanisms of antibiotic resistance in *Staphylococcus aureus*, *Future Microbiol* 2: 323-334.

Paterson GK, Harrison EM, Holmes MA (2014) The emergence of *mecC* Methicillin-resistance *Staphylococcus aureus*. *Trends Microbiol* 22: 42-47.

Quinn PJ, Carter ME, Markey BK, Carter GR (1994) *Clinical Veterinary Microbiology*, 1st edn., Wolfe Publishing. London. 209-284.

Saei HD, Ahmadi M, Mardani K, Batavani RA (2009) Molecular typing of *Staphylococcus aureus* isolated from bovine mastitis based on polymorphism of the coagulase gene in the north west of Iran. *Vet Microbiol* 137: 202-206.

Spohr M, Rau J, Friedrich A, Klittich G, Fetsch A, Guerra B (2011) Methicillin-resistant *Staphylococcus aureus* (MRSA) in three dairy herds in southwest Germany. *Zoonoses Public Health* 58: 252-261.

Stutzenberger, FJ, San Clemente CL (1967) Nephelometric assay of bovine anti-*Staphylocoagulase* serum. *J Bacteriol* 94: 821-825.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol* 28: 2731-2739.

Cite this article as: Biswas S, Chakravarti S, Barui A (2018) Emergence of coagulase positive Methicillin resistant *Staphylococcus aureus* isolated from buffalo mastitis milk samples. *Explor Anim Med Res* 8(2): 190-196.