B-4969
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RESEARCH ARTICLE Indian Journal of Animal Research

Effect of Atorvastatin and Vitamin D against Multi-drug Resistant *Staphylococcus* spp and *Escherichia coli* Isolated from Bovine Mastitis Cases

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ABSTRACT

Background: In the current scenario, the emergence of antimicrobial resistance has made it difficult to treat bacterial infections alone using antibacterial agents.

Methods: Milk samples collected from 100 mastitis affected cows from August 2018 to June 2019 and were stored at 4°C. After species confirmation, isolates were subjected to ABST and screened for resistance genes - *mecA*, *blaTEM* using PCR. Minimum Inhibitory Concentration (MIC) of tetracycline and ampicillin were determined against both *Staphylococcus spp* and *E. coli*, alone and in combination with atorvastatin, vitamin D by using a modified microdilution method.

Result: *Staphylococcus* and *E. coli* isolates showed 80.65% and 85.71%, 100% and 85.71% resistance against tetracycline and ampicillin, respectively. Atorvastatin and vitamin D did not display antibacterial effects as sole agents against both bacterial species. However, there was a significant decrease in the MIC of ampicillin against *E. coli* and *Staphylococcus spp* when combined with atorvastatin and vitamin D but not for tetracycline.

Key words: ABST, Ampicillin, Atorvastatin, Bovine, Mastitis, Resistance, Tetracycline, Vitamin D.

INTRODUCTION

Mastitis is an inflammation of the udder caused by a various microorganisms, is a widespread and economically devastating disease in dairy cattle reported across the world (Abebe *et al*., 2016). A wide variety of organisms are implicated in the occurrence of mastitis, chief among them being *Staphylococcus aureus* and *E. coli* (Aksoy, 2021). The principal treatment for mastitis is administration of antibacterial agents, namely ampicillin, cloxacillin, penicillin G, streptomycin and tetracycline (Bhosale *et al.,* 2014). However, the effective treatment of bovine mastitis mainly depends on the antimicrobial susceptibility of the organisms, the type of mastitis, the type of cattle breed and the treatment regimen (Barkema *et al.,* 2006).

As the udder quarters were infected with penicillin-sensitive pathogens, penicillin G to be used as first-line antibiotic agents (Grave *et al.*, 1999). However, the appearance of penicillin G resistant staphylococci causing bovine mastitis has been reported (Ramasamy *et al.,* 2021). Tetracycline is the most intensively used antibiotics due to its relative safety, low cost and broad-spectrum activity against Gram-positive, Gram-negative and Mycoplasma (Al-Nazawi, 2006). Antibiotics like ampicillin and tetracycline, which were hitherto effective in the treatment of mastitis, have reported significantly reduced efficacy (Mirzaagha *et al*., 2011). Treatment of mastitis has become increasingly difficult and challenging owing to the rapid emergence of antimicrobial resistance (Beuron *et al*., 2014).

Recently, the non-antibiotic drugs such as atorvastatin and vitamin D possess pleiotropic properties, the anti-bacterial effect being one such quality that has received a lot of attention lately (Masadeh *et al*., 2012; Golpour *et al*., 2019). The increasing problem of antibiotic resistance, the discovery of newer antibiotics having almost come to a standstill, has prompted the scientific community to look for ways to strengthen the efficacy of antibiotics through synergistic interaction with non-antibiotic drugs. Because of the above, the purpose of this study was to determine the in-vitro antibacterial effect of atorvastatin and vitamin D alone and in combination with antibiotics against multi-drug resistant *Staphylococcus spp* and *E. coli* isolated from bovine mastitis cases.

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How to cite this article: Sriraam, K.S., Ramasamy, T., Porteen, K., Elamaran, A., Pavithra, M., Jagadeesh, T. and Ramesh, S. (2022). Effect of Atorvastatin and Vitamin D against Multi-drug Resistant *Staphylococcus* spp and *Escherichia coli* Isolated from Bovine Mastitis Cases. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4969.

Submitted: 25-06-2022 **Accepted:** 16-11-2022 **Online:** 23-12-2022

MATERIALS AND METHODS

Sample collection

The study was conducted at the Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College,

Chennai, Tamil Nadu, India from August 2018 to June 2019. A total of 100 milk samples were collected from different cows suspected for mastitis from the Department of Clinics, Madras Veterinary College Teaching Hospital, Chennai. About 15 mL of milk was aseptically transferred to a sterile universal sampler tube and were refrigerated at 4°C.

Isolation and characterization

Selective isolation of *Staphylococcus spp* and *E. coli* was performed using Mannitol Salt Agar (MSA) and Eosin Methylene Blue agar (EMB), respectively. The individual colonies obtained from selective media were stored as glycerol stocks at -20°C. Biochemical kits were used for the characterization of the isolates (HiMedia™). Polymerase Chain Reaction (PCR) was performed for genotypic characterization of the isolates (Fang and Hedin, 2003) including *tuf* gene for *Staphylococcus spp*, *uspA* gene for *E. coli* and *nuc* gene for *S. aureus*.

Resistance genes

PCR was carried out for detection of resistance genes bla_{TEM} for ampicillin, *tet*M and *tet*B for tetracycline. The PCR products were separated using electrophoresis and the gel were visualized on Mega Capt Gel Doc. A 100bp DNA ladder (Thermoscientific™) was employed to determine the size of the PCR products. The list of primers, cycling conditions for identifying genes and resistance genes are given in Table 1-3, respectively.

Antibacterial sensitivity patterns

An antibiotic sensitivity test (ABST) was performed using the modified Kirby-Bauer disc diffusion method. An overnight

Table 1: List of primers used in this study

Gene	Sequence (5′ to 3′)	Size(bp)	Reference
bla_{TEM}	F: ATG AGT ATT CAA CAT TTC CG	847	Bandyopadhyay *et al.*, 2015
	R: GTC ACA GTT ACC AAT GCT TA		
tet(M)	F: AGT GGA GCG ATT ACA GAA	158	Strommenger *et al.,* 2005
	R: CAT ATG TCC TGG CGT GTC TA		
tet(B)	F: CCT CAG CTT CTC AAC GCG TG	634	Lyimo *et al.,* 2016
	R: GCA CCT TGC TGA TGA CTC TT		
mec A	F: AAA ATC GAT GGT AAA GGT TGG C	532	Shahraz *et al.,* 2012
	R: AGT TCT GCA GTA CCG GAT TTG C		
tuf	F: GAA GAA TTA TTA GAA TTA GT	235	Hedge *et al.,* 2013
	R: GTG ATT GAG AAT ACG TCC TC AA		
nuc	F: GTG CTG GCA TAT GTA TCG CAA TTG T	181	
	R: TAC GCC CTT ATC TGT TTG TGA TGC		
uspA	F: CCG ATA CGC TGC CAA TCA GT	884	Chen and Griffiths (1999)
	R: ACG CAG ACC GTA GGC CAG AT		

Table 2: PCR cycling conditions for identification of genes.

Genes targeted	Initial denaturation	Denaturation	Annealing	Extension	Final extension
		94°C for 30 sec	50°C for 30 sec	72°C for 30 sec	72°C for 10 min
tuf	94°C for 5 min		**Repeated for 30 cycles**		
usp A		94°C for 1 min	55°C for 1 min	72°C for 2 min	72°C for 5 min
			Repeated for 30 cycles		
nuc		94°C for 30 sec	54°C for 30 sec	72°C for 30 sec	72°C for 10 min
			Repeated for 30 cycles		

Table 3: Cycling conditions for resistance genes.

Genes targeted	Initial denaturation	Denaturation	Annealing	Extension	Final extension
		94°C for 1 min	55°C for 1 min	72°C for 2 min	72°C for 5 min
mecA	94°C for 5 min		**Repeated for 35 cycles**		
tet (B)	95°C for 5 min	95°C for 30 sec	55°C for 30 sec	72°C for 30 sec	72°C for 2 min
			Repeated for 30 cycles		
tet (M)	94°C for 5 min	94°C for 1 min	50°C for 1 min	72°C for 90 sec	72°C for 5 min
			Repeated for 30 cycles		
bla_{TEM}	96°C for 5 min	96°C for 1 min	58°C for 1 min	72°C for 1 min	72°C for 10 min
			Repeated for 35 cycles		

inoculum of culture was diluted to 0.5 McFarland standards and used for ABST. All the antibiotic discs were purchased from HiMedia™. For evaluation of combinations, 1mg of atorvastatin, vitamin D (separately) was dissolved in 1ml of Dimethyl Sulfoxide (DMSO) and 8 µl (8 µg) and 15 µl (15 µg) of the same solution was added to the tetracycline and ampicillin discs. The discs were allowed to be set for 20 minutes at room temperature and then incubated at 35-37°C for 24 hours. A sterile disc inoculated with the same volume of DMSO served as a control (Haeri *et al*., 2015).

Minimum inhibitory concentration (MIC)

MIC was evaluated using the modified micro-dilution method using the resazurin indicator. A 96 welled plate was used for testing of samples and the resulting colour change was noted. The concentration of the drug in the well was calculated to find MIC (Elshikh *et al*., 2016). The drug solutions used were ampicillin (300 mg/ml), tetracycline (100 mg/ml), atorvastatin (100 mg/ml) and vitamin D (100 mg/ml) in DMSO.

Statistical analysis

Using one way ANOVA, followed by a Post-hoc Duncan test was performed with SPSS software for statistical analysis of the data generated. A p-value <0.005 was considered significant.

RESULTS AND DISCUSSION

100 milk samples were collected from cows with mastitis, 60 samples (60%) showed growth on MSA and/or EMB agar. In that, 40 samples were positive for *Staphylococcus spp.* (66.67%) (Fig 1) and other 20 samples streaked onto EMB agar produced metallic green sheen (33.33%) positive for *E. coli* (Fig 2). Six samples (10%) were produced colonies in both MSA and EMB agar, indicative of polymicrobial infection.The isolates were characterized as *Staphylococcus spp* (72.5%) and *E. coli* (35%) by using commercial biochemical test kits.

Genotypic confirmation of *Staphylococci spp* was carried out through *tuf* gene (Fig 3) and *S. aureus* through *nuc* gene using PCR (Fig 4). Based on results, 77.5% (31/40) isolates were *Staphylococci spp* and among these 41.94% (13/31) isolates were *S. aureus.* The presence of *E. coli* was identified in 35% of isolates (Fig 5).

The ABST targeted for *Staphylococcus spp* isolates revealed 100% resistance against penicillin and ampicillin, 83.87% samples were observed resistant to methicillin and 80.65% were found to be resistant to tetracycline, gentamicin, vancomycin and amikacin. The co-trimoxazole showed 74.19% resistance and 61.29% samples resistant to ciprofloxacin and ceftriaxone. 58.06% and 51.61% noticed resistance to cefotaxime and enrofloxacin respectively.

Based on the sensitivity test, *E. coli* isolates revealed that 85.71% were found to be resistant to tetracycline, gentamicin, cefotaxime, ceftriaxone, co-trimoxazole,

Fig 1: MSA - golden yellow colonies.

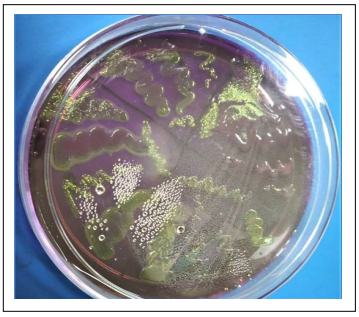

Fig 2: EMB plates - metallic green colonies.

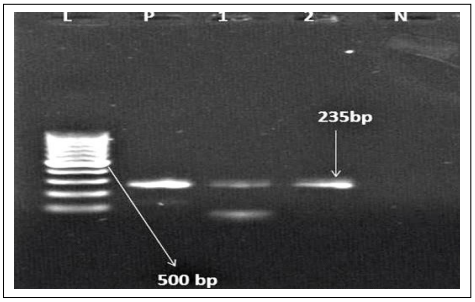

Fig 3: Genotypic confirmation of *tuf* gene.

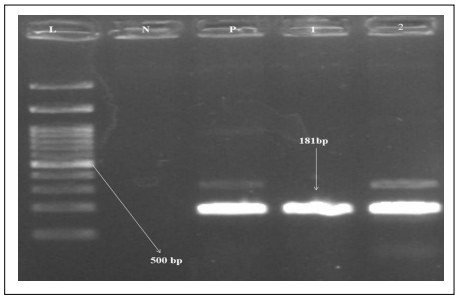

Fig 4: PCR assay for *nuc* gene.

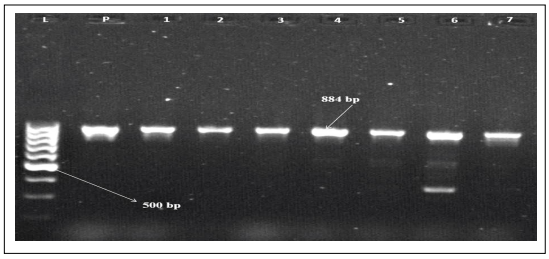

Fig 5: PCR assay for *E. coli* targeting *uspA* gene.

ampicillin, amikacin and ciprofloxacin and 75.43% samples resistant to enrofloxacin. Complete resistance (100%) was observed against penicillin, methicillin and vancomycin. Methicillin resistance was found that 38.46% in *S. aureus* (Fig 6) and 16.67% in other *Staphylococcus spp.* The bla_{TEM} gene were expressed in 16.13% of *Staphylococcus spp* and 14.29% of *E. coli* isolates.

The antibacterial effect of atorvastatin, vitamin D revealed no significant antibacterial activity against both *Staphylococcus spp* and *E. coli* isolates (Fig 7). However, the combination of atorvastatin and vitamin D with ampicillin indicated wider zones of inhibition against both *Staphylococcus spp* and *E. coli* (Fig 8 and 9).

There was increased MIC of tetracycline for *E. coli* (2.53 µg/ml) and *Staphylococcus spp* (2.45 µg/ml) observed. The combination of tetracycline with atorvastatin and vitamin D decreased the MIC to 1.73 µg/ml and 1.51 µg/ml for *Staphylococcus spp* respectively. Similarly, for *E. coli*, the MIC was reduced 1.89 µg/ml with tetracycline-atorvastatin and 1.56 µg/ml with tetracycline-vitamin D combination, which was non significant (Table 4).

The MIC of ampicillin for *E. coli* isolates was 22.62µg/ml and the recommended CLSI (2016) MIC breakpoint for ampicillin against E. coli is ≤8 µg/ml. The increase in MIC of ampicillin for *Staphylococcus spp* isolates was 17.20 µg/ml where as the recommended MIC for ampicillin against *Staphylococcus spp* by CLSI (2016) is ≤4 µg/ml. The MIC of ampicillin in combination with atorvastatin against *Staphylococcus spp* and *E. coli* was 2.49 µg/ml and 2.61 µg/ml, respectively. The combination of ampicillin with vitamin D against both *Staphylococcus spp* and *E. coli* produced an average MIC of 2.742 µg/ml and 1.092 µg/ml, respectively, which was statistically significant (Table 5).

In the present study, *Staphylococcus spp* (77.5%) was the predominant bacteria isolated from the bovine mastitis, followed by *E. coli* (35%). Previous studies in our laboratory have also shown that *Staphylococcus spp* (94%) and *E. coli* (50%) are the most common bacteria usually in mastitis affected milk (Ramasamy *et al*., 2021). Similar findings were also reported by Jeykumar *et al.,* (2013) and Nalband *et al.,* (2020). Among these *Staphylococcus spp*, 41.94% of samples were carried the *S. aureus* (*nuc*) gene, which is the highest prevalence compared to our previous work (12.9%) (Ramasamy *et al*., 2021). This may be due to increased resistance *S. aureus* organism in years progress.

ABST results revealed complete resistance of penicillin against both bacterias. The isolates of *Staphylococcus spp* and *E. coli* were showed 80.65% and 85.71% resistance to tetracycline, respectively. Recent study also reported the *Staphylococcus spp* and *E. coli* were resistant to penicillin and tetracycline with high MIC (Anurag *et al.,* 2021; Ramasamy *et al*., 2021). The increased resistance pattern observed in present study shows that β- lactams and tetracyclines are leading in front for the treatment of mastitis.

The lowest prevalence of beta lactam resistance genes were found in the both *Staphylococcus spp* and *E. coli* isolates from bovine mastitis. None of the tested isolates was positive for *tet* M and *tet* B genes. There was increased

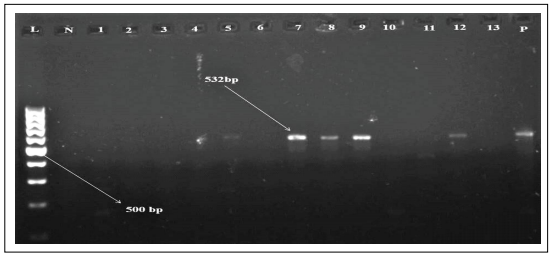

Fig 6: *Staphylococcus aureus* isolates containing *mecA* gene.

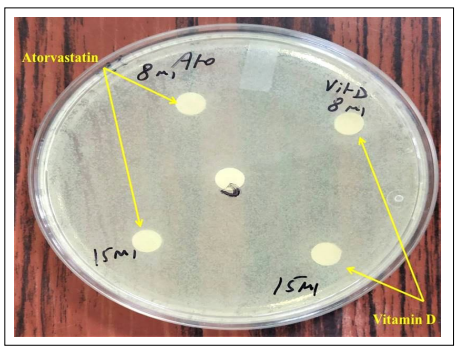

Fig 7: Zone of inhibition of atorvastatin and vitamin D alone.

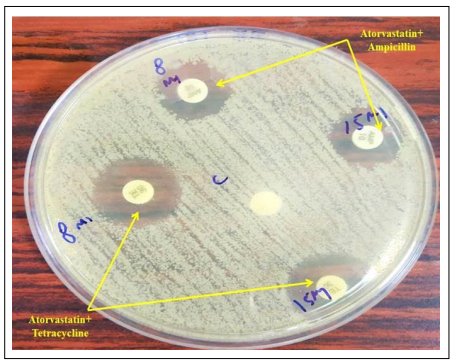

Fig 8: Zone of inhibition of atorvastatin with ampicillin and tetracycline.

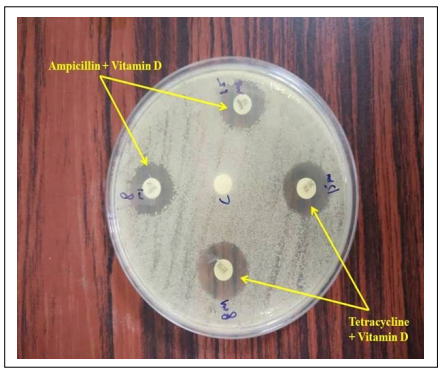

Fig 9: Zone of inhibition of vitamin D with ampicillin and tetracycline.

Table 4: MICs of tetracycline.

Groups	MIC (Mean±SE)	
	E. coli	*Staphylococcus spp*
Tetracycline	2.53±1.08	2.45±0.47
Tetracycline + Atorvastatin	1.89±0.81	1.73±0.37
Tetracycline + Vitamin D	1.56±0.81	1.51±0.34
P value	0.751^{NS}	0.221^{NS}

Table 5: MICs of Ampicillin and its combinations.

Groups	MIC	
	E. coli	*Staphylococcus spp*
Ampicillin	22.62±3.95^{b}	17.20±1.44^{b}
Ampicillin+Atorvastatin	2.61±1.26^{a}	2.49±0.49^{a}
Ampicillin+Vitamin D	1.09±0.60^{a}	2.74±0.73^{a}
P value	0.01**	0.01**

Mean±SE bearing different superscript differs significantly at 1% (p<0.01).

resistance reported for *tet* M (67.70%) and *tet* B (75%) in our previous study (Ramasamy *et al*., 2021). This variation in the resistance genes expression may be due to changes in the study period.

In the current study, the sole antibacterial effect of atorvastatin was not observed. This result was supported by Manalo *et al*., (2017), who observed no antibacterial activity in *in vitro*. This may be due to resistant bacterial isolates from clinical samples used in this study, whereas previous workers were used the laboratory standard bacterial culture. The combination of ampicillin and atorvastatin reduced the MIC of ampicillin against both *Staphylococcus spp* and *E. coli*. *In vitro* and *in vivo* studies of statins have shown antimicrobial effects against both organisms (Ko *et al*., 2018; Choudhary *et al*., 2015; Thangamalai *et al*., 2014).

The ampicillin-vitamin D displayed a synergistic antibacterial effect against both study organisms, with a significant decrease in MIC. Also, there is ample evidence to prove that vitamin D possesses antimicrobial properties (Saputo *et al.*, 2018; Youssef *et al.*, 2011). However, it is still unclear how it is responsible for potentiating the antibacterial effect of another antibiotic.

Though the combination of tetracycline with the non-antibiotic drugs *viz.,* atorvastatin and vitamin D produced non-significant reduction in MIC against previously resistant clinical isolates.. This difference between tetracycline and ampicillin might be because of tetracycline is primarily inhibiting the protein synthesis and ampicillin is a cell wall synthesis inhibitor.

CONCLUSION

The atorvastatin and vitamin D improves the effect of ampicillin against resistant bacteria indicating their role in bacterial cell wall synthesis. However, further studies are required to ascertain the exact mechanism of action insofar as its antibacterial property is concerned.

ACKNOWLEDGEMENT

This study was funded by Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.

Conflict of interet: None.

REFERENCES

Abebe, R., Hatiya, H., Abera, M., Megersa, B., Asmare, K. (2016). Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. BMC Veterinary Research. 12(1): 270. https://doi.org/10.1186/s12917-016-0905-3.

Aksoy, A. (2021). Antimicrobial susceptibility and detection of genes for antimicrobial resistance of *Mycoplasma bovis*, *Staphylococcus aureus* and *Escherichia coli*. Indian Journal of Animal Research. 55: 1240-1245.

Al-Nazawi, M.H. (2006). Resistance and residues of antibacterial in dairy farm and diary production in Al-Hassa region, Saudi Arabia. Journal of Medical Sciences. 6: 198-202.

Anurag, B., Ramasamy, T., Ramesh, S., Sriraam, K.S., Kalaiselvi, L., Deepak, S.J., Gokula Kannan R. and Arunaman, C.S. (2021). Screening of milk borne *Staphylococcus aureus* for resistance against beta lactam antibiotics. Agricultural Science Digest. 41: 113-115.

Bandyopadhyay, S., Samanta, I., Bhattacharyya, D., Nanda, P.K., Kar, D., Chowdhury, J., Dandapat, P., Das, A.K., Batul, N., Mondal, B., Dutta, T.K. (2015). Co-infection of methicillin-resistant *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* and extended spectrum β-lactamase producing *Escherichia coli* in bovine mastitis-three cases reported from India. Veterinary Quarterly. 35(1): 56-61.

Barkema, H., Schukken, Y., Zadoks, R. (2006). Invited Review: The role of cow, pathogen and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science. 89(6): 1877-1895.

Beuron, D.C., Cortinhas, C.S., Botaro, B.G., Macedo, S.N., Gonçalves, J.L., Brito, M. A., Santos, M.V. (2014). Risk factors associated with the antimicrobial resistance of *Staphylococcus aureus* isolated from bovine mastitis. Pesquisa Veterinária Brasileira. 34(10): 947-952.

Bhosale, R., Osmani, R.A., Ghodake, P.P., Shaikh, S.M., Chavan, S.R. (2014). Mastitis: An intensive crisis in veterinary science. International Journal of Pharma Research and Health Sciences. 2(2): 96-103.

Chen, J. and Griffiths, M.W. (1999). Cloning and sequencing of the gene encoding universal stress protein from *Escherichia coli* O157: H7 isolated from Jack in a Box outbreak. Letters in Applied Microbiology. 29(2): 103-107.

Choudhury, S., Kannan, K., Addison, M.P., Darzi, S.A., Singh, V., Singh, T.U., Thangamalai, R., Dash, J.R., Parida, S., Debroy, B., Paul, A. (2015). Combined treatment with atorvastatin and imipenem improves survival and vascular functions in mouse model of sepsis. Vascular Pharmacology. 71: 139-50.

Clinical and Laboratory Standards Institute (CLSI). (2016). Performance standards for antimicrobial susceptibility testing; Twenty-sixth informational supplement. CLSI document M100-S26. Wayne, PA: Clinical and Laboratory Standards Institute.

Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., Banat, I.M. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letters. 38(6): 1015-1019.

Fang, H. and Hedin, G. (2003). Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR assay. Journal of Clinical Microbiology. 41(7): 2894-28999. DOI: 10.1128/JCM.41.7.2894-2899.

Golpour, A., Bereswill, S., Heimesaat, M.M. (2019). Antimicrobial and immune-modulatory effects of vitamin D provide promising antibiotics-independent approaches to tackle bacterial infections-lessons learnt from a literature survey. European Journal of Microbiology and Immunology. 9(3): 80-87.

Grave, T., Greko, C., Nilsson, L., Odensvik, K., Mørk, T., Rønning, M. (1999). The usage of veterinary antibacterial drugs for mastitis in cattle in Norway and Sweden during 1990-1997. Preventive Veterinary Medicine. 42: 45-55.

Haeri, M.R., White, K., Qharebeglou, M., Ansar, M.M. (2015). Cholesterol suppresses antimicrobial effect of statins. Iranian Journal of Basic Medical Sciences. 18(12): 1253-6.

Hegde, R., Isloor, S., Prabhu, K.N., Shome, B.R., Rathnamma, D., Suryanarayana, V.V.S., Yatiraj, S., Prasad, C.R., Krishnaveni, N., Sundareshan, S. and Akhila, D.S. (2013). Incidence of subclinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. Indian Journal of Microbiology. 53(3): 315-320.

Jeykumar, M., Vinodkumar, G., Bashir, B.P., Krovvidi, S. (2013). Antibiogram of mastitis pathogens in the milk of crossbred cows in Namakkal district, Tamil Nadu. Veterinary World. 6(6): 354-356. doi:10.5455/vetworld.2013.354-356.

Ko, H.H., Lareu, R.R., Dix, B.R., Hughes, J.D. (2018). *In vitro* antibacterial effects of statins against bacterial pathogens causing skin infections. European Journal of Clinical Microbiology and Infectious Diseases. 37(6): 1125-1135.

Lyimo, B., Buza, J., Subbiah, M., Smith, W., Call, D.R. (2016). Comparison of antibiotic resistant *Escherichia coli* obtained from drinking water sources in northern Tanzania: A cross-sectional study. BMC Microbiology. 16(1): 254. https://doi.org/10.1186/s12866-016-0870-9.

Manalo, R.V., Josol, V.J., Gloriani, N.G. (2017). The differential effects of atorvastatin co-administered with ampicillin on the bacterial growth and biofilm formation of *Staphylococcus aureus*. Current Medicine Research and Practice. 7(5): 178-83.

Masadeh, M., Mhaidat, N., Alzoubi, K., Al-azzam, S., Alnasser, Z. (2012). Antibacterial activity of statins: A comparative study of atorvastatin, simvastatin and rosuvastatin. Annals of Clinical Microbiology and Antimicrobials. 11: 13. doi: 10.1186/1476-0711-11-13.

Mirzaagha, P., Louie, M., Sharma, R., Yanke, L.J., Topp, E., McAllister, T.A. (2011). Distribution and characterization of ampicillin- and tetracycline-resistant *Escherichia coli* from feedlot cattle fed subtherapeutic antimicrobials. BMC Microbiology. 11: 78. doi: 10.1186/1471-2180-11-78.

Nalband, S.M., Kolhe, R.P., Deshpande, P.D., Jadhav, S.N., Gandhale, D.G., Muglikar, D.M., Kolhe, S.R., Bhave, S.S., Jagtap U.V. and Dhandore C.V. (2020). Characterization of *Escherichia coli* Isolated from bovine subclinical mastitis for virulence genes, phylogenetic groups and ESBL production. Indian Journal of Animal Research. 54: 1265-1271.

Ramasamy, T., Keerthana, S., Srinivasan, M.R., Chandrasekar, D., Porteen, K., Borthakur, A., Elamaran, A., Sriram, P. (2021). Molecular characterization of antibiotic resistance gene pattern of *Staphylococcus aureus* and *Escherichia coli* in mastitis affected dairy cows. Indian Journal of Animal Research. 55: 463-468.

Saputo, S., Faustoferri, R.C., Quivey, R.G. (2018). Vitamin D compounds are bactericidal against *Streptococcus* mutans and target the bacitracin-associated efflux system. Antimicrobial Agents and Chemotherapy. 62(1): e01675-17.

Shahraz, F., Dadkhah, H., Khaksar, R., Mahmoudzadeh, M., Hosseini, H., Kamran, M., Bourke, P. (2012). Analysis of antibiotic resistance patterns and detection of mecA gene in *Staphylococcus aureus* isolated from packaged hamburger. Meat Science. 90(3): 759-763. https://doi.org/10.1016/j.meatsci.2011.11.009.

Strommenger, B., Kehrenberg, C., Kettlitz, C., Cuny, C., Verspohl, J., Witte, W., Schwarz, S. (2005). Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. Journal of Antimicrobial Chemotherapy. 57(3): 461-465.

Thangamalai, R., Kandasamy, K., Sukumarn, S.V., Reddy, N., Singh, V., Choudhury, S., Parida, S., Singh, T.U., Boobalan, R., Mishra, S.K. (2014). Atorvastatin prevents sepsis-induced downregulation of myocardial â1-adrenoceptors and decreased cAMP response in mice. Shock. 41(5): 406-12.

Youssef, D.A., Miller, C.W., El-Abbassi, A.M., Cutchins, D.C., Cutchins, C., Grant, W.B., Peiris, A.N. (2011). Antimicrobial implications of vitamin D. Dermato-endocrinology. 3(4): 220-229.