Resistance Trends of E. Coli Against Selected Beta-Lactam Group of Antimicrobials

Vikas V. Karande[1], M. M. Gatne[2], R.J. Zende[3], R.V. Gaikwad[4], P.L. Dhande[5], S.H. Dalavi[6], S.S.Chavan[7], A.S. Nagvekar[8] and Sujata Bhave[9]

1. Asst. Prof, Veterinary Pharmacology and Toxicology, 2. Professor, Veterinary Pharmacology and Toxicology, Associate Professor, Veterinary Public Health, 4. Professor, Veterinary Medicine, 5. Professor, Anatomy, 6. Associate Professor, Biochemistry, 7. Animal Technician 8. Associate Professor, Veterinary Physiology, 9. Student, Veterinary Public Health. Bombay Veterinary College, Parel MAFSU Mumbai

Corresponding author e-mail: drvikas111081@gmail.com

Paper Received on November 01, 2016, Accepted on January 12, 2017 and Published Online on January 28, 2017

ABSTRACT

In the present study, 750 buffaloes from six buffalo farms were screened for subclinical mastitis and positive samples were screened for antimicrobial susceptibility test. A total of 310 positive subclinical mastitis with prevalence of 41.33 % were subjected to E. coli isolation. Out of 310 milk samples 94 E. coli strains were isolated with prevalence of 30.32%. All the 94 E. coli strains were studied for their antimicrobial resistance pattern against 10 beta lactam antibiotics. The study indicated that greater percentages of isolates were resistant to Cefotaxime (53.19%), followed by Aztreonam (53.19%), Ceftazidime (42.55%), Ceftazidime (42.55%) Ampicillin (32.97%) and Cefepime (19.14%). However, highest sensitivity was observed towards Cefepime + Clavulanic acid (86.17%) followed by Cefepime (80.85%), Amoxicillin + Clavulanic acid (77.65%) and Ampicillin + Sulbactam (71.27%). The sensitivity to other tested beta-lactam antimicrobials was observed below 40%. These findings can be utilized in mastitis treatment programmes and antimicrobials strategies in organized herds.

Keywords: *Mastitis; E. coli; Beta-lactams; Resistance; Sensitivity;*

India ranks first in milk production with annual 146.3 million tonnes in year 2014-15. In spite of this, mastitis has remained one of the most important disease of dairy cattle which lead to heavy economical loss the dairy industry worldwide, affecting animal welfare and having potential public health implications, if untreated or if inadequately treated milk is consumed. Based on the effect on productivity, international trade, animal welfare and zoonotic risk, mastitis ranked highest above all other infectious diseases (*Shpigel, 2001*). Several studies conducted at United States of America (USA) showed that cost related to mastitis on dairy farms is approximately $ 200 per cow per year; with annual loss of $ 2 billion for dairy industry. In India, annual economic loss incurred by dairy industry on account of udder infection is estimated to be Rs. 6053.21 crores of which 70 to 80 per cent has been attributed to subclinical mastitis (*NAAS, 2013*). Average decrease in milk yield due to clinical and subclinical mastitis is estimated to be 50 % and 17.5 per cent, respectively. The most frequently isolated microorganisms causing intramammary infection in bovines are *Staphylococci, Streptococci* and coliforms (*Bradley, 2002; Person et al., 2011*). Farm environment is an important source of coliform mastitis and *Escherichia coli* (*Bradley and Green 2001; Hogan and Smith, 2003; Dogan et al., 2006*). Several virulence factors have been detected in pathogenic *E. coli*, which includes toxins, adhesions, invasins, capsule production, ability to resist serum complement and ion scavenging. *E. coli* isolates with combinations of such virulence factors will be capable of causing disease (*Fernandes et al., 2011*). Antimicrobial resistance (AMR) in bacteria has emerged as a global problem in human and veterinary medicine. The indiscriminate use of antimicrobial agents results in AMR among pathogenic and commensal bacteria

prevalent in food and environment. Among various antimicrobial agents, varieties of â-lactam are currently licensed for use in veterinary medicine and thus provide opportunity for selection pressure in development of â-lactam resistance. They are critically important antibiotics in the treatment of mastitis. But, now-a-days *E. coli* isolates highly resistant to â-lactam antibiotics such as penicillin and ampicillin which are frequently used in the treatment of mastitis without knowing their effectiveness. Thus, resistant strains of *E. coli* are emerging rapidly.

Mastitis being managemental practices associated infection and disease of milk animals for which antimicrobials are use, thus, the antimicrobial resistance pattern of *E. coli* in subclinical and clinical bovine mastitis has to be accessed as dairy farming is the predominant livestock enterprise in India. Considering the losses resulting from mastitis, the recognition of *E. coli* as highly adaptive bacteria in different ecological niches, treatment failure in mastitis and its zoonotic significance, the present study was under taken to investigate the antimicrobial resistance pattern of *E. coli* isolated from subclinical mastitis with special consideration of â-lactam antibiotics.

METHODOLOGY

Sample collection: Farms with minimum 100 animals were included in the study. Six buffalo farms near Mumbai were selected. All the milking animals at farm were sampled and screened for presence of subclinical mastitis by California Mastitis Test (CMT). Clinical mastitis was detected by clinical observation of udder, teats and milk. A total of 750 buffalo were screened by adding 5 ml milk sample to CMT reagent from each quarter of each buffalo. Animals with clinical, subclinical mastitis were sampled. All these samples were transported and processed in the Department of Pharmacology and Toxicology, Bombay Veterinary College, Mumbai, India.

Isolation and Identification of E. coli: All the milk samples were subjected to isolation of organism by following standard method. Milk samples (few drops) added in enrichment media and incubated at 37°C for 24hrs. After enrichment loopful of broth inoculated on EMB agar and further incubated for 24hrs. Colonies showing metallic sheen were examined for confirmation by biochemical testing (Table 1).

Table 1: Biochemical properties of *E. coli*

Biochemical characteristic	Reaction
Gram staining	- ve
Catalase	+ve
Oxidase	-ve
Methyl red	+ve
Voges proskaur test	-ve
Indole test	+ve
Citrate utilization test	-ve

Antimicrobial susceptibility test by disc diffusion method: To study antimicrobial resistance pattern of *E. coli* isolated from buffaloes with mastitis, agar disc diffusion method was used (*Bauer et al., 1966*) and modifications was done as per the recommendation of Clinical and Laboratory Standards Institute (*CLSI, 2014*) against n= different antimicrobials. *E. coli* culture was grown overnight in nutrient broth and then coated on Muller Hinton Agar. Antimicrobial discs of Ampicillin, Ampicillin + Sulbactam, Cefepime, Cefepime + Clavulanic acid, Cefotaxime, Cefotaxime + Clavulanic acid, Amoxicillin + Clavulanic acid, Ceftazidime, Ceftazidime + Clavulanic acid and Aztreonam were procured from Himedia laboratories, Mumbai and included in the present study. Antimicrobial discs were placed aseptically at required distance and incubated at 37°C for 24 hr. Zones of inhibition was measured and results were interpreted as per the manufacturer's instructions (HiMedia Laboratories Mumbai).

RESULTS AND DISCUSSION

Prevalence of subclinical mastitis: The prevalence of mastitis at any livestock farm is directly or indirectly indicator of managemental practices of that farm. Since, mastitis is the environmental associated contagious disease of bovine's especially milking animals and adoption of Good Hygienic Practices in day to day dairy operations would be of paramount importance to reduce its incidence. During present investigation a total of 750 buffaloes were sampled from six farms and screened for subclinical mastitis by California Mastitis Test (CMT). A total 310 samples were found positive with overall prevalence of subclinical mastitis was 41.33 per cent. Farmwise prevalence of subclinical mastitis in present study is depicted in Table 2.

Estimated prevalence of subclinical and clinical mastitis in bovines recorded by earlier researchers revealed wide variations. A cross sectional study carried

Table 2: Farm wise Incidence of Subclinical Mastitis

Place /Farm	No. of Milk Samples tested	Subclinical cases	Prevalenc (%)
Unit 21 Goregaon	205	90	43.90
Unit 19 Goregaon	155	72	46.45
Palghar	90	46	51.11
Safale	97	32	32.98
Dahisar	88	38	43.18
Virar	115	32	27.82
Total	750	310	41.33

out by *Mekibib et al. (2010)* in dairy farms of central Ethiopia also revealed prevalence of clinical and subclinical mastitis together as 71 per cent, out of which 22.4 and 48.6 per cent cases suffered from clinical and subclinical mastitis, respectively. Study of *Rahman et al. (2010)* in dairy cows reared overall prevalence of subclinical mastitis as 51.3 per cent at Government dairy farm in Bangladesh. Findings of *Ayano et al. (2013)* conducted on commercial dairy farms, Ethiopia also recorded prevalence of subclinical mastitis at 41.02 per cent which is almost similar to the observation of present study.

Prevalence of subclinical mastitis in dairy animals in India revealed 10-70 per cent. Comparatively less prevalence of subclinical mastitis in cow (14.17%) was recorded by *Supriya et al. (2010)*. Their results showed prevalence of clinical and subclinical mastitis as 8 per cent and 72 per cent, respectively. *Srinivasan et al. (2013)* and *Ali et al. (2015)* have observed 26.21 per cent and 31.25 per cent prevalence of subclinical mastitis from Namakkal and Indian Veterinary Research Institute (IVRI) respectively. Findings of present research work are in agreement with the aforementioned reports of prevalence of clinical and subclinical mastitis in bovines.

Prevalence of E. coli: All the CMT positive milk samples obtained from animals suffering from subclinical mastitis were processed for isolation of *E. coli* by culture method. *E. coli* strains could be isolated from 94 out of 310 milk samples with prevalence of 30.32 per cent.Thus, present research clearly indicated that *E. coli* is ubiquitous in the dairy farm environment and animal itself may act as an important source of this enteropathogen which may cause mastitis.

E. coli has been successfully isolated from bovine mastitis cases by number of investigators in India (*Ranjan et al., 2011; Kurjogi and Kaliwal, 2011; Hegade et al., 2012; Palaha et al., 2012*) and outside India by many researchers (*Dopfer et al., 1999; Bradley and Green 2001; Lira et al., 2004; Momtaz et al., 2012; Abera et al., 2013; Alekish et al., 2013; Tesfaye et al., 2013 and Mahamoud et al., 2015; Iraguha et. Al., 2015*). All these investigators have recorded prevalence of *E. coli* from bovine mastitis in the range of 6 to 35 per cent. So, present findings are consistent to these findings in which overall prevalence 30.32 per cent was recorded.

Antimicrobial Resistance Pattern: Resistance pattern of *E. coli* was studied against the beta- lactams group of antimicrobials. A total 10 beta lactam antimicrobials were selected including with and without beta-lactamase inhibitors. The resistance pattern shown by *E. coli* isolates in the present study is depicted in Table 3.

The study indicated that greater percentages of isolates were resistant to Cefotaxime (53.19%), followed by Aztreonam (53.19%), Ceftazidime (42.55%),

Table 3: Antimicrobial Resistance pattern of *E. coli*

Antimicrobials	Per cent Sensitivity/ resistance		
	Susceptible	Intermediate	Resistant
Ampicillin	38.29 (36)	28.72 (27)	32.97 (31)
Ampicillin + Sulbactam	71.27 (67)	19.14 (18)	9.57 (9)
Cefepime	80.85 (76)	(0)	19.14 (18)
Cefepime + Clavulanic acid	86.17 (81)	8.51 (8)	4.25 (4)
Cefotaxime	37.23 (35)	9.57 (9)	53.19 (50)
Cefotaxime + Clavulanic acid	71.27 (67)	19.14 (18)	9.57 (9)
Amoxicillin + Clavulanic acid	77.65 (73)	10.63 (10)	11.70 (11)
Ceftazidime	9.57 (9)	47.87 (45)	42.55 (40)
Ceftazidime + Clavulanic acid	28.72 (27)	52.12 (49)	19.14 (18)
Aztreonam	4.25 (4)	42.55 (40)	53.19 (50)

Ampicillin (32.97%) and Cefepime (19.14%). However highest sensitivity was observed towards Cefepime + Clavulanic acid (86.17%) followed by Cefepime (80.85%), Amoxicillin + Clavulanic acid (77.65%) and, Ampicillin + Sulbactam (71.27%). Whereas the sensitivity to other tested beta-lactam antimicrobials was observed below 40 per cent (Plate 1 and Plate 2).

Momtaz et al. (2012) carried out study to detect the virulence factors, serogroups, and antibiotic resistance properties of Shiga toxin-producing *E. coli* (STEC), by using 268 bovine mastitic milk samples which were diagnosed using CMT. The disk diffusion method showed that the STEC strains had the highest resistance to penicillin (100%), followed by tetracycline (57.44%), while resistance to cephalothin (6.38. In the study of *Mahmoud et al. (2015),* the antimicrobial sensitivity indicated that the most effective antibiotics were Lincospectine (56.6%), Danofloxacin (56.6%), Enrofloxacin (40%) and, ceftiofur (40%), while the lowest effective antibiotics were oxytetracycline and ampicillin. *Kurjogi and Kaliwal (2011)* conducted antibiogram studies for the isolates from clinical and subclinical mastitis by using 14 antibiotics like kanamycin (76.20%), Cloxacillin (75.30%), rifampicin (48.26%), ampicillin (60.86%), penicillin-G (25.61%), carbenicillin (25.41%), chloramphenicol (67.07%), cephalothin (43.57%), tetracycline (78.35%), trimethoprim (38.73%), polymyxin-B (20.08%), streptomycin (57.13%), gentamicin (49.77%) and, amikacin (39.02%) which were used frequently for the treatment of mastitis. Tetracycline was found to be more effective antibiotic among all the tested antibiotic. In the present study *E. coli* resistance to Amoxicillin + Clavulanic acid was 31.91 per cent. *Rangel and Marin (2009)* from Brazil, reported similar resistance pattern (31%) from the *E. coli* mastitis. *Alekish et al. (2013)* reported 84.5 per cent resistance to ampicillin by *E. coli* isolates obtained from bovine clinical mastitis. *Najeeb et al. (2013)* recorded 58.69 per cent resistance against penicillin from mastitic isolates.

CONCLUSION

The aim of the present study was to assess the antimicrobial resistance pattern of *E. coli* obtained from subclinical mastitis. Worldwide, mastitis is one of the most important and costly infectious diseases of the dairy industry. It is unique disease having multiple etiologies. Coliform mastitis is one of the forms of mastitis caused by *E. coli.* It is highly alarming to quote that most of isolates were resistant to many antibiotics. The study indicated that greater percentages of isolates were resistant to Cefotaxime however highest sensitivity was observed towards Cefepime + Clavulanic acid followed by Cefepime, Amoxicillin + Clavulanic acid and Ampicillin + Sulbactam. Present study revealed that AMR with special reference to â- lactams is common in *E. coli* strains as resistance to Cefotaxime was found very high and resistance to Cefepime/Clavulanic acid

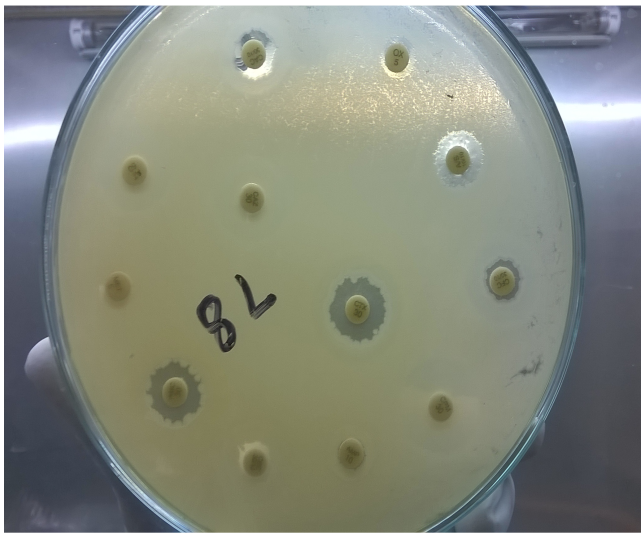

Plate 1: Resistant E.coli to Ampicillin, Ampicillin+Sulbactam, Amoxycillin, Amoxicillin+ Clavulanic acid, Ceftazidime, Cefotaxime

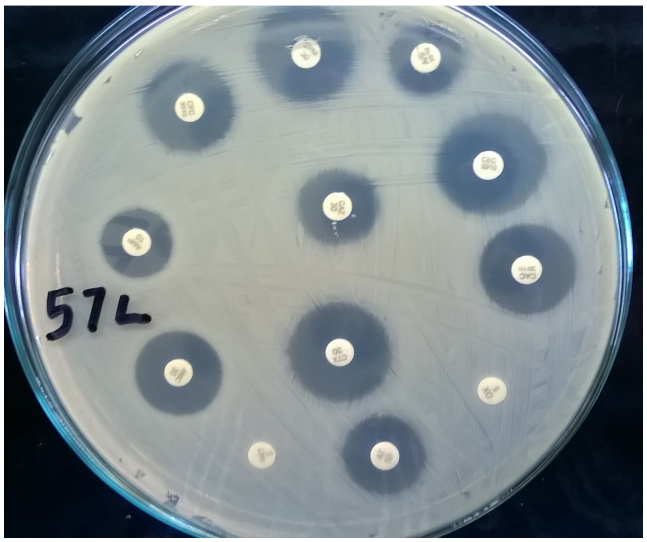

Plate 2: Showing resistance to Ceftazidime, Ampicillin and Ampicillin + Sulbactam

was comparatively low. Hence, it can be interpreted that beta-lactams along with Clavulanic acid or other beta-lactamase inhibitors may be more useful in the mastitis treatments.

Thus, it has concluded that *Escherichia coli* have potential to cause subclinical mastitis and they are ubiquitous in the farm environment and judicious use of antimicrobials in veterinary practice is recommended to reduce the risk associated with developing resistance of *E. coli* against beta-lactam antimicrobials.

REFERENCES:

Abera, B., Lemma, D. and Iticha I. (2013). Study of bovine mastitis in Asella government dairy farm of Oromia Regional state, South Eastern Ethiopia. *Intl. Journal of Current Research and Academic Review,* **1** (2): 134 – 145.

Alekish, O. K., AL-Qudah, K.M. and AL-Saleh A. (2013). Prevalence of antimicrobial resistance among bacterial pathogens isolated from bovine mastitis in northern Jordan. *Revue Med. Vet.***164** (6) :319 – 326.

Ayano, A. A., F. Hiriko, F., Simyalew, A. M. and Yohannes, A. (2013). Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district. *Journal of Veterinary Medicine and Animal Health*. **5**(3): 67-72.

Bauer, A.W., Kirby, W.M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*. **45**: 493–496.

Bradley A.J. (2002). Bovine mastitis: An evolving disease. *The veterinary Journal*. **163** :1-13.

Bradley, A. J. and Green M.J. (2001). Adaptation of *Escherichia coli* to the bovine mammary gland. *Journal of Clinical Microbiology* **39** (5) :1845–1849.

CLSI. (2014). Performance standards for antimicrobial susceptibility testing – 24 the informational supplement M100-S24, PP 66-68

Dogan, B., S. Klaessig, M. Rishniw, R. A. Almeida, S. P. Oliver,K. Simpson, Y.H. Schukken, H. (2006). Adherent and invasive *Escherichia coli* are associated with persistent bovine mastitis. *Veterinary Microbiology.* **116**: 270–282

Dopfer, D.,H. W. Barkema, T. J. Lam, Schukken Y. and Gaastra W. (1999). Recurrent clinical mastitis caused by *Escherichia coli* in dairy cows. *Journal of Dairy Science*. **82**(1):80-5.

Fernandes, J. B. C., Zanardo, L. G., Galvao, N.N., Carvalho, L. A., Nero, M. A. and Moreira S. (2011). *Escherichia coli* from clinical mastitis: serotypes and virulence factors. *Journal of Veterinary Diagnostic Investigation,* **23**(6) : 1146– 1152 .

Hegde, R., Isloor, S., Prabhu, K.N., Shome, B.R., Rathnamma, V., Suryanarayana, S. Yatiraj, S., Prasad, R., Krishnaveni, N., Sundareshan, N., Akhila D.S. and Gomes A.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.

Hogan J. and Smith K.L. (2003) Coliform mastitis. *INRA, EDP Sciences, and Veterinary. Research*. **34** : 507–519.

Iraguha, B., Hamudikuwanda, H. & Mushonga, B. (2015). Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda, Journal of the South African Veterinary Association [On-line] Available: http:// dx.doi.org/ 10.4102/jsava. v86i1.1228 9835568177 [December 8, 2016]

Kurjogi, M. M. and B. B. Kaliwal B.B. (2011). Prevalence and antimicrobial susceptibility of bacteria isolated from bovine mastitis. *Advances in Applied Science Research,* **2** (6):229-235

Lira, W. M., C. Macedo and J. M. Marin (2004). The incidence of shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. *Journal of Applied Microbiology.* **97**: 861–866.

Mahmoud, A. K. A., A. M. Khadr, T. M. Elshemy, H. A. Hamoda and M. I. Ismail (2015). Some studies on *E. coli* mastitis in cattle and buffaloes. Alexandria. *Journal of Veterinary Science*. **45**: 105-112.

Mekibib, B., M. Furgasa, F. Abuna, B. Megersa and A. Regassa (2010). Bovine mastitis**:** Prevalence, risk factors and major pathogens in dairy farms of Holeta town, Central Ethiopia. *Veterinary world.* **3**(9):397-403.

Momtaz, H., S.D. Farhad S., T. Taktaz, A. Rezvani, and S. Yarali (2012). Shiga toxin-producing *Escherichia coli* isolated from bovine mastitic milk: serogroups, virulence factors and antibiotic resistance properties. *The Scientific World Journal*. [on-line] pp 1-9. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3507047/pdf/TSWJ2012-618709.pdf. [Decemeber 8, 2016]

NAAS (2013). Mastitis management in diary animals. Policy paper No. 61, National academy of agriculture Science, New Delhi : PP 38-39

Palaha R., N. Chaudhary and H. Kumar (2012). Detection of *Escherichia coli* from the udder of the dairy farmbuffaloes in Phagwara region, Punjab, India. *Veterinary World*, **5**(9): 522-525.

Persson, Y., A. J. Nyman and U. G. Andersson (2011). Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Veterinaria Scandinavica*, **53**:36.

Rahman, M. M., M. R. Islam, M. B. Uddin and M. Aktaruzzaman. 2010. Prevalence of subclinical mastitis indairy cows reared in Sylhet District of Bangladesh. International Journal of Biological Research. **1**(2): 23-28

Ranjan, R., M.K. Gupta and K. K. Singh (2011). Study of bovine mastitis in different climatic conditions in Jharkhand, India. *Veterinary World*, **4**(5) : 205-208.

Shpigel N. Y. (2001). Clinical and theupeutic aspect of coliform mastitis in dairy cows under intensive management. DVM. Veterinary Medicine University of Helsinki, Finland.

Srinivasan, P., D. Jagadeswaran, R. Manoharan, T. Giri, G.A. Balsubramaniam and P. Balchandran (2013). Prevalence and etiology of subclinical mastitis among buffaloes (*Bubalusbubalus*) in Namakkal. India. *Pakistan Journal of Biological Sciences*. **1**;**16** (23):1776-80

Supriya., V., Saxena V. and Lather, D. (2010). Prevalence of subclinical mastitis in organized cow herd in Haryana *Veterinary world*, **49:** 64-65.

Tenover, F. C.(2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine*. **119** (6A) : S3- S10.

Tesfaye A., Yohannes, A., Hunde, A., Tezera, T., Zelalem, G. and Sadik, T. (2013). Mastits: Prevalence, risk factors and antimicrobial sensitivity patterns of bacterial isolates in dairy cattle at Holeta farm in Ethiopia. *African Journal of Agriculture*. **8** (23) : 2837-2842.

Patricia Rangel and José Moacir Marin (2009). Analysis of *Escherichia coli* isolated from bovine mastitic milk. *Brazilian Journal of Veterinary Research*. **29**(5):363-368.

Najeeb, M. F., Anjum, A. A., Ahmad, M. U., Khan, M. A., Ali, M. A. and Sattar, M. K. (2013). Bacterial etiology of subclinical mastitis in dairy goats and multiple drug resistance of the isolates *Journal of Animal and Plant Science*. **23**(6):2013 pp 1541-1544`

• • • • •