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COMPARISON OF BACTERIAL PATHOGENS ASSOCIATED WITH DIFFERENT TYPES OF BOVINE MASTITIS AND THEIR ANTIBIOTIC RESISTANCE STATUS IN BANGLADESH

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ABSTRACT

Background: Mastitis is one of the most prevalent complex diseases of mammals with high economic impact in dairy industry worldwide. Most of the published inland reports on bovine mastitis are mainly based on sub-clinical mastitis and some extent to clinical types.

Objectives: The main objectives of this study are (a) to compare the bacterial pathogens associated with sub-clinical, clinical and chronic mastitis, and (b) to detect the antibiotic sensitivity and resistance status of the isolated bacteria from different types of mastitis.

Materials and Methods: A total of 539 quarter milk samples, collected from lactating cows of Rajshahi and Mymensingh districts were subjected to standard bacterial culture and biochemical tests during the period of 2010-2011. Antibiogram test was done on bacteria isolated from sub-clinical (n = 444), acute (n = 35) and chronic (n = 60) mastitis cases include *Staphylococcus spp.*, *Streptococcus spp.*, *Bacillus spp.* and *E. coli* in 78.54%, 80% and 71.67% milk samples as a single and 21.46%, 20% and 28.33% as mixed infection, respectively.

Results: The *Staphylococcus spp.* was recorded as major pathogen for all the sub-clinical (42.15%), acute (45.71%) and chronic (41.67%) mastitis cases. The right hind quarters were found significantly ($p < 0.05$) more affected with sub-clinical, acute and chronic types of mastitis than other three quarters in cows. The highest sensitivity (up to 100%) was recorded with gentamicin, ciprofloxacin, oxytetracycline and enrofloxacin against all the tested four organisms isolated from sub-clinical, clinical and chronic mastitis cases. Antibiotic resistance was highly prevalent, especially streptomycin (70-100%), amoxicillin (30-100%) and ampicillin (0-100%) against the four isolated bacteria of three different types of mastitis.

Conclusions: It may be concluded that there is a need to establish a nationwide plan for monitoring the resistance of antibiotics and ensure the cautious use of antibiotics in the veterinary medical practices.

Keywords: Bovine mastitis, Clinical mastitis, Subclinical mastitis, Chronic mastitis, Mastitic bacterial pathogens and Antibiogram

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INTRODUCTION

There is a heavy shortage and high demand of milk due to inadequate production, population growth and increasingly urbanized population in Bangladesh. To meet the increasing demand, improvements of genetic potential of the indigenous zebu cattle through cross-breeding program with high-grade exotic breeds have been launched in Bangladesh. The cross-bred dairy cattle are genetically highly susceptible to diseases including mastitis. Bovine mastitis is a multi-etiological and commonly prevalent most costly complex disease in dairy industries. Bangladesh does not have a well-established Veterinary medical services (VMS) infrastructure up to rural levels. As a result, most of the VMS are performed by non-vet graduates and quack which results massive indiscriminate and improper use of antibacterial drugs in animal health that lead to development of resistant to the causal bacteria. However, the prevalence and risk factors of bovine mastitis,[1-9] isolation and identification of bacterial pathogens associated with clinical[10-12] and sub-clinical mastitis with antibiogram[13-18] pattern have been reported from Bangladesh. Therefore, this paper describes the comparison of bacterial pathogens associated with sub-clinical, acute and chronic bovine mastitis with their sensitivity and resistance status in the smallholder and commercial dairy farms in Bangladesh.

MATERIALS AND METHODS

Sources of milk samples

This research study on different types of mastitis was conducted in a total of 151 cross-bred lactating dairy cows in the districts of Rajshahi and Mymensingh during the period from 2010 to 2011. Milk samples (n = 444) were collected from 111 cross-bred (Shahiwal × Deshi) apparently healthy lactating cows of Rajshahi Dairy and Breed Development Farm (RDBDF), Rajabari hat, Rajshahi and 95 Milk samples from clinical mastitis cases were collected from 40 cross-bred (HF × Deshi) lactating cows of adjacent villages of BAU Campus (AVBAU), Mymensingh. Relevant information about the farm, breed and history of individual animal were recorded.

Milk samples were collected aseptically for bacteriological studies as suggested earler.[19] Prior to sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry. The milk samples were collected in sterile polypropylene containers and brought to the laboratory for bacterial cultural and antibiogram studies.

Categorization of mastitis

Three types of mastitis were used in this study that include clinical mastitis (inflammation with visual signs of inflammation in the udder and changes in milk), sub-clinical mastitis (inflammation of the udder without any visible abnormalities of either the milk or the udder) and chronic mastitis (clinical mastitis cases that remain infected for more than 100 days) in lactating dairy cows.[20]

Media reagents and chemicals

The media and reagents were either obtained from Hi-media, Mumbai or prepared in the laboratory as per the standard procedures.[21]

Identification of isolates

In the present study, bacteriological examination was carried out for the detection of mastitis in which from each sample approximately 0.01 ml of milk sample was cultured on nutrient broth, nutrient agar, blood agar, MacConkey's agar and Eosine Methylene Blue agar plates and the plates were incubated at 37°C for 24 to 48 hours. The staining and cellular morphological features of organisms were ascertained by microscopic examination of Gram stained smears. The bacteria isolated were identified on the basis of their cultural, morphological and biochemical characteristics.[21]

Antibacterial susceptibility testing

Antibiotic sensitivity test was done by Kirby- Bauer's disc. The antibiotic discs (M/s Hi Media Laboratories Ltd., Mumbai) viz., Ampicillin 10 µg, Amoxycillin 10 µg, Enrofloxacin 5 µg, Doxycycline 30 µg, Gentamicin 120 µg, Streptomycin 10 µg, Oxytetracycline 30 µg, Chloramphenicol 30 µg, Ciprofloxacin 5 µg and Erythromycin 15 µg were placed on the surface of charged agar plates aseptically at equidistant from each other. The plates were incubated at 37°C overnight. The susceptibility of the isolates to different drugs was observed by measuring zone of inhibition.[22] Of all bacterial isolates, each of ten isolates of *Staphylococcus spp., Streptococcus spp., Bacillus spp.* and *Escherichia coli* were selected randomly for testing the antibiotic sensitivity status against the antibiotics.

Staining methods

Gram's staining method was followed to study the morphological and staining characteristics bacteria and to provide information about the presumptive bacterial identification.[23] A small colony was picked up from different agar plates with a bacteriological loop, smeared on separate glass slide and fixed by gentle heating. Crystal violate was then applied on each smear to stain for two minutes and then washed with running water. Few drops of Gram's iodine solution was then added to act as a mordent for one minute and then again washed with running water. Acetone alcohol was then added for few second. After washing with water, safranin was added and allowed to stain for two minutes. The slides were then washed with running water, blotted and dried in air and then examined under microscope with high power objects (100 ×) using immersion oil.

Statistical analysis

Data were analyzed by Chi square (χ^2) test and Z test for comparison of proportion to observe the significant influence of the bacteria for causing mastitis of cows using IBM SPSS (Statistical Package for Social Science) statistics 20.0 software.

RESULTS

Bacteriological investigation

Sub-clinical mastitis

Bacteriological examination of milk samples of 444 apparently healthy quarters (111 cows) revealed that 261(58.78%) quarters had different infections, of which 205 (78.54%) quarters

had single bacterial infections and 56 (21.46%) quarters had mixed bacterial infections (Table 1). Infection was observed in 114 front and 147 hind quarters of total 261 quarters (Table 2).

Table 1. Comparison of bacterial pathogens isolated from different types of bovine mastitis

SN	Organism isolated	Sub-clinical mastitis (n = 444) Quarter +ve No. (%)	Sub-clinical mastitis (n = 444) Isolates No. (%)	Acute mastitis (n = 35) Isolates No. (%)	Chronic mastitis (n = 60) Isolates No. (%)
1.	*Staphylococcus* spp.	261 (58.78)	110 (42.15)	16 (45.71)	25 (41.67)
2.	*Streptococcus* spp.	261 (58.78)	039 (14.94)	04 (11.43)	07 (11.66)
3.	*Bacillus* spp.	261 (58.78)	017 (06.51)	02 (05.71)	06 (10.00)
4.	*Escherichia coli*	261 (58.78)	039 (14.94)	06 (17.14)	05 (08.33)
5.	*Staphylococcus* spp.+ *Streptococcus* spp.	261 (58.78)	009 (03.45)	01 (02.86)	04 (06.67)
6.	*Staphylococcus* spp. + *Bacillus* spp.	261 (58.78)	013 (04.98)	01 (02.86)	04 (08.33)
7.	*Staphylococcus* spp. + *Escherichia coli*	261 (58.78)	015 (05.75)	02 (05.71)	03 (05.00)
8.	*Bacillus* spp. + *Streptococcus* spp.	261 (58.78)	011 (04.21)	01 (02.86)	02 (03.33)
9.	*Bacillus* spp. + *Escherichia coli*	261 (58.78)	008 (03.07)	02 (05.71)	03 (05.00)
	Overall (Single infection)	261 (58.78)	205 (78.54)*	28 (80.00)*	43 (71.67)*
	Overall (Mixed infection)	261 (58.78)	056 (21.46)	07 (20.00)	17 (28.33)

n = No. of quarter milk samples tested *Significantly ($p < 0.05$) higher

Acute mastitis

Over 35 milk samples of acute mastitic quarters revealed that 35 quarters had different infection having 28 (80%) quarters single bacterial infections and 7 (20%) quarters mixed bacterial infections (Table 1). Among acutely infected 35 quarters, front and hind quarters were 10 (28.57%) and 25 (42.46%) respectively (Table 2).

Chronic mastitis

Sixty milk samples examined from chronic mastitic quarters of which, 40 cows showed that all had different infections of which single and mixed bacterial infections were found 43

(71.67%) and 17 (28.33%) quarters respectively (Table 1). Again, of the 60 quarters, 20 Front quarters and 40 Hind quarters were infected with chronic mastitis (Table 2).

Table 2. Quarter-wise prevalence of different types of mastitis in lactating dairy cows

SN	Quarter side	Acute mastitis (n = 35) +ve quarter No. (%)	Chronic mastitis (n = 60) +ve quarter No. (%)	SC mastitis (n= 261) +ve quarter No. (%)
①	Left front	05 (14.28)	10 (16.67)	60 (22.99)
②	Right front	05 (14.28)	10 (16.67)	54 (22.99)
	Sub-total	10 (28.57)	20 (33.33)	114 (43.68)
③	Left hind	10 (28.57)	17 (28.33)	71 (27.20)
④	Right hind	15 (42.86)*	23 (38.33)*	76 (29.12)*
	Sub-total	25 (71.43)	40 (66.67)	147 (56.32)

SC = Sub-clinical *Significantly ($p < 0.05$) higher

Antibiogram study of the isolated bacteria

Antibiotic sensitivity pattern of *Staphylococcus spp. and Streptococcus spp.*

The antibiograms of various isolates of Staphylococci and Streptococci from the three types of mastitis were found to be 100% sensitive to gentamicin, ciprofloxacin, erythromycin, chloramphenicol, enrofloxacin and oxytetracycline whereas 70% resistant to ampicillin and streptomycin. In SCM, 70% resistant to amoxycillin and in acute and chronic mastitis, 100% resistant to amoxycillin. In SCM and acute mastitis, nature of being resistance towards doxycycline is 30% whereas in chronic mastitis, isolates were 70% resistant to doxycycline (Table 3)

Antibiotic sensitivity pattern of *Bacillus spp*.

Various isolates of *Bacillus spp*. in three cases of mastitis were found to be 100% sensitive to gentamicin, ciprofloxacin, enrofloxacin and oxytetracycline. Again, in both SCM and acute mastitis isolates were 70% resistant towards ampicillin, amoxicillin and streptomycin but 100% resistant in chronic mastitis. However, 30% resistant to erythromycin and chloramphenicol in case of SCM and same observation was found in acute case only for erythromycin. 100% sensitivity was found to chloramphenicol and doxycycline in acute case and only doxycycline in SCM. Again in chronic mastitis, various isolates were 70% resistant to erythromycin, chloramphenicol and doxycycline. (Table 3)

Table 3. Antibiotic resistance status of bacteria isolated from mastitic udder of lactating cows

SN Antibiotics	No. of isolate tested	Types of mastitis	*Escherichia coli* No. (%)	*Bacillus* spp. No. (%)	*Streotococcus* spp. No. (%)	*Staphylococcus* spp. No. (%)
① Streptomycin	10	SC	7 (70.0)	7 (70.0)	7 (70.0)	7 (70.0)
	10	Acute	7 (70.0)	7 (70.0)	7 (70.0)	7 (70.0)
	10	Chronic	7 (70.0)	10 (100)	7 (70.0)	7 (70.0)
② Doxycycline	10	SC	3 (30.0)	0	3 (30.0)	3 (30.0)
	10	Acute	0	0	3 (30.0)	3 (30.0)
	10	Chronic	3 (30.0)	7 (70.0)	7 (70.0)	7 (70.0)
③ Oxytetracycline	10	SC	0	0	0	0
	10	Acute	0	0	0	0
	10	Chronic	0	0	0	0
④ Enrofloxacin	10	SC	0	0	0	0
	10	Acute	0	0	0	0
	10	Chronic	0	0	0	0
⑤ Amoxicillin	10	SC	3 (30.0)	7 (70.0)	7 (70.0)	7 (70.0)
	10	Acute	3 (30.0)	7 (70.0)	10 (100)	10 (100)
	10	Chronic	7 (70.0)	10(100)	10 (100)	10 (100)
⑥ Ampicillin	10	SC	0	7 (70.0)	7 (70.0)	7 (70.0)
	10	Acute	0	7 (70.0)	7 (70.0)	7 (70.0)
	10	Chronic	0	10 (100)	7 970.0)	7 (70.0)
⑦ Chloramphenicol	10	SC	0	3 (30.0)	0	0
	10	Acute	0	0	0	0
	10	Chronic	0	7 (70.0)	0	0
⑧ Erythromycin	10	SC	0	3 (30.0)	0	0
	10	Acute	0	3 (30.0)	0	0
	10	Chronic	0	7 (70.0)	0	0
⑨ Ciprofloxacin	10	SC	0	0	0	0
	10	Acute	0	0	0	0
		Chronic	0	0	0	0
⑩ Gentamicin	10	SC	0	0	0	0
	10	Acute	0	0	0	0
	10	Chronic	0	0	0	0

SC = Sub-clinical

Antibiotic sensitivity pattern of *Escherichia coli*

In three cases, the anti-biograms of various isolates of *E. coli* were found to be 100% sensitive to gentamicin, ciprofloxacin, erythromycin, chloramphenicol, enrofloxacin, oxytetracycline and ampicillin, again 30% sensitive to streptomycin. Moreover, in case of acute mastitis the isolates were 100% sensitive to doxycycline but in both SCM and chronic mastitis, 30% resistant to doxycycline. Again, in both SCM and acute mastitis, the anti-biograms of various isolates of *E. coli* were 30% resistant to amoxicillin but in chronic mastitis, 70% resistant to amoxicillin (Table 3).

DISCUSSION

Many infectious agents have been implicated as causes of mastitis but *Staphylococcus* spp., *Streptococcus* spp., *E. coli* and *Bacillus spp.* were isolated from the mastitic milk samples of cows in this study which corroborate the findings of the earlier reports.[3,10,13,24-27] Single infection is significantly ($p < 0.05$) higher in acute (80%), in chronic (71.67%) and in SCM (78.54%) than mixed infection in acute (20.0%), in chronic (28.33%) and in SCM (21.46%) respectively. These findings consistent with the earlier reports of 78.18% single infection and 21.82% mixed infections,[13] 61.36% single infection and 38.63% mixed infections,[3] 60.0% single infection and 40.0% mixed infections.[26] However, Shike *et al.*[28] reported 7 (31.82%) single and 15 (68.18%) mixed infection in the sub-clinical infection, and 9 (42.86%) pure and 12 (57.14%) mixed infection respectively, from the clinical cases.

Variability on the frequency distributions of different species of bacterial isolates in different milk samples was found. *Staphylococcus spp.* has been isolated as the main pathogens of mastitic cows. This finding, in agreement with the results of earlier different reports with slight variation.[3,13,25,27,29-33]

The bacterial infections were found in all the four quarters but hind quarters were more infected, RH (Right hind) quarters were mostly infected and this finding consistent with earlier report of Sudhan *et al.*[34] who reported that the right hind quarter was the most affected (38.18%) compared with the other quarters.

In *in-vitro* antibiotic sensitivity test under this study of four different types of bacterial isolates, a major variation was noticed regarding sensitivity against the ten different antibiotics.

The antibiograms of various isolates of Staphylococci and Streptococci from sub-clinical, acute and chronic mastitis were found to be 100% sensitive to gentamicin, ciprofloxacin, erythromycin, chloramphenicol, enrofloxacin and oxytetracycline and 70% resistant to ampicillin and streptomycin.

In SCM various isolates of staphylococci and streptococci were 70% resistant to amoxicillin and in acute and chronic mastitis, 100% resistant to amoxicillin. In SCM and acute mastitis, nature of being resistance towards doxycycline is 30%, whereas in chronic mastitis, isolates of staphylococci and streptococci are 70% resistant to doxycycline. Overall study shows that isolates of both staphylococci and streptococci were resistant to amoxicillin, ampicillin, streptomycin and doxycycline. The results are in conformity with the earlier reports.[13,24,26,30,35-37]

Isolates of *Bacillus* spp. in three cases of mastitis were found to be 100% sensitive to gentamicin, ciprofloxacin, enrofloxacin and oxytetracycline. In both SCM and acute mastitis isolates were 70% resistant towards ampicillin, amoxicillin and streptomycin but 100% resistant in chronic mastitis. However, 30% resistant to erythromycin and chloramphenicol in case of SCM and same observation was found in acute case only for erythromycin. The chloramphenicol and doxycycline showed 100% sensitivity in acute case whereas only with doxycycline in SCM. Again in chronic mastitis, various isolates were 70% resistant to erythromycin, chloramphenicol and doxycycline. The *Bacillus* spp. was found resistant to amoxicillin, ampicillin, streptomycin and doxycycline. These results are in consistent with the earlier reports.[13,24,26]

In all the three types of mastitis cases, the antibiograms of various isolates of *E. coli* were found to be 100% sensitive to gentamicin, ciprofloxacin, erythromycin, chloramphenicol, enrofloxacin, oxytetracycline and ampicillin. Isolates of *E. coli* from three types of mastitis were 70% resistant to streptomycin. Moreover, in case of acute mastitis the isolates were 100% sensitive to doxycycline but in both SCM and chronic mastitis, 30% resistant to doxycycline. Again, in both SCM and acute mastitis, the antibiograms of various isolates of *E. coli* were 30% resistant to amoxicillin but in chronic mastitis, 70% resistant to amoxicillin. Overall *E. coli* were found resistant to amoxicillin, streptomycin and doxycycline. These findings are in agreement with the results of the earlier reports.[13,24,26,38]

Overall in this study, gentamicin was the most effective drug, which consistent with earlier reports,[30,38,39] followed by ciprofloxacin, enrofloxacin, oxytetracycline, erythromycin and chloramphenicol but amoxicillin, ampicillin, streptomycin and doxycycline were least effective. The variation in the sensitivity of common antibiotics could be the result of extensive and indiscriminate use of these in the treatment of udder infection.

CONCLUSIONS

Different types of mastitis in cross-bred cows may occur by either single or mixed infections and in both of the cases *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp. and *E. coli* are established as the major etiological agents. From this study it has been identified that single infection is higher than mixed infections where the mixed bacterial infections are the major causes of different types of mastitis in cows. Indiscriminate and long term use of different antibiotics may results in the development of antibiotic resistance bacteria and it has a potentiality to cause health hazards in human. So, from the clinical and economic point of view it is necessary to find out the bacteria not only resistance but also sensitive to specific antibiotics. Cultural, morphological and biochemical tests help to isolate, identify and finding out the frequency distribution of causal agents. On the other hand, antibiogram studies represent the sensitivity status of the isolates to specific antibiotics. Therefore, the findings of the present study showed that gentamicin, ciprofloxacin, enrofloxacin and oxytetracycline in optimum doses would be the drug of choice to resolve the most cases of mastitis.

Overall this study shows that in sub-clinical, acute and chronic mastitis cases, gentamicin will be the best drug of choice. Conversely, the study also recommends that amoxicillin, ampicillin,

streptomycin and doxycycline should not be preferred as there is a resistance of the isolated pathogens toward these drugs.

CONFLICT OF INTEREST

There is no conflict of interests. No funding has been received for any part.

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REFERENCES

01. Rahman MS, Nooruddin M and Rahman MM (1997). Prevalence and distribution of mastitis in cross-bred and exotic dairy cows. *Bangladesh Veterinarian* 14: 1-4
02. Rahman MA, Bhuiyan MMU, Kamal MM and Shamsuddin M (2009). Prevalence and risk factors of mastitis in dairy cows. *Bangladesh Veterinarian* 26: 54-60
03. Rahman MM, Islam MR, Uddin MB and Aktaruzzaman M (2010). Prevalence of subclinical mastitis in dairy cows reared in Sylhet District of Bangladesh. *International Journal of Bio Research* 1: 23-28
04. Islam MA, Rahman AKMA, Rony SA and Islam MS (2010). Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari Milk shed area of Sirajgonj. *Bangladesh Journal of Veterinary Medicine* 8: 157-162
05. Sarker SC, Parvin MS, Rahman AK and Islam MT (2013). Prevalence and risk factors of sub-clinical mastitis in lactating dairy cows in north and south regions of Bangladesh. *Tropical Animal Health Production* 45: 1171-1176
06. Barua M, Prodhan MAM, Islam K, Chowdhury S, Hasanuzzaman M, Imtiaz MA and Das GB (2014). Sub-clinical mastitis prevalent in dairy cows in Chittagong district of Bangladesh: detection by different screening tests. *Veterinary World* 7: 483-488
07. Rahman MM, Munsi MN, Kabir MH, Ekram MF, Rahman MT and Saha S (2014). Prevalence of sub-clinical mastitis in cows at Anwara, a coastal Upazila of Chittagong district in Bangladesh. *Journal of Veterinary Advances* 4: 594-598
08. Begum MIA, Hossain MS, Ershaduzzaman M, Islam MN and Rana MS (2015). Study on prevalence and risk factors of subclinical mastitis in lactating dairy cows in Rajshahi and Rangpur division of Bangladesh. *Wayamba Journal of Animal Science* 7: 1129-1137
09. Biswas D and Sarker T (2017). Prevalence of sub-clinical mastitis at Banaripara upazilla, Barisal. *Bangladesh Journal of Veterinary Medicine* 15: 21-26
10. Mahbub-E-Elahi ATM, Rahman MA, Rahman MM, Rahman MM, Rahman MM and Prodhan MAM (1996). Isolation and identification of bacteria from different quarters of mastitis affected dairy cows in Bangladesh. *Bangladesh Veterinaary Journal* 30: 63-65
11. Mia MT, Hossain MK, Rumi NA, Rahman MS, Mahmud MS and Das M (2016). Detection of bacteria species from clinical mastitis in dairy cows at Nilphamari district and their antibiogram studies. *Asian Journal of Medical and Biological Research* 2: 656-663

12. Hossain S, Reza MA, Hasan MN, Sorwar MG and Billah M (2016). Impact of clinical mastitis in dairy farming at Keshabpur upazilla in Jessore in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 14: 59-64
13. Kader MA, Samad MA, Saha S and Taleb MA (2002). Prevalence and etiology of sub-clinical mastitis with antibiotic sensitivity of isolated organisms among milch cows in Bangladesh. *Indian Journal of Dairy Science* 55: 218-223
14. Islam MA, Islam MZ, Islam MA, Rahman MS and Islam MT (2011). Prevalence of sub-clinical mastitis in dairy cows in selected areas of Bangladesh. *Bangladesh Journal of Veterinary Medicine* 9: 73-78
15. Hasan MA, Rahman MM, Rahman MK, Ali MZ and Islam MA (2013). Studies on bovine sub-clinical mastitis with special emphasis to bacterial pathogens and antibiogram at Dinajpur district. *International Journal of Animal and Fisheries Science* 1: 1-7
16. Haque ME, Islam MA, Akter S and Saha S (2014). Identification, molecular detection and antibiogram profile of bacteria isolated from California Mastitis Test positive milk samples of cross-bred cows of Satkhira district in Bangladesh. *GSTF International Journal of Veterinary Science* 1: 59-63
17. Kayesh M, Talukder M and Anower ANM (2014). Prevalence of sub-clinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal district in Bangladesh. *International Journal of Biological Research* 2: 35-38
18. Hasan MT, Islam MR, Runa NS, Hasan MN, Uddin AHMM and Singh SK (2016). Study on bovine sub-clinical mastitis on farm condition with special emphasis on antibiogram of the causative bacteria. *Bangladesh Journal of Veterinary Medicine* 14: 161-166
19. Honkanen-Buzalski T (1995). *Laboratory Handbook on Bovine Mastitis*. NMC Inc., Madison, WI, pp: 111-114.
20. Samad MA (2008). *Animal Husbandry and Veterinary Medicine*. Volume 2. LEP Publication No. 11, BAU Campus, Mymensingh, Bangladesh
21. Cruickshank R, Duguid JP, Marmion BP and Swain RHA (1975). *Medical Microbiology*. Vol. 2, 12thedn., Crurchill Livingstone, New York
22. Bauer AW, Kirby WM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical pathology* 45: 493-496
23. Merchant IA and Packer RA (1967).*Veterinary Bacteriology and Virology*. 7thedn., The Iowa University Press, Ames, Iowa, USA
24. Elango A, Doraisamy KA, Rajarajan G and Kumaresan G (2010). Bacteriology of sub clinical mastitis and antibiogram of isolates recovered from cross bred cows. *Indian Journal of Animal Research* 44: 280 - 284
25. Kurjogi MM and Kaliwal BB (2011). Prevalence and antimicrobial susceptibility of bacteria isolated from bovine mastitis. *Advances in Applied Science Research* 2: 229-235
26. Harini H and Sumathi BR (2011). Screening of bovine milk samples for sub-clinical mastitis and antibiogram of bacterial isolates. *Veterinary World* 4: 358-359
27. Mohanty NN, Das P, Pany SS, Sarangi LN, Ranabijuli S and Panda HK (2013). Isolation and antibiogram of *Staphylococcus*, *Streptococcus* and *E. coli* isolates from clinical and subclinical cases of bovine mastitis. *Veterinary World* 6: 739-743
28. Shike DD, Keskar DV, Jagadish S, Bhalero DP and Sharma LK (1998). Clinical and subclinical mastitis in cross-bred cows: aetiology and antimicrobial sensitivity. *Indian Veterinary Journal* 75: 458-459

29. Hegde R, Isloor S, Prabhu KN, Shome BR, Rathnamma D, Suryanarayana VV, Yatiraj S, Prasad CR, Krishnaveni N, Sundareshan S, Akhila DS, Gomes AR, Hegde NR (2013). Incidence of sub-clinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. *Indian Journal of Microbiology* 53: 315–320
30. Tamiru F, S Alemu and ATsega, 2013. Aerobic microorganisms isolated from mastitic bovine milk and their antimicrobial susceptibility profiles, Ethiopia. *Am-Euras Journal of Agriculture and Environmental Science* 13: 920-925
31. Saidi R, Khelef D and Kaidi R (2013). Subclinical mastitis in cattle in Algeria: Frequency of occurrence and bacteriological isolates. *Journal of the South African Veterinary Association* 84: 1-5
32. Jeykumar M, Vinodkumar G, Bashir BP and Krovvidi S (2013). Antibiogram of mastitis pathogens in the milk of crossbred cows in Namakkal district, Tamil Nadu. *Veterinary World* 6: 354-356.
33. Padhy A, Dalai N, Shekhar S, Sahu AR, Sahoo S and Kashyap DK (2014). A Microbial, Antibiogram and Haematological Study of Mastitic Cows. *Journal of Cell and Tissue Research* 14: 4681-4684
34. Sudhan NA, R Singh, M Singh, JS Soodan, 2005. Studies on the prevalence, etiology and diagnosis of subclinical mastitis among crossbred cows. Indian Journal of Animal Research 39: 127-130
35. Adesola AE (2012). Antimicrobial resistance pattern of *Streptococci* and *Staphylococci* isolated from cases of bovine clinical mastitis in Nigeria. *Nature and Science* 10: 96-101
36. Jain B, Tewari A, Bhandari BB and Jhala MK (2012). Antibiotic resistance and virulence genes in *Streptococcus agalactiae* isolated from cases of bovine subclinical mastitis. *Veterinary Archive* 82: 423-432
37. Mubarack HM, Doss A, Vijayasanthi M, Venkataswamy R (2012). Antimicrobial Drug Susceptibility of *Staphylococcus aureus* from Subclinical Bovine Mastitis in Coimbatore, Tamilnadu, South India. *Veterinary World* 5: 352-355
38. Gitau GK, Wabacha JK, Mulei CM, Ndurumo S and Nduhiu JM (2011). Isolation rates and antimicrobial sensitivity patterns of bovine mastitis pathogens in periurban area of Nairobi, Kabete, Kenya. *Ethiopian Veterinary Journal* 15: 1-13
39. Awandkar SP, Bhikane AU and Kulkarni MB (2013). Antibiotic resistance trends in clinical bovine mastitis. *Biolife* 1: 139-143