Identification and Antibigram of Mastitis Pathogens Isolated from Bovine and Caprine Clinical Mastitis in Wayanad District- Kerala

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Abstract

The present study included 30 milk samples from animals affected with clinical mastitis for antibiotic sensitivity tests from TVCC, Pookode, and Ambulatory clinic in Wayanad. 18 were bovine milk samples and 12 were goat milk samples. Most of the cases were presented with swelling in the udder, yellowish milk, flakes of pus, serosanguinous discharge, fibrosis of the udder, low milk yield, and edema. Based on the culture and biochemical characteristics, the organism was identified. Out of 30 samples, 13 (43.33 percent) was positive for staphylococcus aureus, 7 (23.33%) samples for streptococcus agalactiae, 3 (10%) samples for *E coli*, 2 (6.66%) samples for Enterobacter, 1 (3.33%) sample for klebsiella, rest 4 (13.33%) samples had no growth. Antibiotic sensitivity test showed 66.66 percent samples were sensitive to ceftriaxone/tazobactam, 63.33 percent samples sensitive to cefoperazone, 56.66 percent samples sensitive to enrofloxacin, 53.33 percent samples sensitive to Amoxicillin-clavulanate, 50 percent samples sensitive to tetracycline, 40 percent samples sensitive to gentamicin, 26.66 percent samples sensitive to ceftizoxime, 20 percent samples sensitive to co-trimazole and Amoxicillin. The results of the present study demonstrated the growing resistance of frequently used antimicrobials in the case of mastitis.

Keywords: Clinical Mastitis; Identification; Antibiotic Sensitivity; Antibigram; Bovine Mastitis; Caprine Mastitis

Introduction

Mastitis is the inflammation of the parenchyma of the mammary gland, characterized by physical, chemical, and bacteriological changes in milk and pathological changes in glandular tissues [1]. Mastitis is one of the complex disease conditions among ruminants. Three major types of mastitis are clinical mastitis, subclinical mastitis, and chronic mastitis. In clinical mastitis symptoms are obvious. They include changes in the consistency and colour of the milk. Glandular changes of the udder result in swelling, heat, pain, and induration of the udder. *Staphylococcus aureus* is one of the most important causes of clinical mastitis [2]. It is estimated that an economic loss of over Rs 6000 crore occurs, out of which Rs 1700 crore are due to clinical mastitis and Rs 4300 crore is due to subclinical mastitis [3]. An important reason for the failure of

antimicrobial therapy is the indiscriminate usage of easily available antibiotics without subjecting them to proper antibiotic sensitivity tests.

Materials and Methods

A total of 30 milk samples of animals positive for clinical mastitis were collected from TVCC Pookode and Ambulatory clinic in the Wayanad district. Out of which 18 samples were bovine milk and 12 samples were caprine milk. All the milk samples were streaked on Brain heart infusion agar, followed by enrichment in nutrient broth, and then streaked on Muller Hinton agar with antibiotic discs. Antibiotic discs used included Gentamicin (10mcg), Ceftriaxone/Tazobactam (30mcg), Tetracycline (10mcg), Cefoperazone (75mcg), Amoxyclav (30mcg), Co-trimoxazole (25mcg), Amoxicil-

lin (30mcg), Ceftizoxime (30mcg), Enrofloxacin (10mcg). Minimal inhibition values were determined by the disc diffusion method. Further, each bacterial isolate's staining and cellular morphological features were ascertained by examination of gram-stained smears. Isolated bacteria were confirmed on the basis of their cultural and other biochemical characteristics like IMViC, catalase and oxidase tests.

Results and Discussion

Out of 30 samples of clinical mastitis, 18 were bovine milk samples, 12 were caprine milk samples. 10 samples obtained had already been subjected to antibiotic treatment. Symptoms observed were low milk yield, swelling of the udder; yellowish milk, flakes of pus, serosanguinous discharge, fibrosis, and edema of the udder. Growth on media was observed for 26 samples and there was no growth for 4 samples. Of the cultural isolates obtained 16 (61.5%) were Gram-positive organisms and the remaining 10 (38.4%) were Gram-negative organisms. The predominant organism identified was *Staphylococcus aureus* (43.33%), followed by *Streptococcus agalactiae* (23.33%), *Escherichia coli* (10%), *Enterobacter sp.* (6.66%), and *Klebsiella sp.* (3.33%). Antibigram study revealed that there is high sensitivity for Ceftriaxone/Tazobactam (66.66%) and Cefoperazone (63.33%) followed by Enrofloxacin (56.66%), Amoxclav (53.33%), Tetracycline (50%), Gentamicin (40%), Ceftizoxime (26.66%), Amoxicillin and Co-trimoxazole (20%).

Clinical mastitis is one of the most challenging disease conditions affecting both bovines and caprines in the dairy industry. The economic loss contributed by this condition is significant [3]. The high prevalence of *Staphylococcus aureus* in this study is in accordance with the study conducted by other researchers in India [4-6]. There is a gradual change in the trend of antibiotic sensitivity towards the organisms causing mastitis. Earlier antibiotics like gentamicin, enrofloxacin, and tetracycline were found to be more sensitive [7-9,10]. The present study indicated that there is a drop in the sensitivity towards gentamicin, enrofloxacin, and tetracycline. This change in the trend can be attributed to the growing resistance of the microbes. Indiscriminate usage of antibiotics by the dairy farmers without proper guidance and treatment of mastitis without prior antibiogram tests can be the reasons behind this emergence and change in the resistance pattern.

Figure 1: Mastitis in Cow.

Figure 2: Mastitis in Goat.

Figure 3: Antibigram.

Figure 4: IMViC test +++ (Staphylococcus aureus).

Figure 6: Proportion of gram-stained organism.

Figure 5: Organisms obtained.

Figure 7: Antibiotic sensitivity.

Sl. No	Species	Symptoms	Antibiotic Usage	Gram +/-	Catalase/ Oxidase	Sensitivity
1	cattle	Swelling of the udder, thick pus	No	Gram +(strep.agal)	C -ve O -ve	Enrofloxacin, gentamicin, cef-tazo, cefoperazone
2	cattle	Fever, watery milk, edema of udder	No	G+ (staph.aureus)	C +ve O -ve	Enro, genta, cef-tazo, cefoperazone
3	cattle	Yellowish thick pus, udder fibrosed	No	G-ve <i>E. coli</i>	C +ve O -ve	Amoxicillin, Amox clav, tetracycline, Enro, cef- tazo, ceftizoxime
4	cattle	Thick milk, drop in milk production	No	G-ve <i>E. coli</i>	C +ve O -ve	Amox clav, Enro, Tetracycline, cef-tazo, cefoperazone
5	Cattle	Swelling of the udder, serous discharge	Yes (intacef)	G-ve enterobacter	C +ve O -ve	Tetracycline, gentamicin, amox clav, cef tazo, cefoperazone
6	cattle	watery milk, hot to touch	yes	G-ve <i>E. coli</i>	C +ve O -ve	Gentamicin, ceftizoxime, cefoperazone, amox clav

7	Goat	Serosanguinous discharge, udder fibrosed	No	G+ve Staph aureus	C +ve O-ve	Amox clav, Enro, Tetracycline, cef tazo, cefoperazone
8	Goat	Swelling of the udder, rosy milk	No	G+ve Strep agal	C -ve O-ve	Amoxicillin, Enro, cef tazo, gentamicin, Co-trimazole
9	Goat	Edematous udder, pain envinced on plapation, yellowish milk	Yes (enro, genta, intacef)	Nil	Nil	No growth
10	Goat	Swelling of the udder, thick pus discharge	No	G+ve Staph aures	C +ve O-ve	Amox clav, Tetracycline, cef tazo, cefoperazone, ceftizoxime
11	Cattle	Slight fever, calved a day before, reduced milk yield	No	Nil	NIL	No growth
12	Goat	Edematous udder, low milk yeild	Yes (genta)	G+ve Strep agal	C-ve O-ve	Amox clav, enro, cef tazo , cefoperazone
13	cattle	No milk let down from hind quarter. Milk from fore quarter creamy consistency	No	G-ve klebsiella	C +ve O-ve	Enro, Genta, Tetracycline, cef tazo, co-trimazole, cefoperazone
14	Goat	Serosaguinous discharge, gangrenous udder	Yes (OTC)	G+ve Staph aures	C +ve O-ve	Enro, Amox clav, clindamycin,cef tazo, cefoperazone, ceftizoxime
15	cattle	Watery milk, drop in milk production	No	G-ve Staph aures	C +ve O -ve	Enro, Tetracycline,cef-tazo, cefoperazone
16	cattle	Thick creamy milk, edematous udder	No	G+ve Strep agal	C-ve O-ve	amox clav, cef tazo, ceftizoxime, amoxicillin
17	cattle	Reduced feed intake, recently calved, slightly watery milk	yes	NIL	NIL	NO Growth
18	Goat	Swelling in the udder, flakes of pus	No	G-ve Staph aures	C +ve O-ve	Genta, Co-trimazole, tetracycline, cef tazo, cefoperazone
19	cattle	Rosy milk, udder painful to touch, reduced milk yield	yes	+ve Staph aures	C +ve O-ve	Cef tazo, tetracycline, ceftizoxime
20	cattle	Reduced feed intake, reduced milk	yes	NIL	NIL	No Growth
21	cattle	Slight edema of the udder, creamy milk	No	G-ve Staph.aures	C +ve O-ve	Enro, Genta, Tetracycline, cef tazo, co-trimazole, cefoperazone
22	cattle	Fever, rosy milk, reduced milk	NO	G+ve Step agal	C-ve O-ve	Enrofloxacin, gentamicin, cef-tazo, cefoperazone
23	goat	Highly edematous udder, flakes of pus	No	G+ve Staphy aures	C +ve O-ve	Enro, Amox clav, clindamycin,cef tazo, cefoperazone, ceftizoxime
24	Goat	Reduced feed intake, no milk, blood discharge found	yes	G+ve Staph aures	Ca +ve O-ve	Enro, tetracycline, amox clav, co trimazole, cef tazo, cefoperazone

25	goat	Pain evinced on palpation, watery milk	NO	G-ve enterobacter	C +ve O-ve	Enro, genta, tetracycline, cef tazo, ceftizoxime
26	cattle	Swelling of udder, rosy milk, thick pus	NO	G+ve Strep agal	C-ve O-ve	Enro, amoxicillin, amox clav, tetracycline, cef tazo, cefoperazone
27	goat	Edematous udder of udder, rosey milk	yes	G+ve Strep agal	C-ve O-ve	Tetracycline, amox clav, genta, cef tazo
28	cattle	Fibrosed udder, no milk, serosanguinous secerions	NO	G+ve Staph aures	C +ve O-ve	Amox, amox clav, tetracycline, enro, ceferazone, ceftizoxime
29	cattle	Creamy pus, hot to touch, swelling of udder	NO	G-ve Staphy aures	C +ve O-ve	Enro, genta, amox clav, ceferazone, co-trimazole
30	goat	Swollen udder, watery milk, reduced feed intake	NO	G+ve Staph aures	C +ve O-ve	Amox, amox clav, enro, co-trimazole, cef-tazo

Table a

Conclusion

Bovine mastitis is a global problem as it adversely affects animal health, the quality of milk, and the economics of milk production. Every country including developed ones suffers huge financial losses from this condition. The emergence of resistant microbes has a huge impact, not only on animal health but also on human health. Indiscriminate use of antibiotics on dairy animals has also contributed to the growing resistance of these antibiotics in humans as well. Diagnosis should be meticulous and strict antibiotic sensitivity tests should be carried out before prescribing any therapeutic instructions. This scenario can be altered only by scrupulous usage of antimicrobials in the future.

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Nil.

Conflict of Interest

I declare that there were no conflicts of interest encountered.

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