Pathogens isolated from clinical mastitis in Murrah buffaloes and their antibiogram

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Abstract

Aim: To determine the etiology of clinical mastitis in Murrah buffaloes and to develop an antibiogram of organisms isolated.

Materials and Methods: A total of 564 quarter milk samples of 144 buffaloes suffering from clinical mastitis received in Veterinary College Central Laboratory were inoculated on blood agar, MacConkey's lactose agar and sabouraud dextrose agar. Bacteria isolated were characterized to the species level and subjected to *in-vitro* antimicrobial sensitivity testing.

Results: Out of 564 quarters examined for mastitis, 320 (56.73%) quarters were found culturally positive showing isolation of *Staphylococcus aureus* 140 (38.04%), *Streptococcus dysgalactiae* 112 (30.43%), *Streptococcus agalactiae* 13 (3.53%), Escherichia coli 74 (20.10%) and *Corynebacterium pyogenes* 29 (7.88%). On carrying antibiogram staphylococci and streptococci revealed high sensitivity towards chloramphenicol, gentamicin, amikacin and enrofloxacin. Streptococci showed high sensitivity towards ceftriaxone and cefaperazone also. *E. coli* was found highly sensitive to chloramphenicol and gentamicin. *C. pyogenes* was sensitive to the majority of antibiotics.

Conclusion: *S. aureus* was the most predominant bacteria isolated from mastitis cases and irrespective of the isolates chloramphenicol was found to be most sensitive when tested *in-vitro*.

Keywords: antibiogram, *Escherichia coli*., mastitis, staphylococci, streptococci.

Introduction

India ranks first in milk production with 121.8 million tonnes of milk production [1] of which more than 55% comes from buffaloes. Haryana has the world's best dairy type buffalo, the Murrah, capable of milk yields as high as 35 kg a day. Mastitis is an inflammation of mammary glands causing heavy economic losses to the dairy industry throughout the world. These losses in India have been increased from Rs. 6053.21 crores [2] to Rs. 7165.51 crores per annum [3].

At present it has been established that mastitis can be caused by over 250 microorganisms [4] and detection of these pathogens is considered essential for the definitive diagnosis of mastitis. Treatment with antibiotics is one of the most important components to control mastitis. Due to indiscriminate use of antibiotics, antibiotic resistance has been developed against most of the commonly used drugs leading to treatment failure. Therefore, definitive diagnosis of etiological agents and their antimicrobial sensitivity prior to treatment will help in selecting suitable and cost effective antibiotic to treat an animal properly.

Therefore, the present study was planned to determine the etiological agent(s) responsible for causation of clinical mastitis in Murrah buffaloes and to determine their antimicrobial sensitivity to institute proper line treatment and adoption of control measures.

Materials and Methods

Ethical approval

State project on mastitis is under operation in College Central Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. The milk samples used in the current study were directly received from animal owners. As per University rules for these samples approval of Institutional Animal Ethics Committee is not required.

Place of study

The study was conducted in College Central Laboratory, College of Veterinary Sciences, LUVAS, Hisar.

Source of milk samples

A total of 564 quarter milk samples were received directly from animal owners of 144 buffaloes suffering from clinical mastitis in a sterile container from different parts of Haryana and nearby villages of Rajasthan.

Bacteriological examination

Immediately after receiving, the milk samples were inoculated in 0.01 ml volume on 5% sheep blood agar (BA), MacConkey's lactose agar (MLA) and sabouraud dextrose agar plates with the help of a 4 mm diameter platinum loop. The plates

were incubated aerobically at 37°C for 24-48 h. Sub-cultures of the resulting growth were made on BA for purification of isolates and identified on the basis of Grams reaction, morphology and colony characteristics. All the isolates were characterized up to species' level following standard microbial procedures of National Mastitis Council [5].

The Gram-positive cocci were subjected to catalase test to differentiate between staphylococci and streptococci. To rule out possibility of micrococci, all the catalase positive cocci were subjected to oxidase test and oxidation fermentation test. On the basis of coagulase test, haemolysis on 5% BA and Staph™ latex test kit (HiMedia, Mumbai), staphylococci were identified up to species' level.

The organisms that on preliminary examination were found to be streptococci, were further characterized for different species using Strep™ latex test kit (HiMedia, Mumbai, India) as per instructions of the manufacturer. Other organisms like *Corynebacterium pyogenes* were identified on the basis of colony characteristics, Grams staining reaction, morphology and catalase test, whereas *Escherichia coli* were differentiated on the basis of growth on eosin methylene blue agar, MLA and IMViC test.

Antimicrobial susceptibility testing

Different strains of various organisms isolated from udder infections were subjected to *in-vitro* drug sensitivity testing, using 14 antimicrobials by disc-diffusion method [6]. With the help of a platinum loop, a small amount of test culture was transferred into a tube of brain heart infusion broth and incubated for 2-5 h at 37°C, so as to obtain turbidity. With the help of a sterile cotton swab, the broth culture was then evenly spread by smearing over the surface of BA/Mueller-Hinton agar plates. The antimicrobial discs were placed on the agar and gently pressed. These were then, incubated at 37°C for 24 h. The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer. The results were recorded as sensitive, intermediate and resistant. Different antimicrobials used were ampicillin (25 mcg), ceftriaxone (30 mcg), amoxicillin (10 mcg), streptomycin (25 mcg), cloxacillin (30 mcg), chloramphenicol (30 mcg), neomycin (30 mcg), tylosin (15 mcg), enrofloxacin (10 mcg), cefoperazone (75 mcg), amikacin (30 mcg), penicillin-G (10 units), gentamicin (30 mcg), and oxytetracycline (30 mcg) (Hi media).

Result

Prevalence of mastitis on the basis of bacteriological examination was found to be 56.73% quarter wise. Out of 564 quarters examined for mastitis, 320 quarters were found culturally positive of which 48 quarters were showing mixed dual bacterial infection, therefore accounting for total 368 isolates (320 + 48). These organisms were identified as *Staphylococcus aureus* 140 (38.04%), *Streptococcus dysgalactiae* 112 (30.43%), *Streptococcus agalactiae* 13 (3.53%), E. coli 74 (20.10%) and *C. pyogenes* 29 (7.88%). None of the milk samples showed growth of fungi. The frequency of isolation of different bacterial species is depicted in Table-1.

The results of antimicrobial sensitivity testing have been shown in Table-2. Staphylococci (140) revealed antibiotic sensitivity in descending order to amikacin (88.57%), gentamicin (86.42%), chloramphenicol (84.28%), enrofloxacin (82.14%), cefoperazone (81.42%), ceftriaxone and streptomycin (79.28%), oxytetracycline (77.14%), tylosin (72.85%), ampicillin (67.85%), cloxacillin (63.57%), neomycin (61.42%), amoxicillin (52.14%) and least towards penicillin-G (29.28%).

In case of streptococci (125) irrespective of species the maximum sensitivity 97.00% was seen towards chloramphenicol followed by ceftriaxone, cefoperazone, and enrofloxacin (95.20%), amikacin, gentamicin and ampicillin (92.00%), oxytetracycline (87.20%), tylosin and neomycin (84.80%), streptomycin (82.40%), amoxicillin (72.00%), cloxacillin (69.00%) and penicillin-G (56.00%).

Variable sensitivity was shown by *E. coli* (74) isolates with maximum sensitivity towards chloramphenicol (94.59%) and least towards cloxacillin (8.10%). In the present study sensitivity of *E. coli* isolates towards gentamicin, ceftriaxone, enrofloxacin and amikacin were in the range of 74.32-82.43%. However, sensitivity to other antibiotics was comparatively low. Only 17.56% isolates were sensitive to tylosin.

All 29 (100%) isolates of *C. pyogenes* were sensitive to streptomycin, chloramphenicol, cloxacillin, amikacin, gentamicin, amoxicillin, enrofloxacin, ceftriaxone and cefoperazone whereas 89.65% were sensitive for ampicillin, neomycin and tylosin. Least sensitivity was shown towards oxytetracycline (77.41%) and penicillin-G (68.96%).

Table-1: Relative frequency of organisms isolated from 564 milk samples of 144 buffaloes.

Parameters	Buffalo (144)	Percentage
Total quarters	576	-
Quarters examined	564	-
Quarters culturally positive	320	56.73
Quarters culturally negative	244	43.27
Total isolates	368	-
Staphylococci	140	38.04
Streptococci	125	33.96
E. coli	74	20.10
C. pyogenes	29	7.88
Mixed infections	48*	15.00

*Staphylococci+Streptococci (17),
Streptococci+*C. pyogenes* (6), Staphylococci+*E. coli* (13),
Staphylococci+*C. pyogenes* (6), Streptococci+*E. coli* (6),
C. pyogenes=Corynebacterium pyogenes,
E. coli=Escherichia coli

Table-2: Antibiogram pattern of isolates obtained from 564 quarters milk samples.

Organism (isolates)	P	S	O	C	A	N	Cx	Ex	G	Ak	Am	Ctr	Cf	Tl
S. aureus (140)	29.28	79.28	77.14	84.28	67.85	61.42	63.57	82.14	86.42	88.57	52.14	79.28	81.42	72.85
Streptococci spp. (125)	56.00	82.40	87.20	97.00	92.00	84.80	69.00	95.20	92.00	92.00	72.00	95.20	95.20	84.80
E. coli (74)	13.51	68.91	60.81	94.59	27.02	35.13	8.10	78.57	82.43	78.37	17.56	74.32	60.81	17.56
Corynebacterium (29)	68.96	100	79.31	100	89.65	89.65	100	100	100	100	100	100	100	89.65
Total (368)	38.91	19.88	77.44	91.91	69.56	66.29	57.13	87.22	88.58	88.58	55.70	85.32	83.42	67.11

S. aureus=Staphylococcus aureus, *E. coli*=Escherichia coli, A=Ampicillin (25 mcg), Ctr=Ceftriaxone (30 mcg), Am=Amoxycillin (10 mcg), S=Streptomycin (25 mcg), Cx=Cloxacillin (30 mcg), C=Chloramphenicol (30 mcg), N=Neomycin (30 mcg), Tl=Tylosin (15 mcg), Ex=Enrofloxacin (10 mcg), Cf=Cefoperazone (75 mcg), Ak=Amikacin (30 mcg), P=Penicillin-G (10 units), G=Gentamicin (30 mcg), and O=Oxytetracycline (30 mcg)

Discussion

Prevalence of different microorganisms

Among the various microorganisms isolated, *S. aureus* was the most prevalent followed by *Streptococcus* spp., which was in accordance with reports of several workers [7-13]. Sumathi *et al*. [14] also reported staphylococci as the most important and prevalent mastitis causing organism globally, including India. High prevalence of *S. aureus* points to poor milking time hygiene as this pathogen is mainly spread during and between two milking via milkers' hands and towels. *S. aureus* is also of public health importance as enterotoxigenic strains can cause food poisoning in human beings. Moreover, the existence of high concentration of *S. aureus* in milk also indicates the relatively poor quality of milk and milk products. These results also show that in India among streptococci, *S. agalactiae* and *S. dysgalactiae* are the most frequently encountered organisms followed by other species of streptococci. In developed countries like USA and UK, a dramatic decrease in the incidence of contagious mastitis pathogens has been achieved by implementing five-point mastitis control programme. In these countries infections caused by the *S. agalactiae* and *S. dysgalactiae* have been eradicated or reduced to very low levels, and the effect on staphylococcal infection is similar though the decline in infection level is slow. However over the same period there was no change in the prevalence of mastitis pathogens as no controls measures were adopted in India. In contrast to our study, Sumathi *et al*. [14], Dubal *et al*. [15], Ranjan *et al*. [16], Harini and Sumathi [17], Kurjogi and Kaliwal [18], Abd-Elrahman [19] and Jeykumar *et al*. [20] observed *E. coli* being second in prevalence following staphylococci. However, Marimuthu *et al*. [21] found 21% of organisms isolated to be *Bacillus* spp. which was totally absent in our study and other studies mentioned earlier. Isolation of *E. coli* is indicative of poor hygienic conditions of animal environment as *E. coli* infects the udder via teat canal from the environment. Isolation of *C. pyogenes* is indicative of poor care and managemental practices. *C. pyogenes* alone establishes itself readily in mammary tissue causing subclinical mastitis. The situation worsens when *C. pyogenes* infect the udder along with other bacteria leading to chronic mastitis ultimate loss of the quarter.

Antibiogram of *S. aureus* isolates

Results of antibiotic sensitivity pattern for *S. aureus* isolates showed amikacin to be the most sensitive among all the antibiotics tested which was in accordance with Ranjan *et al*. [16]; higher than reported by Pankaj *et al*. [8], whereas on the lower side as compared with Kurjogi and Kaliwal [18]. However, *S. aureus* was the least sensitive against penicillin-G in our study that was similar to studies reported by Mohanty *et al*. [10] and Ranjan *et al*. [16] conducted around the country. The resistance of *S. aureus* to penicillin-G in our study may be attributed to the production of beta lactamase, an enzyme that inactivates penicillin and closely related antibiotics. The probable reason for the development of resistance by these organisms to penicillin-G is conventional, prolonged and indiscriminate usage of this drug in the field conditions. The reasons for higher sensitivity against penicillin-G reported by Pankaj *et al*. [8] and Charaya *et al*. [9] might be that both these studies were conducted at organised farm where rational use of antibiotics is done.

Antibiogram of streptococci

Chloramphenicol has been found most sensitive (97%) towards *Streptococcus* isolates irrespective of the species. Antibiogram pattern reported by Pankaj *et al*. [8] revealed higher sensitivity towards penicillin-G, neomycin, cloxacillin, gentamicin, amoxicillin, ceftriaxone and cefoperazone. However, few studies [8,16,18] showed lesser sensitivity of *Streptococcus* isolates towards penicillin-G, gentamicin, streptomycin, ampicillin and amikacin as compared to present study. While comparing sensitivity of enrofloxacin, we found Ranjan *et al*. [16] and Mohanty *et al*. [10] to be on higher side whereas Pankaj *et al*. [8] and Abd-Elrahman [19] on lower side.

Antibiogram of *E. coli* isolates

E. coli is a major pathogen responsible for the causation of coliform mastitis that is an acute and potentially lethal type of mastitis. Selection of suitable antimicrobial for acute *E. coli* mastitis depends primarily on the sensitivity of the organism to the selected drug and the ability to maintain an effective concentration in plasma. Out of antibiotics tested chloramphenicol outstand with sensitivity of 94.59%. Similar pattern were observed by Dubal *et al*. [15], Ranjan *et al*. [16], Charaya *et al*. [9] and Mohanty

et al. [10] while Kurjogi and Kaliwal [18] and Abd-Elrahman [19] reported it to be on lower side. However, the least sensitivity was shown towards penicillin-G (13.51%) that was in accordance with report of Mohanty *et al.* [10]. For successful treatment of coliform mastitis, along with suitable antimicrobial therapy, fluids, calcium, bicarbonates, antihistamines are suggested. However, the efficacy of therapy, particularly for antimicrobials, is unproven in coliform mastitis.

Antibiogram of *C. pyogenes* isolates

In our study more than 80% *C. pyogenes* isolates revealed sensitivity towards all the antibiotics except penicillin-G. Similar to our study, Dutta *et al.* [22] also recorded high sensitivity towards ampicillin but contrary to our study they found less sensitivity towards cloxacillin.

Conclusion

In conclusion, the present study indicated considerable prevalence of the disease and pathogens from clinical mastitis in Hisar and its surroundings. Appropriate treatment and control strategies should be formulated to eradicate or reduce major pathogens *S. aureus*, *S. dysgalactiae*, *S. agalactiae* and *E. coli*. Mastitis is the single largest cause of antimicrobial use in dairy farms. Extensive and indiscriminate use of antibiotics against these bacteria results into emergence of drug resistant mutants which cause treatment failure in animals have also become a potential health risk for humans, as they are able to cause direct and indirect transmission of infection. Therefore, establishing an antibiogram of pathogens is very important from the clinical and economic points of view. Therefore, dairy farm owners need to be advised to get antimicrobial susceptibility testing prior to the institution of proper line of treatment.

Authors' Contributions

GC, AS and MS planned the study design. GC and MS carried out the examination part. Analysis of the result was made by AS. GC and MS prepared the manuscript. AS, AK and PG finalized the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Competing Interests

The authors declare that they have no competing interests.

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