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ORIGINAL ARTICLE

Antibiogram profile of Group B Streptococci isolated from bovine mastitis cases

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Abstract

Mastitis in bovines has become extremely complex and is one of the costliest disease in dairy industry. One important reason for treatment failure is assumed to indiscriminate use of antibacterial without testing in vitro sensitivity of causal organisms. This practice results in development of resistance to commonly used antimicrobials. To chalk out suitable antibiotic therapy, isolation and antibiotic sensitivity studies are always essential. Keeping these points in view the present study was undertaken to select a suitable antibiotic for treatment for Streptococcal mastitis. A total of 96 Streptococcal isolates were recovered from 261 milk samples collected from in and around Bangalore city, including fourteen milk samples from clinical mastitis cases which was confirmed by *tuf* gene based PCR. Out of 96 Streptococcal isolates, sixteen isolates including one reference strain were found to be *S. agalactiae* based on 16S rRNA and *sip* gene based PCR. The in vitro antibiogram study was conducted with fourteen antibiotics which were chosen according to their common use in research and veterinary practice. Investigation had shown that antibacterial susceptibility against *S. agalactiae* in following order, of sensitivity Ampicillin/ Sulbactum (94%), Chloramphenicol (81%), Gentamicin (69%), Ampicillin and Penicillin G (50%), Amoxycillin/Sulbactum and Streptomycin (37%), Piperacillin/Tazobactam (31%), Methicillin and Cefotaxime (25%), Enrofloxacin (19%), Ceftriaxone/Sulbactum and Ceftriaxone (12%) and Oxacillin (0%). Ampicillin/Sulbactum combination proved to be the drug of choice in this study.

Keywords: *Streptococcus;* antibiogram; bovine mastitis; sensitivity; antimicrobials.

Introduction

India is the largest milk producer of the world producing more than 100 million tons of milk per annum accounting to about 13 per cent of global production and 57 per cent of total Asia's production with 185 million cattle and 98 million buffaloes (Livestock Census, 2007). Bovine mastitis is characterized by physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissue of the udder. Streptococcus species are one of the most important causative agents of mastitis. Usually the mastitis caused by streptococci is of the subclinical type, so early detection of such mastitis cases is of paramount importance. Among *Streptococcus* species, *S. agalactiae, S. dysgalactiae and S. uberis*are the predominant group of organisms isolated from mastitis next to *Staphylococcus* species.

Bovine mastitis is the single most common cause for antibacterial use in lactating dairy cattle (Kaneene and Miller, 1992). Treatment of this disease is also the most common cause of illegal antibacterial residues in marketed milk (Erskine, 1996). Antibacterial therapy of bacterial-induced diseases in cattle has been incriminated as a catalyst for resistance in bacteria isolated from treated animals. Additionally, antibacterial use has been suggested as a selective force in determining the bacterial ecology of bovine mastitis (Myllys *et al.,* 1994). Numerous studies have determined the antibacterial susceptibility patterns of mastitis pathogens isolated from clinical studies or submissions to diagnostic laboratories (Brown and

Scasserra, 1990; Davidson, 1980; McDonald *et al.,* 1976). Overall, susceptibility patterns for various bacteria are similar between studies, but few studies have compared trends in susceptibility patterns over a period of several years from the same laboratory and geographical region (Erskine *et al.,* 2002). The purpose of this document is to determine if the resistance is emerging, or progressing, ideally the resistance observed historically would be compared with that of the present. Unfortunately, the continuity between studies for this comparison is tenuous at best. Most studies have reported susceptibility data determined from the disk diffusion (Kirby-Bauer) method. However, differences among specific techniques used to test isolates by the disk diffusion method do not allow valid comparisons among studies. Other studies reported susceptibility data obtained from serial broth dilution or minimal inhibitory concentration (MIC) testing. Additionally, numerous studies did not offer data by bacterial species, often grouping coliforms and streptococci together. As can be seen NMC Annual Meeting Proceedings (2004) 401 from Table 4 (Rossitto *et al.,* 2002), a 30-fold difference in MIC values among species of streptococci for the same drug can exist. The proportion of resistant isolates identified by disk diffusion methods can also vary widely for the same drug when compared among streptococcal and coliform species. An attempt to compare results of antibacterial susceptibility among studies lacking species differentiation can lead to erroneous assumptions. Therefore, studies lacking delineation of species were not used in the assessment of emerging resistance.

Materials and Methods

Collection of milk samples

Milk samples were collected from one organized and two unorganized sectors located in and around Bangalore. Milk was collected in 10 mL sterile tubes following strict aseptic measures and was immediately transported to laboratory in refrigerated condition.

Isolation of *Streptococcus* species from milk

A total of 72 milk samples based on EC and SCC were subjected for bacteriological examination. About 0.1 mL of milk sample having SCC more than 5,00,000 cells/mL and EC more than 6.5 mS/cm were inoculated in *Streptococcus* Selection Broth, with 10 per cent CO_2 tension for six hours to obtain sufficient growth of the organisms. Then, the growth from *Streptococcus* Selection broth was streaked on to Blood agar plates incubated at 37° C for 48 hours under 10 per cent CO_2 tension to obtain pure culture. These pure cultures were again streaked onto BHI agar for further identification procedures.

Identification of the isolates

Pure cultures thus obtained were subjected for the primary test like catalase test. Further, catalase negative cultures were streaked onto nutrient agar slants and preserved at 4°C. From these slants the pure cultures were subjected for various biochemical tests as per the standard procedures confirming *Streptococcus*.

Preparation of inoculums

Single colony of *S. agalactiae* were picked from a fresh isolation plate and inoculated in corresponding tubes containing one ml of BHI broth. The broth was incubated for six hours at 37°C until there was visible growth.

Preparation of McFarland 0.5 turbidity standard

McFarland 0.5 standard was prepared by adding 0.05 mL of one per cent w/v $BaCl_2 .2H_2O$ in PBS to 9.95 mL of one per cent v/v H_2SO_4 in PBS. The growth of all the isolates was adjusted to McFarland 0.5 turbidity standard using sterile PBS. This gives a 10^8 cfu/mL suspension.

Antibiotic sensitivity test

The antibiotic sensitivity tests were performed according to Collee *et al.* (1996). As per the instructions, all *S. agalactiae, S. dysgalactiae* and *S. uberis* isolates including reference strains to be tested were incubated in one ml BHI broth for 24 hours at 37°C with 10 per cent CO_2. After incubation, 0.5 mL of the bacterial suspension was taken in sterile serological tubes separately. The growth of all the cultures was adjusted to McFarland 0.5 turbidity standard using sterile PBS. Then, the bacterial suspension was plated on Mueller-Hinton agar, with the help of a sterile cotton swab. The following antibiotic test discs (Table 1) were laid on the dried inoculums with the help of a dispenser. The plates were incubated at 37°C for 24 hours with 10 per cent CO_2. Sterile disc was also used as control. The zone of inhibition was estimated using antibiotic clearance zone measuring scale in mm.

Results

The antibiotic sensitivity pattern of the *S. agalactiae* isolates and one reference strain (AD1) are shown in Figure 1 and Table 2. The antibiogram of *S. agalactiae* isolates revealed highest sensitivity to Ampicillin with Sulbactum (94 %) followed by

Chloramphenicol (81 %), Gentamicin (69 %), Ampicillin (50 %), Penicillin G (50 %), Amoxycillin with Sulbactum and Streptomycin (37 %), Piperacillin with Tazobactam (31 %), Methicillin (25 %) and Cefotaxime (25 %). The least sensitivity was recorded against Ceftriaxone with Sulbactum (12 %) and Ceftriaxone (12 %) followed by Enrofloxacin (19 %). All the isolates exhibited resistance to Oxacillin (0 %).

Discussion

All the isolates were tested for the sensitivity to various antibiotics that are routinely used for treatment of mastitis in bovines. Highest sensitivity was detected against Ampicillin with Sulbactum (94%) followed by Chloramphenicol, Gentamicin, Ampicillin, Penicillin G, Amoxycillin with Sulbactum and Streptomycin, Piperacillin with Tazobactam, Methicillin and Cefotaxime. This clearly indicated that the highest sensitivity can be obtained when semi synthetic pencillin like Ampicillin and Amoxycillin arecombined with β-lactamase inhibitors *viz.,* Sulbactum, Tazobactum which would fairly reduce action of β-lactamase enzyme and increase their efficacy. As far as the sensitivity to Ampicillin is concerned, results are in

Table 1: Details of antibiotics used for antibiogram profile

S. No:	Antibiotics	Code	Concentration
1	Ampicillin	AMP	10 mcg / disc
2	Amoxycillin / Sulbactam	AMS	30/15mcg / disc
3	Ampicillin / Sulbactam	AS	10/10 mcg / disc
4	Chloramphenicol	C	30mcg / disc
5	Ceftriaxone / Sulbactam	CIS	30/15mcg / disc
6	Ceftriaxone	CTR	30mcg / disc
7	Cefotaxime	CTX	30mcg / disc
8	Enrofloxacin	EX	10mcg / disc
9	Gentamicin	GEN	10mcg / disc
10	Methicillin	M	5mcg / disc
11	Oxacillin	OX	1mcg / disc
12	Penicillin	P	10 units / disc
13	Piperacillin / Tazobactam	PT	100/10mcg / disc
14	Streptomycin	S	10mcg / disc

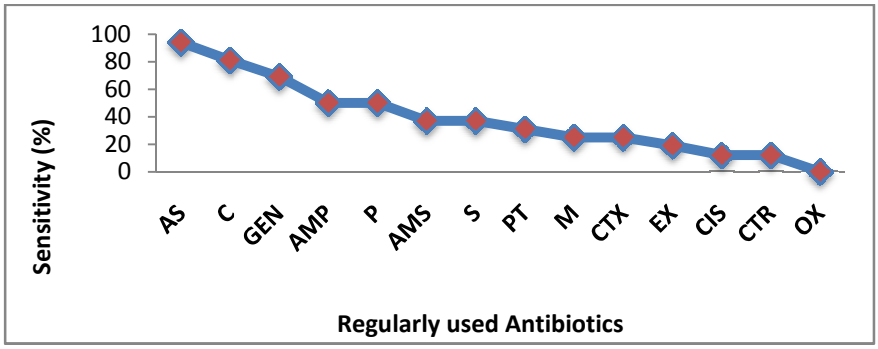

Fig 1: Antibiotic sensitivity pattern of *S. agalactiae* isolated from bovine mastitis

Figure-1: Ampicillin / Sulbactam showing highest sensitivity followed by other commonly used antibiotics in the Veterinary field for treating Bovine mastitis case in India.

Discription of Antibacterials: AS (Ampicillin / Sulbactam), C (Chloramphenicol), AMP (Ampicillin), CIS (Ceftriaxone / Sulbactam), AMS (Amoxycillin / Sulbactam), CTX (Cefotaxime), CTR (Ceftriaxone), EX (Enrofloxacin),GEN(Gentamicin), M (Methicillin), OX (Oxacillin), P (Penicillin), PT (Piperacillin/Tazobactam) and S (Streptomycin).

Table 2: Antibiogram profile of *Streptococcus agalactiae* isolates

AS	C	AMP	CIS	AMS	CTX	CTR	Control	EX	GEN	M	OX	P	PT	S
(11-15 mn	(17-21 mn	(18-27 mn	(24-30 mn	(29-37 mn	(25-28 mn	(24-27 mn	(Sterile dis	(22-30 mn	(12-15 mn	(9-14 mm)	(18-24 mn	(19-28 mn	(17-18 mn	(11-15 mn
27	18	35	20	35	27	23	-	28	22	<10	14	10	14	14
S	S	S	R	S	S	R		S	S	R	R	R	R	S
30	26	31	31	37	34	29	-	23	17	17	0	32	31	13
S	S	S	S	S	S	S		S	S	S	R	S	S	S
20	19	23	11	34	20	11	-	0	13	0	0	15	14	0
S	S	S	R	S	R	R		R	S	R	R	R	R	R
19	17	15	<10	25	17	0	-	18	12	0	0	15	<10	<10
S	S	R	R	R	R	R		R	S	R	R	R	R	R
19	14	19	0	24	17	10	-	17	12	<10	0	18	13	10
S	R	S	R	R	R	R		R	S	R	R	R	R	R
28	23	<10	27	34	27	24	-	21	16	18	0	30	27	<10
S	S	R	S	S	S	S		R	S	S	R	S	S	R
14	21	23	11	35	24	10	-	17	14	11	0	16	11	11
S	S	S	R	S	R	R		R	S	S	R	R	R	S
20	17	21	<10	34	25	11	-	19	15	16	0	25	16	<10
S	S	S	R	S	S	R		R	S	S	R	S	R	R
18	15	16	19	28	19	20	-	15	0	12	0	21	19	0
S	R	R	R	R	R	R		R	R	R	R	R	R	R
25	19	21	18	25	21	0	-	20	12	0	0	19	22	0
S	S	S	R	R	R	R		R	S	R	R	S	S	S
15	20	15	14	21	15	14	-	16	14	0	0	16	14	11
S	S	R	R	R	R	R		R	R	R	R	S	S	S
17	21	13	11	24	15	13	-	17	12	0	0	16	16	12
S	S	R	R	R	R	R		R	S	R	R	S	S	R
0	16	0	0	24	10	0	-	16	17	0	0	10	11	0
R	R	R	R	R	R	R		R	R	R	R	S	S	R
15	23	20	15	21	18	15	-	0	12	0	0	0	16	11
S	S	S	R	R	R	R		R	S	R	R	R	S	S
14	21	0	23	25	19	21	-	24	14	0	0	0	23	0
S	S	R	R	R	R	R		S	R	R	R	R	R	R
17	21	17	10	25	21	10	-	11	15	0	0	16	20	12
S	S	R	R	R	R	R		R	R	R	R	S	R	S

agreement with earlier reports (Nkang *et al*., 2010). On the contrary to high sensitivity to Chloramphenicol recorded in the present study, Culebras *et al*. (2002) have reported high resistance to Chloramphenicol. It is believed that the differences in antimicrobial use, prophylactic practice and serotype frequency may result in regional differences in the susceptibility of GBS (*S. agalactiae*) to antibiotics (Gray *et al*., 2007; Tazi *et al*., 2008).

The least sensitivity was recorded against Ceftriaxone with Sulbactum (12%) and Ceftriaxone (12%) followed by Enrofloxacin (19%). The sensitivity pattern to these antibiotics is in contrast with the results obtained for the clinical mastitis samples which were processed routinely in the laboratory. Wherein, the highest sensitivity was recorded against Enrofloxacin and Ceftriaxone. This might be due to the use of BHI broth instead of *Streptococcus* selection broth and lack of requisite conditions such as 10 per cent CO_2 tension, during processing of routine samples, resulting in failure to recover *Streptococcus* from the samples. High resistance detected against Enrofloxacin and Ceftriaxone may be due to the indiscriminate use of these antibiotics for the treatment of mastitis. All the *Streptococcus* isolates exhibited resistance to Oxacillin (100%). The phenotypic resistance demonstrated against Oxacillin is a forewarning sign of development of resistance to Oxacillin and related group of penicillin antibiotics. Nevertheless, further genotypic studies are needed to confirm the presence of genes responsible for the resistance against antibiotics. The antibiogram confirmed high prevalence of streptococci isolated from bovine mastitis, which are resistant to commonly used chemotherapeutics at the field. This observation can be linked to the virulence of streptococci. However, there was no remarkable difference in the sensitivity pattern between the isolates obtained from clinical and subclinical cases.

Conclusion

Scientific evidence does not support a widespread, emerging resistance among mastitis pathogens to antibacterial drugs. Although resistance to antibacterial drugs among mastitis pathogens has been well documented for nearly four decades, evidence has not been presented to suggest that this is either an emerging or progressing phenomenon. Controlled studies have not determined, on a pharmacodynamics basis, which drug therapeutic regimens may increase this risk, or for that matter, help to decrease it. Monitoring should be continued, preferably by studies that follow data over a course of time and not one point in time. Data derived for MIC testing would be preferred for this purpose because of a more consistent and quantitative nature compared with the disk diffusion method.

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