

Original Research Article

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Antimicrobial Sensitivity Testing of Bacterial Isolates from Subclinical Mastitis in Buffaloes

Rakesh Kumar^{1*}, Yudhbir Singh Rana² and Anshu Sharma³

¹Department of Animal Husbandry & Dairying, Haryana, India

²Department of Veterinary Medicine, LUVAS, Hisar, Haryana, India

³Incharge College Central Laboratory, COVSc, LUVAS, Hisar, Haryana, India

*Corresponding author

ABSTRACT

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In the present investigation, *in-vitro* antimicrobial sensitivity of 46 isolates from milk of mastitis affected buffaloes towards 21 antimicrobials revealed variation in their sensitivity pattern. Overall all strains of staphylococci and streptococci were sensitive to penicillin G, chloramphenicol, cloxacillin, ampicillin, kanamycin, enrofloxacin, neomycin, oleandomycin, ampiclox, spiramycin, novobiocin and ciprofloxacin antibiotics.

Introduction

Mastitis is a one of the most economically important disease of dairy animals, which not only causes heavy economic losses in terms of milk quality and quantity but also poses public health hazard due to persistence of antibiotic residues in the milk. Due to the wide and indiscriminate use of antimicrobial agents for treatment of Mastitis, there are chances of emergence of resistant bacterial strains and change in sensitivity pattern of microbes; Therefore, it is necessary to perform repeated drug trials with newer formulations. The selection of antimicrobial for a particular pathogen should be based on culture and sensitivity testing.

Materials and Methods

A total of 235 quarter milk samples collected from 59 apparently healthy lactating Murrah buffaloes from Buffalo Research Centre, CCS Haryana Agricultural University, Hisar were the source of material for the study.

Different strains of various organisms isolated from udder infections were subjected to *in-vitro* chemotherapeutic sensitivity using 21 antimicrobials by disc diffusion method as described by Bauer *et al.*, (1966). A small amount of growth from isolated colonies of organisms with the help of platinum loop was transferred in to tubes of trypticase soya broth and incubated for two to five hours at 37°C so

as to obtain a turbidity, equivalent to that obtained by adding 0.5 ml of 0.048 M BaCl₂ (1.17% BaCl₂. 2H₂O) to 99.5 ml of 0.36 NH₂SO₄ (1% v/v). The broth culture with the help of sterile cotton swab was then evenly spread by smearing over the surface of blood agar and Nutrient agar plates.

The discs were then placed on the agar and pressed gently with a sterile forceps so as to have a uniform close contact with the medium.

The plates were then kept at low temperature for three to five minutes to allow pre-diffusion of antibiotics. The plates were then incubated at 37°C for 18-24 hrs and observed for sensitivity by measuring the zone of inhibition. Results were recorded on the basis of table provided by the manufacturer for zone size interpretation as sensitive (S) and resistant (R).

Results and Discussion

Results of *in-vitro* antimicrobial sensitivity of all bacterial isolates from SCM cases of mastitis against 21 antimicrobial agents are presented in the table below:

All the 35 strains of staphylococci were sensitive to penicillin G, chloramphenicol, cloxacillin, ampicillin, kanamycin, enrofloxacin, neomycin, oleandomycin, ampiclox, spiramycin, novobiocin and ciprofloxacin. More than 80 per cent of these strains showed sensitivity towards oxytetracycline, tetracycline, erythromycin, amoxicillin, amoxyclav, co-trimoxazole and streptomycin, however sensitivity to nitrofurantoin and polymyxin-B was 62.85 and 74.28 per cent, respectively.

All the 9 strains of diphtheroids were found sensitive to oxytetracycline, tetracycline, chloramphenicol, cloxacillin, enrofloxacin, neomycin, oleandomycin, amoxyclav,

ampiclox, and novobiocin. More than 80 percent of these strains were found sensitive to penicillin G, ampicillin, kanamycin, amoxicillin, spiramycin, ciprofloxacin and streptomycin. Sensitivity towards nitrofurantoin, polymyxin-B, erythromycin and co-trimoxazole ranged between 44.44 and 77.77 per cent.

Both the strains of streptococci showed sensitivity towards nitrofurantoin, penicillin G, oxytetracycline, tetracycline, chloramphenicol, cloxacillin, ampicillin, kanamycin, enrofloxacin, neomycin, oleandomycin, erythromycin, amoxicillin, amoxyclav, co-trimoxazole, spiramycin, novobiocin, ciprofloxacin and streptomycin. Sensitivity towards polymyxin-B was found to be 50 per cent of these strains.

The studies of *in-vitro* antimicrobials sensitivity testing of different bacterial isolates from SCM cases of mastitis against 21 antimicrobial agents showed that all the strains of staphylococci were sensitive to penicillin G, chloramphenicol, cloxacillin, ampicillin, kanamycin, enrofloxacin, neomycin, oleandomycin, ampiclox, spiramycin, novobiocin and ciprofloxacin. More than 80 per cent of these strains showed sensitivity towards oxytetracycline, tetracycline, erythromycin, amoxicillin, amoxyclav, co-trimoxazole and streptomycin, however sensitivity to nitrofurantoin and polymyxin-B was 62.85 and 74.28 per cent, respectively. Similar results for chloramphenicol have been reported by Dahiya and Kapur (1984), Saxena *et al.*, (1993), Mallikarjunaswami and Krishnamurthy (1997), Saxena (2000) and Bulla *et al.*, (2003). RamaniPushpa *et al.*, (1998), Gentilini *et al.*, (2000), Datta and Ranganekar (2001) and Kumari *et al.*, (2002) suggested highest sensitivity of these isolates to gentamycin. Saxena *et al.*, (1993) found penicillin-G least sensitive against udder isolates.

Antimicrobial sensitivity of microorganisms (46) isolated from subclinical cases of mastitis

Antimicrobial agent	Organism and number tested						
		Staphylococci (35)		Streptococci (2)		Diphtheroids (9)	
Nitrofurantoin (NF) (300 mcg)	S	22	(62.85)	2	(100.00)	6	(66.66)
	R	13	(37.15)	0	(00.00)	3	(33.33)
Penicillin-G (P) 10 units	S	35	(100.00)	2	(100.00)	8	(88.88)
	R	0	(0.00)	0	(00.00)	1	(11.11)
Oxytetracycline (O) (30 mcg)	S	34	(97.14)	2	(100.00)	9	(100.00)
	R	1	(2.85)	0	(00.00)	0	(0.00)
Tetracycline (T) (30 mcg)	S	33	(94.28)	2	(100.00)	9	(100.00)
	R	2	(5.71)	0	(00.00)	0	(0.00)
Chloramphenicol (C) (30 mcg)	S	35	(100.00)	2	(100.00)	9	(100.00)
	R	0	(0.00)	0	(0.00)	0	(0.00)
Cloxacillin (Cx) (10 mcg)	S	35	(100.0)	2	(100.00)	9	(100.00)
	R	0	(0.00)	0	(0.00)	0	(0.00)
Ampicillin (A) (10 mcg)	S	35	(100.00)	2	(100.00)	8	(88.88)
	R	0	(0.00)	0	(0.00)	1	(11.11)
Kanamycin (K) (30 mcg)	S	35	(100.00)	2	(100.00)	8	(88.88)
	R	0	(0.00)	0	(0.00)	1	(11.11)
Polymyxin B (Pb) (300 mcg)	S	26	(74.28)	1	(50.00)	4	(44.44)
	R	9	(25.71)	1	(50.00)	5	(55.55)
Enrofloxacin (Ex) (10 mcg)	S	35	(100.00)	2	(100.00)	9	(100.00)
	R	0	(0.00)	0	(0.00)	0	(0.00)

Neomycin (N) (30 mcg)	S	35 (97.22)	2 (100.00)	9 (100.00)
	R	0 (0.00)	0 (00.00)	0 (0.00)
Oleandomycin (Ol) (15 mcg)	S	35 (100.00)	2 (100.00)	9 (100.00)
	R	0 (0.00)	0 (0.00)	0 (0.00)
Erythromycin (E) (15 mcg)	S	32 (91.42)	2 (100.00)	7 (77.77)
	R	3 (8.57)	0 (0.00)	2 (22.22)
Amoxicillin (Am) (30 mcg)	S	34 (97.14)	2 (100.00)	8 (88.88)
	R	1 (2.85)	0 (0.00)	1 (11.11)
Amoxyclav (Ac) (30 mcg)	S	34 (97.14)	2 (100.00)	9 (100.00)
	R	1 (2.85)	0 (0.00)	0 (0.00)
Ampiclox (Ax) (30 mcg)	S	35 (100.00)	2 (100.00)	9 (100.00)
	R	0 (0.00)	0 (0.00)	0 (0.00)
Co-trimoxazole (Co) (25 mcg)	S	31 (88.57)	2 (100.00)	7 (77.77)
	R	4 (11.42)	0 (0.00)	2 (22.22)
Spiramycin (Sr) (30 mcg)	S	35 (100.00)	2 (100.00)	8 (88.88)
	R	0 (0.00)	0 (0.00)	1 (11.11)
Novobiocin (Nv) (30 mcg)	S	35 (100.00)	2 (100.00)	9 (100.00)
	R	0 (0.00)	0 (0.00)	0 (0.00)
Ciprofloxacin (Cf) (10 mcg)	S	35 (100.00)	2 (100.00)	8 (88.88)
	R	0 (0.00)	0 (0.00)	1 (11.11)
Streptomycin (S) (10 mcg)	S	34 (97.14)	2 (100.00)	8 (88.88)
	R	1 (2.85)	0 (0.00)	1 (11.11)

S-Sensitive, R=Resistant

However higher sensitivity against various antibiotics have been reported by Dahiya and Kapur (1984), Tuteja (1999) and Bulla *et al.*, (2003) whereas Kalorey *et al.*, (1983), Bhalerao *et al.*, (2000) and Saxena (2000) reported low efficacy of various antimicrobials to staphylococci.

All the strains of diphtheroids were found sensitive to oxytetracycline, tetracycline, chloramphenicol, cloxacillin, enrofloxacin, neomycin, oleandomycin, amoxycylav, ampiclox, and novobiocin. Both the strains of streptococci showed sensitivity towards nitrofurantoin, penicillin G, oxytetracycline, tetracycline, chloramphenicol, cloxacillin, ampicillin, kanamycin, enrofloxacin, neomycin, oleandomycin, erythromycin, amoxycillin, amoxycylav, co-trimoxazole, spiramycin, novobiocin, ciprofloxacin and streptomycin. Sensitivity of other microbials were in the range of 44.44 to 77.77 per cent. Almost similar findings were recorded by Verma and Mishra (1977). Lower sensitivity of ampiclox (56.25%) was observed by Saxena (2000), Bertoldini *et al.*, (1985) and Tuteja (1999) also reported the similar sensitivity to spiramycin and co-trimoxazole.

These results indicate the variability in the antimicrobial sensitivity pattern for microbes isolated from SCM cases and suggest the importance of epidemiologic knowledge of a dairy herd to guide initial therapy.

In-vitro antimicrobial sensitivity of 46 bacterial isolates from SCM cases of mastitis was determined against 21 antimicrobial agents. All the strains of staphylococci were sensitive to penicillin G, chloramphenicol, cloxacillin, ampicillin, kanamycin, enrofloxacin, neomycin, oleandomycin, ampiclox, spiramycin, novobiocin and ciprofloxacin. More than 80 per cent of these strains showed sensitivity towards oxytetracycline, tetracycline, erythromycin,

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