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Research Article

SENSITIVITY PATTERN OF COMMONLY USED ANTIBIOTICS FOR TREATMENT OF ENDOMETRITIS IN BUFFALOES

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ABSTRACT

Present study was conducted to rule out the antibiotic sensitivity pattern of different bacterial flora cultured from cervico-vaginal mucus samples which were collected from buffaloes suffering with endometritis. Overall, 61 cervico-vaginal discharges were collected from buffaloes suffering with endometritis out of which 13 samples were free from bacterial growth whereas 48 samples exhibited bacterial growth in nutrient broth. The *in vitro* antibiotic sensitivity test for each turbid sample was done by disc diffusion method and was spread on Mueller-Hinton agar (MHA) along with 14 different antibiotic discs. Antibiotic discs used were ciprofloxacin (Cip), enrofloxacin (Ex), levofloxacin (LE), ofloxacin (Of), gentamicin (Gen), amoxicillin (AMX), penicillin (P), ceftriaxone (CTR), cephalexin (CN),ampicillin (AMP), oxytetracycline (O), streptomycin (S), cloxacillin (COX) and metronidazole (MT). The highest sensitivity was recorded for enrofloxacin followed by ciprofloxacin, levofloxacin and ofloxacin whereas, the highest resistance was recorded for metronidazole followed by penicillin, cloxacillin and amoxycillin.

Keywords: Antibiotics, Buffaloes, Cervico-vaginal discharge, Repeat breeder, Resistance, Sensitivity

Buffaloes are one of the major milk producers after cattle in Himachal Pradesh and are producing 380.5 thousands tons of milk every year in spite of their small population of 6.46 lakhs in the state (Twnteeth livestock census 2019). The milk production in buffaloes is mainly influenced by reproductive status and various disease conditions which alter the uterine involution process after calving. Various infectious diseases which affect uterus are clinical endometritis, subclinical endometritis, puerperal metritis and clinical metritis (Sheldon *et al.*, 2006). The microorganisms enter the vagina during the parturition and mating process. Manipulation of the calf during the calving process results in entrance of microorganisms in uterus (Peters and Ball, 2004).

The infections caused by these microbes affect high yielder and are associated with decreased conception rate, increased inter-calving interval, increased culling rate and more economical losses and require prompt diagnosis and treatment (Giuliodori *et al.*, 2013). In buffaloes, the world wide incidence of clinical endometritis has been reported to vary from 8.0-47.90 per cent (Saxena *et al.*, 2006; Singh *et al.*, 2008). In Himachal Pradesh, an incidence of 30.1 and 12.67 per cent has been reported in cattle and buffaloes, respectively (Bala, 2017).

Endometritis causes huge economic loss to the dairy farmers and is being caused by different causative agents, so proper diagnosis and identification of these organisms is required (Kumar *et al.*, 2018). The available diagnostic methods are rectal palpation, isolation of causative agents in samples, visual inspection of cervical vaginal discharge, ultrasonography, endometrial cytology and biopsy of affected endometrium (Sheldon *et al.*, 2006; Dolezel *et al.*, 2008). In veterinary field, numerous antibiotics are used to treat such infections. The improper extensive use of these antibiotics for the treatment of infections can cause emergence of drug resistance to bacterial strains, so it is mandatory to check their efficiency time to time (Barman *et al.*, 2013; Kumar *et al.*, 2018). The sensitivity test of bacterial culture of cervico-vaginal discharge is one of the common and best methods to evaluate the efficacy of these agents to reduce the wastage of antibiotics and to prevent extra money incurred by farmers for treatment of affected animals.

MATERIALS AND METHODS

Overall, 61 cervico-vaginal discharges were collected in sterilized vials to check the sensitivity of different antibiotics under field conditions. The *in vitro* antibiotic sensitivity test for each sample was done by disc diffusion method described by Kirby *et al.* (1965). Initially, the cervico-vaginal mucus (CVM) was cultured in nutrient broth by keeping the sample in the incubator overnight at 37 °C. The nutrient broth containing samples which turned turbid were considered positive for infection. Out of 61, 48 (78.69%) samples were found positive for infection whereas 13 (21.31%) samples were found negative. The cultured broth was tested for turbidity and was spread plated in Mueller-Hinton (Hi Media, India) agar plates along with 14 different antibiotic discs (Hi Media, India) of known concentrations. Antibiotic discs ciprofloxacin (Cip), enrofloxacin (Ex), levofloxacin (LE), ofloxacin (Of), gentamicin (Gen), amoxicillin (AMX), penicillin (P), ceftriaxone (CTR), cephalexin (CN), ampicillin (AMP), oxytetracycline (O), streptomycin (S),

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Table 1
Sensitivity of bacteria in cervical mucus of endometritic buffaloes against various antibacterial drugs in different districts of Himachal Pradesh, India

Sr. No.	District		Sensitivity																				
			Cip			Ex			Gen			Of			AMX			P			MT		
			HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R
1.	Kangra (n=8)		5	3	0	7	1	0	1	6	1	7	1	0	2	3	1	0	3	5	0	0	6
		%	62.5	37.5	0	87.5	12.5	0	12.5	75.0	12.5	87.5	12.5	0	33.3	50.0	16.7	0	37.5	62.5	0	0	100
			100		0	100		0	87.5		12.5	100		0	83.3		16.7	34.5		62.5	0		100
			N=8			N=8			N=8			N=8			N=6			N=8			N=6		
2.	Bilaspur (n=11, NG=2)		5	3	1	7	1	1	0	8	1	6	2	1	0	0	5	0	0	9	0	0	5
		%	55.6	33.3	11.1	77.8	11.1	11.1	0	88.9	11.1	60	20	20	0	0	100	0	0	100	0	0	100
			88.9		11.1	88.9		11.1	88.9		11.1	80		20	0		100	0		100	0		100
			N=9			N=9			N=9			N=9			N=5			N=9			N=5		
3.	Mandi (n=14, NG=7)		6	1	0	6	1	0	4	3	0	4	2	1	1	1	5	0	1	6	0	0	7
		%	85.7	14.3	0	85.7	14.3	0	57.1	42.9	0	57.1	28.6	14.3	14.3	14.3	71.4	0	14.3	85.7	0	0	100
			100		0	100		0	100		0	85.7		14.3	28.6		71.4	14.3		85.7	0		100
			N=7			N=7			N=7			N=7			N=7			N=7			N=7		
4.	Una (n=9, NG=2)		4	2	1	3	2	2	2	4	1	3	3	1	0	0	7	0	0	7	0	1	6
		%	57.1	28.6	14.3	42.9	28.6	28.6	28.6	57.1	14.3	42.9	42.9	14.3	0	0	100	0	0	100	0	14.3	85.7
			85.7		14.3	71.5		28.6	85.7		14.3	85.8		14.3	0		100	0		100	14.3		85.7
			N=7			N=7			N=7			N=7			N=7			N=7			N=7		
5.	Hamirpur (n=19, NG=2)		16	1	0	14	2	1	6	10	1	10	5	2	2	9	6	6	6	5	0	1	16
		%	94.1	5.9	0	82.3	11.8	5.9	35.3	58.8	5.9	58.8	29.4	11.8	11.8	52.9	35.3	35.3	35.3	29.4	0	5.9	94.1
			100		0	94.1		5.9	94.1		5.9	88.2		11.8	64.7		35.3	70.6		29.4	5.9		94.1
			N=17			N=17			N=17			N=17			N=17			N=17			N=17		
Himachal Pradesh Overall (n=61) NG=13		%	36	10	2	37	7	4	13	31	4	30	13	5	5	13	24	6	10	32	0	2	40
			75.0	20.8	4.2	77.1	14.6	8.3	27.1	64.6	8.3	62.5	27.1	10.4	11.9	31.0	57.1	12.5	20.8	66.7	0	4.8	95.2
			95.8		4.2	91.7		8.3	91.7		8.3	89.6		10.4	42.9		57.1	33.3		66.7	4.8		95.2
			N=48			N=48			N=48			N=48			N=42			N=48			N=42		

Sr. No.	District		Sensitivity																				
			CTR			CN			O			S			COX			AMP			LE		
			HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R
1.	Kangra (n=8)		2	6	0	3	3	0	0	4	2	0	4	2	0	2	4	0	1	5	4	2	0
		%	25.0	75.0	0	50.0	50.0	0	0	66.7	33.3	0	66.7	33.3	0	33.3	66.7	0	16.7	83.3	66.7	33.3	0
			100.0		0	100.0		0	66.7		33.3	66.7		33.3	33.3		66.7	16.7		83.3	100.0		0
			N=8			N=6			N=6			N=6			N=6			N=6			N=6		
2.	Bilaspur (n=11, NG=2)		1	8	0	3	0	2	2	3	0	0	1	4	0	0	5	0	0	5	3	2	0
		%	11.1	88.9	0	60	0	40	40	60	0	0	20	80	0	0	100	0	0	100	60	40	0
			100.0		0	60		40	100		0	20		80	0		100	0		100	100		0
			N=9			N=5			N=5			N=5			N=5			N=5			N=5		
3.	Mandi (n=14, NG=7)		2	1	4	0	3	4	0	4	3	3	3	1	0	1	6	1	2	4	0	3	4
		%	28.6	14.3	57.1	0	42.9	57.1	0	57.1	42.9	42.9	42.9	14.3	0	14.3	85.7	14.3	28.6	57.1	0	42.9	57.1
			42.9		57.1	42.9		57.1	57.1		42.9	85.7		14.3	14.3		85.7	42.9		57.1	42.9		57.1
			N=7			N=7			N=7			N=7			N=7			N=7			N=7		
4.	Una (n=9, NG=2)		2	4	1	1	5	1	0	0	7	1	4	2	0	1	6	0	1	6	5	2	0
		%	28.6	57.1	14.3	14.3	71.4	14.3	0	0	100	14.3	57.1	28.6	0	14.3	85.7	0	14.3	85.7	71.4	28.6	0
			85.7		14.3	85.7		14.3	0		100	71.4		28.6	14.3		85.7	14.3		85.7	100		0
			N=7			N=7			N=7			N=7			N=7			N=7			N=7		
5.	Hamirpur (n=19, NG=2)		9	7	1	7	6	4	7	10	0	4	11	2	3	9	5	5	10	2	15	2	0
		%	52.9	41.2	5.9	41.2	35.3	23.5	41.2	58.8	0	23.5	64.7	11.8	17.6	52.9	29.4	29.4	58.8	11.8	88.2	11.8	0
			94.1		5.9	76.5		23.5	100		0	88.2		11.8	70.5		29.4	88.2		11.8	100		0
			N=17			N=17			N=17			N=17			N=17			N=17			N=17		
Himachal Pradesh Overall (n=61) NG=13		%	16	26	6	14	17	11	9	21	12	8	23	11	3	13	26	6	14	22	27	11	4
			33.3	54.2	12.5	33.3	40.5	26.2	21.4	50.0	28.6	19.0	54.8	26.2	7.1	31.0	61.9	14.3	33.3	52.4	64.3	26.2	9.5
			87.5		12.5	73.8		26.2	71.4		28.6	73.8		26.2	38.1		61.9	47.6		52.4	90.5		9.5
			N=48			N=42			N=42			N=42			N=42			N=42			N=42		

CIP=Ciprofloxacin (5 mcg/disc), EX=Enrofloxacin (10mcg/disc), GEN=Gentamicin (10mcg/disc), OF=Ofloxacin (5 mcg/disc), AMX=Amoxicillin (10 mcg/disc), O=Oxytetracycline (30 mcg/disc), P=Penicillin (10 units/disc), MT=Metronidazole (5 mcg/disc), CTR=Ceftriaxone (10 mcg/disc), CN=Cephalexin(30 mcg/disc), S=Streptomycin (10 mcg/disc), COX=Cloxacillin (10 mcg/disc), AMP=Ampicillin (10 mcg/disc), LE=Levofloxacin (5 mcg/disc), (HS=Highly sensitive, S=Sensitive, R=Resistant, NG=No growth

cloxacillin (COX) and metronidazole (MT) were carefully placed on the surface of inoculated plates and incubated at 37°C overnight. The diameter of the zone of inhibition was taken into consideration for determining sensitivity of the sample against the specific antibiotics.

RESULTS AND DISCUSSION

The perusal of Table 1 indicated that bacteria of endometritic buffaloes had shown highest sensitivity pattern for ciprofloxacin (95.8%) followed by enrofloxacin (91.7%), levofloxacin (90.5%) and ofloxacin (89.6%). A similar trend of antibiotics sensitivity pattern was recorded in cows of Himachal Pradesh by Kumar *et al.* (2018) where highest sensitivity was recorded for ciprofloxacin (96%) followed by enrofloxacin (92%) and ofloxacin (89%). However, Dutt *et al.* (2017) recorded highest sensitivity for enrofloxacin (60%) followed by chloramphenicol and gentamicin (33.3%) and least sensitivity was recorded for ceftriaxone and cefoperazone (13.3%). In another study on buffaloes, antibiotic sensitivity was highest for gentamicin, followed by chloramphenicol, enrofloxacin, cephalexin, tetracycline, ampicillin, co-trimoxazole and furazolidone (Prajapati *et al.*, 2005).

These results were also in accordance to the results recorded by Bala (2017) where highest sensivity was found for flouroquinolones with highest sensitivity recorded for ciprofloxacin (88.24%) followed by enrofloxacin (88.23%), ofloxacin (82.35%) and levofloxacin (73.33%).

In our study, the least sensitivity was recorded for metronidazole (4.8%). Similar results were recorded by Bala (2017) for metronidazole. Mane *et al.* (2009) recorded a sensivity of 93.75 per cent for ciprofloxacin and 84.37 per cent for gentamicin which were in agreement to our study. In present study, low sensitivity was recorded for penicillin (33.3%) which was higher to the sensitivity pattern (17.65%) recorded by Bala (2017) The recorded low sensitivity for penicillin might be due to extensvie and indiscriminate use of penicillin over a long period of time . However, in a study by Arora *et al.* (2000) gentamicin and pefloxacin (94.3%) were most effective drugs for the treatment of endometritis but in our study moderate sensitivity for gentamicin was found. Similarly, in another study gentamicin, enrofloxacin and chlortetracycline (32%) were found moderately sensitive and highest sensitivity (64%) was recorded for ceftriaxone (Udhayavel *et al.*, 2013).

Over the period the increased resistance of microbes to the commonly used antibiotics such as penicillin, metronidazole, amoxicillin, ampicillin and cloxacillin may be attributed to improper and indiscriminate use of these antibiotics over a long period of time (Arora *et al.*, 2000, Barman *et al.*, 2013; Kumar *et al.*, 2018). So these antibiotics should not be considered as first choice for the treatment of bovines suffering with endometritis (Bala, 2017; Kumar *et al.*, 2018).

REFERENCES

Arora, A.K., Singh, J., Pangaonkar, G.R. and Nanda, A.S. (2000). Bacteriological studies on the genital tract in repeat breeder bovines. *Int. J. Anim. Sci.* **15**: 205-207.

Bala, I. (2017). Clinical endometritis and its therapeutic management in bovines of Himachal Pradesh. M.V.Sc. thesis submitted to Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya Palampur, India.

Barman, P., Yadav, M.C. Bangthai, A. and Kumar, H. (2013). Antibiogram of bacteria isolated from bovine endometritis. *Vet. Res. Int.* **1**: 20-24.

Dolezel, R., Vecera, M., Palenik, T., Cech, S. and Vyskocil, M. (2008). Systematic clinical examination of early postpartum cow and treatment of puerperal endometritis did not have beneficial effect on subsequent reproductive performance. *Vet. Med.* **53(2)**: 59-69.

Dutt, R., Singh, G., Singh, M., Sharma, M., Dalal, J. and Chandolia, R.K. (2017). Diagnosis of subclinical endometritis in Murrah buffaloes through cytobrush technique. *Int. J. Curr. Microbiol. App. Sci.* **6(11)**: 494-499.

Giuliodori, M.J., Magnasco, R.P. Becu-Villalobos, D., LacauMengido, I.M., Risco, C.A. and de la Sota, R.L. (2013). Clinical endometritis in an Argentinean herd of dairy cows: risk factors and reproductive efficiency. *J. Dairy Sci.* **96**: 210-218.

Kumar, P., Singh, M.M. and Sharma. A. (2018). Antibiogram of bacteria isolated from Cervico-vaginal discharge of endometritic cows in Himachal Pradesh. *Indian J. Anim. Sci.* **88(12)**: 1358-1361.

Kirby, W.M.M., Bauer, A.W.,Sherris, J.C. and Turck, N. (1965). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**: 493-496.

Mane, P.M., Dhoble, R.L., Chaudhari, R.J., Dawane, S.C. and Kolpe, A.B. (2009). Bacterial spectrum, antibiotic sensitivity pattern of bacterial isolates and conception rate in repeat breeders. *Intas Polivet.* **10**: 32-35.

Prajapati, S.B., Ghodasara, D.J., Joshi, B.P., Prajapati, K.S.and Jani, V.R. (2005). Aetiopathological study of endometritis in repeat breeder buffaloes. *Buff. J.* **21(2)**:145-65.

Peters, A.R. and Ball, P.J.H. (2004). Reproduction in cattle (3rd Edn), Blackwell Publishing, Oxford, UK.

Saxena, G., Rani, S., Danodia, H.K. and Purohit, G.N. (2006). Pathological conditions in genital tract of female buffaloes (*Bubalus bubalis*). *Pakistan Vet. J.* **26(2)**: 91-93.

Sheldon, I.M., Lewis, G.S., LeBlanc, S., and Gilbert, R.O. (2006). Defining postpartum uterine disease in cattle. *Theriogenology.* **65**: 1516-1530.

Singh, J., Dadarwal, D., Honparkhe, M.and Kumar,A. (2008). Incidences of various etiological factors responsible for repeat breeding syndrome in cattle and buffaloes. *Int. J. Vet. Med.* **6(1)**: 220-229.

Twenteeth livestock census. (2019). All India eport.http://dahd.nic.in/dahd/WriteReadData/Livestock.pdf.

Udhayavel, S., Malmarugan,S., Palanisamy,K. and Rajeswar, J.(2013). Antibiogram pattern of bacteria causing endometritis in cows. *Vet. World.* **10**:100-102.