



Antibiogram of bacteria isolated from cervico-vaginal discharge of endometritic cows in Himachal Pradesh

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ABSTRACT

The present study was designed to assess the antibiotic sensitivity pattern of different bacterial flora isolated from 120 cervico-vaginal mucus (CVM) samples which were collected from cows suffering from endometritis. Out of 120 discharge samples, 105 exhibited bacterial growth and 15 samples were bereft of bacteria in nutrient broth. The *in vitro* antibiotic sensitivity test for each turbid sample was done by disc diffusion method. Antibiotics used were ciprofloxacin (Cx), enrofloxacin (Ex), gentamicin (G), ofloxacin (Of), ampicillin (Am), oxytetracycline (O), co-trimoxazole (Co), tetracycline (T), penicillin (P) and metronidazole (Mt). Highest sensitivity was recorded for ciprofloxacin followed by enrofloxacin and ofloxacin, whereas, highest resistance was recorded for penicillin followed by metronidazole and tetracycline. The antibiogram could be useful to the practitioners in choosing the most efficient antibacterial products.

Key words: Antibiogram, Cervico-vaginal discharge, Endometritic cows, Himachal Pradesh

After calving, most of the bacteria which are isolated from the uterus of normal cow are responsible for different types and degrees of infection depending on the health status of the animals (Aberé and Belete 2016). On the basis of severity of infection and time of development of infection in the uterus, these infections are divided into 5 categories, viz. puerperal metritis, clinical metritis, clinical and subclinical endometritis and pyometra (Sheldon *et al.* 2009). These infections affect high yielder animals and are associated with decreased conception rate, extended interval to pregnancy, increased intercalving interval, increased culling rate and more economical losses (Giuliodori *et al.* 2013) and require prompt diagnosis and treatment. Uterine infections alter the uterine environment resulting in impairment of sperm transport, sperm death and interfere with hostile environment of the uterus for subsequent development and maintenance of conceptus (Azawi 2008).

Among the uterine infections, endometritis is one of the major causes of repeat breeding in cattle. It is a localized inflammation of uterine lining and is associated with acute and chronic infection due to pathological bacterial infection which may occur during coitus or artificial insemination (Sheldon and Dobson 2004). Clinical endometritis affects approximately 20% of lactating dairy cows (Galvao 2011),

with the incidence ranging from 5.0 to 30% in some herds (Galvao *et al.* 2009, Bala 2017). It is caused by different causative agents and cause huge economical loss to farmers hence proper diagnosis and identification of these organisms is required. Many methods are available for diagnosis of endometritis such as inspection of vaginal discharge, rectal examination, transrectal ultrasonography, bacterial isolation from uterine discharge, uterine biopsy and endometrial cytology (Dolezel *et al.* 2008). The infections of uterus caused by bacteria are treated with wide range of antibiotics. However, the efficacy of such therapeutic agents is required to be evaluated from time to time due to continuous emergence of drug resistant bacterial strains (Barman *et al.* 2013). The culture sensitivity test of cervico-vaginal discharge is considered as one of the best methods to evaluate the efficacy of these agents which further prevents the wastage of antibiotics and reduce extra burden in terms of money on farmers for treatment of affected animals.

MATERIALS AND METHODS

Cervico-vaginal discharges (CVD) (120) 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 of Kirby and Bauer (1965). Initially, the 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 120 samples, 105 samples were positive for infection. The 18 h old broth culture was tested for

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Table 1. Sensitivity of various antibiotics against bacteria isolated from cervical mucus of repeat breeder cows suffering from endometritis in different districts of Himachal Pradesh.

District	n (%)	Sensitivity																													
		Cx			Ex			G			Of			Am			O			Co			T			P			Mt		
		HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R	HS	S	R
Lahaul and Spiti (n=18)	18	11	5	2	10	5	3	11	5	2	11	5	2	-	-	-	11	5	2	-	-	-	9	3	6	-	-	-	0	3	15
Chamba (n=21)	21	14	7	0	12	7	2	5	14	2	7	10	4	-	-	-	-	-	-	5	13	3	2	11	8	0	2	19	0	6	15
Mandi (n=15, NG=5)	10	8	2	0	8	2	0	6	2	2	5	3	2	3	7	0	2	6	2	-	-	-	-	0	2	8	0	2	8	0	2
Shimla (n=12, NG=3)	9	6	3	0	6	3	0	4	2	3	5	2	2	4	5	0	2	6	1	-	-	-	0	3	6	1	1	7	0	2	7
Kangra (n=14, NG=3)	11	8	2	1	8	2	1	6	4	1	8	2	1	2	4	2	8	1	2	-	-	-	3	3	5	1	2	8	0	2	6
Kinnaur (n=9, NG=2)	7	5	1	1	4	1	2	4	3	0	3	3	1	-	-	-	-	-	-	0	1	6	1	4	2	0	2	5	0	2	5
Bilaspur (n=4)	4	2	1	0	2	1	1	1	3	0	1	3	0	-	-	-	-	-	-	1	1	2	1	2	1	0	1	3	0	1	3
Hamirpur (n=23)	23	19	4	0	17	6	0	9	14	0	13	9	1	-	-	-	-	-	-	25	50	25	50	25	25	0	25	75	25	75	75
Kullu (n=4, NG=2)	2	0	2	0	1	1	0	1	1	0	1	1	0	-	-	-	0	2	0	0	1	1	0	0	2	0	0	2	0	0	2
Overall (n=120, NG=15)	n	105	105	105	105	105	105	105	105	105	105	105	105	27	27	27	50	50	50	57	57	57	105	105	105	87	87	87	79	79	79
		HS=74 (71%)	HS=68 (65%)	HS=68 (65%)	HS=74 (71%)	HS=68 (65%)	HS=68 (65%)	HS=74 (71%)	HS=68 (65%)	HS=68 (65%)	HS=74 (71%)	HS=68 (65%)	HS=68 (65%)	HS=09 (33%)	HS=09 (33%)	HS=09 (33%)	HS=22 (44%)	HS=22 (44%)	HS=22 (44%)	HS=16 (28%)	HS=16 (28%)	HS=16 (28%)	HS=23 (22%)	HS=23 (22%)	HS=23 (22%)	HS=02 (0.2%)	HS=02 (0.2%)	HS=02 (0.2%)	HS=00 (0.2%)	HS=00 (0.2%)	HS=00 (0.2%)
		S=27 (25%)	S=28 (27%)	S=28 (27%)	S=27 (25%)	S=28 (27%)	S=28 (27%)	S=27 (25%)	S=28 (27%)	S=28 (27%)	S=27 (25%)	S=28 (27%)	S=28 (27%)	S=16 (59%)	S=16 (59%)	S=16 (59%)	S=21 (42%)	S=21 (42%)	S=21 (42%)	S=29 (51%)	S=29 (51%)	S=29 (51%)	S=42 (40%)	S=42 (40%)	S=42 (40%)	S=14 (13%)	S=14 (13%)	S=14 (13%)	S=18 (23%)	S=18 (23%)	S=18 (23%)
		R=04 (04%)	R=09 (08%)	R=09 (08%)	R=04 (04%)	R=09 (08%)	R=09 (08%)	R=04 (04%)	R=09 (08%)	R=09 (08%)	R=04 (04%)	R=09 (08%)	R=09 (08%)	R=02 (07%)	R=02 (07%)	R=02 (07%)	R=07 (14%)	R=07 (14%)	R=07 (14%)	R=12 (21%)	R=12 (21%)	R=12 (21%)	R=40 (38%)	R=40 (38%)	R=40 (38%)	R=17 (85%)	R=17 (85%)	R=17 (85%)	R=61 (77%)	R=61 (77%)	R=61 (77%)

Cx, Ciprofloxacin; Ex, enrofloxacin; G, gentamicin; Of, ofloxacin; Am, amoxicillin; O, oxytetracycline; Co, co-trimoxazole; T, tetracycline hydrochloride; P, penicillin; Mt, metronidazole; HS, highly sensitive; S, sensitive; R, resistant; NG, no growth.

turbidity and was spread plated in Mueller-Hinton (Hi Media, India) agar plates along with 10 different antibiotic discs (Hi Media, India) of known concentrations. Antibiotic discs of ciprofloxacin (Cx), enrofloxacin (Ex), gentamicin (G), ofloxacin (Of), ampicillin (Am), oxytetracycline (O), co-trimoxazole (Co), tetracycline (T), penicillin (P) and metronidazole (Mt) were carefully placed on the surface of inoculated plates and incubated at 37°C for overnight. The diameter of the zone of inhibition was taken into consideration for determining sensitivity of the sample against the specific antibiotic.

RESULTS AND DISCUSSION

The sensitivity pattern of different samples is shown in Table 1. Among antibiotics, fluoroquinolones like ciprofloxacin, enrofloxacin and ofloxacin, highest sensitivity of 71, 65 and 52% was recorded, respectively and other antibiotics such as oxytetracycline and gentamicin were moderately effective against bacteria causing uterine infection recording sensitivity of 44 and 45% respectively. These results were in agreement to the findings of Barman *et al.* (2013) where most of the organisms were highly sensitive to enrofloxacin and moderately sensitive to ampicillin, oxytetracycline, and gentamicin. A similar pattern of antibiotics was also found for fluoroquinolones and gentamicin (Sharma 2005, Kumar *et al.* 2014, Bala 2017). Highest sensitivity of 92% and 90% was recorded for ciprofloxacin and gentamicin, respectively in another study (El-Kader and Shehata 2001).

However, in another study on buffaloes, antibiotic sensitivity was highest for gentamicin, followed by chloramphenicol, enrofloxacin, cephalixin, tetracycline, ampicillin, co-trimoxazole and furazolidone (Prajapati *et al.* 2005). Arora *et al.* (2000) found gentamicin and pefloxacin (94.3%) as the most effective drugs for the treatment of endometritis however in present study moderate sensitivity for gentamicin was observed. Similarly in another study, gentamicin, enrofloxacin and chlortetracycline were found moderately sensitive (32%) and highest sensitivity (64%) was recorded for ceftriaxone (Udhayavel *et al.* 2013).

Penicillin and metronidazole were least sensitive antibiotics and highest sensitivity pattern recorded for them was 2 and 0% while moderate sensitivity pattern recorded was 13 and 23%, respectively. Our findings were in agreement with Rao and Seshagiri (1997) and Barman *et al.* (2013), who also reported least sensitivity of 3.13 and 0% with penicillin, respectively.

The resistance of these organisms to the commonly used antibiotics, viz. penicillin, metronidazole, nitrofurantoin and furazolidone may be attributed to large scale and indiscriminate use of these antibiotics over a long period of time (Arora *et al.* 2000, Barman *et al.* 2013) and they should not be first choice for the treatment of cows suffering with endometritis (Kumar *et al.* 2014, Bala 2017).

A variety of therapeutic methods are available for the treatment of endometritis with varied success. These

methods involves use of different therapeutic agents such as antibiotics through intrauterine and systemic routes (Foldi *et al.* 2006), hormones either PGF₂α or estradiol 17β (Akhtar *et al.* 2009), normal saline douching of uterus with or without antibiotics (Mohammad and Sayed 2007), immunomodulators (Kumar *et al.* 2004), antiseptic solution like Lugol's Iodine (Sharma 2005, Sood *et al.* 2012, Alyasiri *et al.* 2015), plant derived antiseptics extracts from *Nigella sativa*, *Quercus infectoria* (Alyasiri *et al.* 2015), neem (Barman *et al.* 2009, Kumar *et al.* 2013). Among all these therapeutic agents, antibiotics play a major role for the treatment of uterine infections either through intrauterine or systemic route. However, the emergence of bacterial resistance is the key factor responsible for their restricted use over a period of time (Barman *et al.* 2013). The use of accurate antibiogram is a required necessity to solve such problems. Although non concordance is found between *in vivo* and *in vitro* susceptibility which may be caused by *in vitro* instability of germs, technique errors and different requirement to culture environment (Udhayavel *et al.* 2013), the antibiogram could be useful to the practitioners in choosing the most efficient antibacterial products.

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