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Bacterial Pathogens and Antibiotic Susceptibility Patterns of Cervico-Vaginal Discharges in Cross Bred Repeat Breeding Heifer Cows

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A B S T R A C T

The type of bacterial isolation and their susceptibility patterns to various antibiotics are necessary to eliminate the uterine infections. The present study was designed to isolate and compare the bacterial pathogens and antibiotic susceptibility in Cervico-vaginal discharges of normal cyclic, repeat breeder and heifer cows. For this purpose 43 cows without any significant detectable pathologic disorders of reproductive tract were selected. The cows were divided into three groups viz. Group A (n=11) normal cyclic, Group B (n=16) repeat breeder and Group C (n=16) heifers cows. Cervico - vaginal discharges were collected with the help of AI gun covered with sheath. 54.5 (6/11), 68.7 (11/16) and 12.5% (2/16) bacterial growth was found in Group A, B and C, respectively. Only single isolates of bacteria were found in Group A while 72.7 and 50% were found in Group B and C, respectively. Further, double isolates were found in 27.3 and 50% Group B and C. *E. coli* and *Streptococcous* were highly (>50%) sensitive to ceftriaxone, ciprofloxacin, amoxyclav and tetracycline while *Bacillus* to ciprofloxacin and tetracycline followed by ciprofloxacin, tetracycline and amoxyclav against *Staphylococcus.* In conclusion higher isolates of *Staphylococcous* were found in Normal cyclic cows while *Bacillus* in repeat breeding cows. Equal isolates of *Bacillus*, *E. coli* and *Streptococcus* were found in heifer cows.

Introduction

Well managed and profitable dairy farming requires animal with good reproductive performance (Nebel and Jobst, 1998). However, various reports of decreasing profit in dairy farming have been identified where improvements can be made (Bishop, 1964). Of them, postpartum uterine infection is the majorcause of economic loss in dairy cows (Fox *et al.*, 2002; Sriskandan *et al.*, 2000). It may be due to a longer calving interval, costs of extra services, treatment and increased culling rate (Drillich *et al.*, 2005; Gilbert *et al.*, 2005). Fertility of cow is affected by many nonspecific and specific pathogens of the genital tract. Uterine environment favors the growth of anaerobic organisms which in turn promotes the growth of various pathogenic aerobes that act synergistically to cause bacterial infection (Huszenicza *et al.*, 1999). A higher percentage of cows (80 to 100%) found positive for bacterial contamination of the uterus in the first 2

weeks postpartum (Foldi *et al.,* 2006). Most of these bacterial contaminations get eliminated during the first 5 weeks after parturition, but the remaining causes the uterine infection in some cows (Noakes *et al.,* 2009; Sheldon *et al.,* 2006). Common bacteria isolated from cows with uterine infections are *Trueperella pyogenes*, *Prevotella* sp., *Fusobacterium necrophorum* and *Escherichia coli* (Sheldon *et al.,* 2006; Lewis, 1997; Smith *et al.,* 1998; Overton *et al.,* 2003and Sheldon *et al.,* 2004).

Bacillus, Streptococcus, Enterococcus and coagulase negative *Staphylococci* are the most frequently isolated uterine bacteria that can act as opportunistic pathogens or contaminants (Westermann *et al.,* 2010; Werner *et al.,* 2012).

The presence of pathogenic bacteria in the uterus causes inflammation and histological lesion of endometrium.

Intra uterine antibiotics are one component often used in the treatment of uterine infections. For adequate treatment of the infection there is a need of rapid microbiological diagnosis.

Major advantages of intrauterine treatment with antibiotics are reduced interval of treatment, rapid recovery rate and improved conception rate but their unselective use has led to development of resistant bacterial strains making further use of such therapy useless (Arora *et al.,* 2000). The effectiveness of such therapeutic agents needs to be evaluated repeatedly to treat drug resistance bacterial strains. Therefore present study was planned to analyze the presence of bacterial pathogens in uterine environment of normal cyclic, repeat breeder and heifers cows with an aim to isolate / identify the bacteria and also to investigate the antibiotic sensitivity to suggest the treatment for the control of such infection.

Materials and Methods

A total of 43 cows without any detectable pathologic disorders of reproductive tract were selected from the University Dairy Farm. On the basis of number of AI and their parity cows were divide into three groups, Group A - normal cyclic (n=11), Group B- repeat breeder (n=16) and Group C-heifers (n=16). Cervico - vaginal discharge (CVD) was collected in sterilized vials from the cows in heat with the help of AI gun covered with sheath and immediately shifted to the lab of Microbiology department, GADVASU, Punjab, India. Collected cervico vaginal discharges were inoculated by streaking on BHI (Brain-heart infusion) and blood agar media.

The inoculated media was incubated at 37°C and observed after every 12 hours till 48 hours post inoculation for the incidence of any progress. Physical characteristics of the isolates were noted and cultures were refined by sub culturing in BHI broth and were chilled. The characterization of each isolate was done on the basis of staining behavior, size, motility, biochemical and cultural tests. Overnight incubation of representative samples (n=13) of isolated colonies was done in nutrient broth at 37^{0} C.

To cover the Muller- Hinton agar medium evenly with bacterial suspension we used a sterile cotton swab that was dipped in the bacterial suspension and then rolled over its surface. Seven different antibiotic discs were positioned over the surface of the agar plate. For this purpose, discrete antibiotic discs (Himedia, Mumbai) containing Amoxyclav 30 µg, Cefotaxim 30 µg, Ampicillin 10 µg, Tetracycline 30 µg, Ciprofloxacin 5 µg, Cefuroxime 30 mg and Ceftriaxone 30 µg per discs were used. Sensitivity or resistance to antibiotic was checked by measuring the diameter of inhibition zone found according to Bauer-Kirby scale.

Results and Discussion

Percentage of isolation and the type of bacteria isolated were different among groups. In Group A, 6 out of 11 normal cycling (54.5%), 11 out of 16(68.7%) repeat breeders in Group B and 2 out of 16 (12.5%) heifersin Group C were found positive for bacterial growth. In Group B, 8 out of 11 positive samples showed single isolates and 3showed double isolates. Among the positive repeat breeder cows (Group B), 8 were found positive for endometritis while rest of them didn't show any change in cervco vaginal discharge. Among 23 isolated colonies, Group B showed maximum number (14) followed by Group A (6) and Group C (3).

Maximum number of double isolates was also shown by Group B indicating the higher percentage of mixed infections in repeat breeders (Table 1).

There were total 14colonies isolated in repeat breeder cows (Group B) and bacteria were 57.1 % *Bacillus* sp., 14.3% each of *E coli*, *Staphylococcus* sp. and *Streptococcus* sp. (Figures 1 and 2). It indicates that main cause of infection in (Group B) was *Bacillus* sp. Infection with *Staphylococcus* sp.was highest (50 %) in normal cycling cows (Group A) whereas, infection was equal i.e. 33.3 % with all three bacteria (*Bacillus* sp., *E coli* and *Streptococcus* sp.) in heifers (Group C). During the present study 68.7 % repeaters were found positive for bacterial isolates, which was higher than normal cycling (54.5 %) and heifers (12.5 %). The predominant bacteria isolated from repeaters were *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp. and *E coli*. Among the four bacteria found in 23 isolated colonies, *Bacillus* sp. was found in higher percentage (43.5 %) (Table 2).

The result of antibiotic sensitivity test in the current study indicated that maximum number of isolates was highly sensitive to Ciprofloxacin (100%) and Tetracycline (90.25%), moderately sensitive to Ceftriaxone (56.5%) and Amoxyclav (49%) and least sensitive to Cefotaxim (26%), Cefuroxime (25%) and Ampicillin (15%) among group of bacteria isolated (Table 3).

Results indicate higher percentage of bacterial growth in repeat breeder cows as compared to normal cycling cows and heifers. Higher positivity of 89, 91.12 and 91.11% in repeat breeder groups were reported in previous years (Zahid, 2004; Shukla and Sharma, 2005 and Zaman *et al.,* 2015). A similar study revealed higher percentage of bacteria isolated from the mucopurulent vaginal discharge of repeat breeding cows (69.6 %) compared to normal cyclic discharge (30.4%) (Gani *et al.,* 2008).

In a study, 16.9% repeat breeders positive for bacterial isolation shows clinical abnormality of vaginal discharge and 45.8% repeat breeder cows having no clinical signs (Singh *et al.,* 1998). Previous reporting of bacterial isolations in relation to services indicates they were higher in cows with repeated services of 5 times or more than heifers and normal cycling cows (Rahman *et al.,* 1984).

A study revealed 56.10% cows with single type of organism and 43.90% samples yielded more than one type of organisms from repeat breeder cows (Zaman *et al.,* 2015). Various reports of mixed infections (Chandrakar *et al.,* 2002; Das *et al.,* 1996; Dholakia *et al.,* 1987 and Abd El-Kader and Shehata, 2001) from the vaginal discharges suggest that pathogens act synergistically to cause bacterial infection (Huszenicza *et al.,* 1999).

Table.1 Bacterial examination of normal cycling-, repeat breeder-cows and heifers

Group	No. of examined animals	Presence of bacteria				No. of colonies	No. of animals with			
		Present		Absent			Single isolate		Double isolate	
		No.	%	No.	%		No.	%	No.	%
Normal cycling cows	11	6	54.5	5	45.5	6	6	100	-	0
Repeat breeder cows	16	11	68.7	5	31.3	14	8	72.7	3	27.3
Heifers	16	2	12.5	14	87.5	3	1	50	1	50
Total	43	19	44.1	24	55.9	23	15	65.2	4	17.4

Table.2 List of microorganisms isolated from 23 cows and heifer

Bacteria	Cows	Percent
E. coli	5	21.7
Bacillus	10	43.5
Staphylococcus	5	21.7
Streptococcus	3	13.1
Total	23	100

Table.3 Percentage of susceptibility of the isolated strain to the different antibiotics

Antibiotic	Bacteria			
	E.coli (n=4)	*Bacillus* (n=7)	*Staphylococcus* (n=3)	*Streptococcus* (n=4)
Ceftriaxone	100 (4)	42.85 (3)	33.33 (1)	50 (2)
Ciprofloxacin	100 (4)	100 (7)	100 (3)	100 (4)
Amoxyclav	50 (2)	28.57 (2)	66.67 (2)	50 (2)
Tetracycline	75 (3)	85.71 (6)	100 (3)	100 (4)
Ampicillin	0 (0)	14.28 (1)	0 (0)	0 (0)
Cefotaxim	25 (1)	28.57 (2)	0 (0)	25 (1)
Cefuroxime	25 (1)	0 (0)	0 (0)	25 (1)

Fig.1 Showing isolated bacteria from the cervico vaginal discharge of positive animals. a) *Bacillus*, b) *E. coli*, c) *Streptococcus*, d) *Staphylococcus*

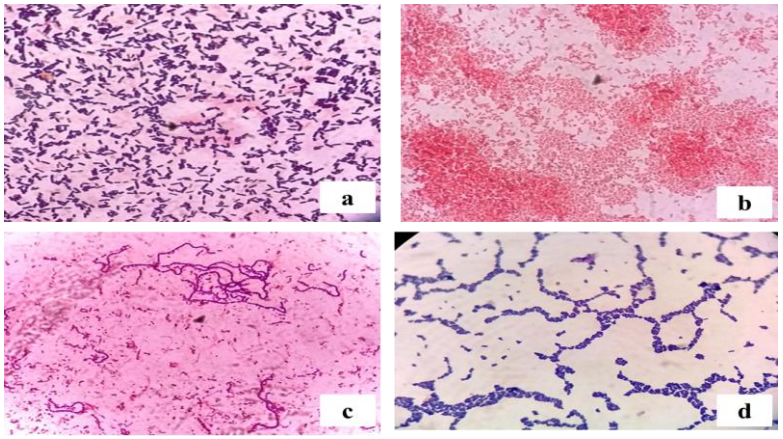
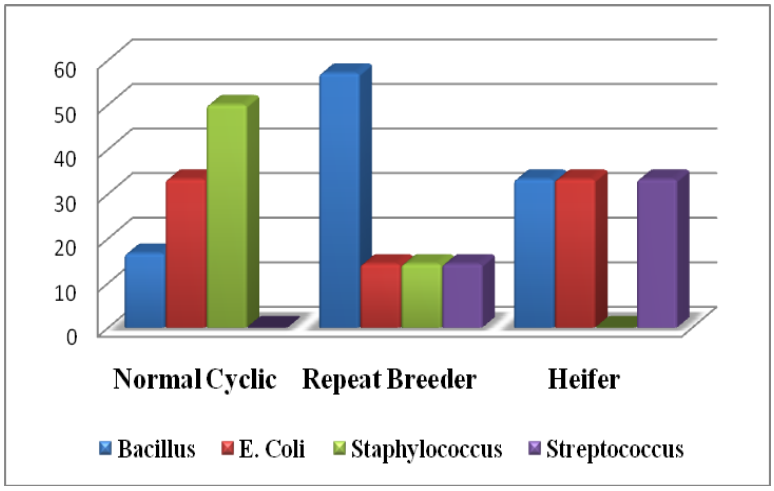


Fig.2 Diagram percentage isolation of different bacteria form group A, B and C



The susceptibility of bacteria *Bacillus* sp. and *E. coli* are more or less effective against 7 antibiotic agents and Amoxicillin, Oxytetracycline and Ciprofloxacin are moderate to highly sensitive to the all of isolates (Gani *et al.,* 2008). Previously it was reported that bacterial isolates was highly sensitive to Ciprofloxacin (Zaman *et al.,*2015, Bhattacharya,2004) but effect of Gentamicin was not the same, this may be due to the over use of Gentamicin during the last few years (Sharma *et al.,* 1993). Based on in vitro drug sensitivity Ciprofloxacin was used in all repeat breeding cows through intrauterine route at the uniform dose rate of 1320 mg daily for three consecutive days (Singla *et al.,* 2004) and they were artificially inseminated in next estrus and the pregnancy diagnosis was done per rectally after 45 days. Ciprofloxacin was also found highly sensitive (60-100%) for four types of isolates followed by Tetracycline (three isolates, 60-100%), Ceftriaxone (two isolates, 60-100 %) and Amoxyclav / Cefotaxim (one isolate, 60%) during the present study. The antibiotic susceptibility patterns needs to be evaluated time and again to decide the best available treatment for the uterine infections and to overcome the problem of drug resistance.

It can be concluded that Bacillus sp. followed by *Staphylococcus* sp. and *E. coli* were the common opportunistic bacteria in normal cyclic, repeat breeder and heifers cows that can any time turn into synergistic pathogen and cause the affections of female genital tract. And Ciprofloxacin and Tetracycline was come out to be highly sensitive drug.

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