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Metritis Associated Aerobic Bacteria in Crossbred Dairy Cows and its Multiple Drug Resistance in Organized Dairy Farm, Maharashtra, India

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A B S T R A C T

A clinical investigation was conducted with the objective to isolate and identify the uterine aerobic bacteria in post partum crossbred dairy cows suffering from metritis and antibiotic sensitivity testing using disc diffusion test. Fourteen post partum crossbred dairy cows found positive for clinical metritis at Research cum Development Project (RCDP) on Cattle, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri Maharashtra State were investigated in the present study. The biochemical characterization results indicated that *E. coli* was isolated from 4 samples; while 3 each sample was found positive for *Klebsiella* spp. and *Staphylococcus* spp. From the remaining 4 metritic samples, no growth was observed either on MacConkey or blood agar. Antibiotic sensitivity pattern revealed that highest resistance was observed in Ampicillin and Amoxycillin (90.0% each), followed by Co-trimoxazole and Enrofloxacin (70.0% each), Tetracycline (50.0%), Ceftriaxone and Gentamicin (40.0% each). While all the isolates were found sensitive to Chloramphenicol and Ciprofloxacin antibiotics. Multiple drug resistance was observed in 7 isolates. Earlier research findings at RCDP on Cattle, MPKV, Rahuri revealed that Enrofloxacin was the drug of choice for treatment of *E. coli* associated Metritis. But present indicated the development of resistance of *E. coli* to Enrofloxacin antibiotic. The paradigm shift in resistance pattern may be due to consistent use of Enrofloxacin for treatment of Metritis resulting into development of resistant mutants.

Introduction

Efficient fertility of lactating dairy cows has always been the approach to cost-effectively profitable dairy farming. Most of the dairy farmers are not able to achieve targets of reproductive performance like 365-day inter-calving interval to be maintained in breeding herds and so bear economic closes. Reproductive performance of dairy cattle is declining. The causes of infertility in dairy animals are many and are complex, among which post-partum uterine infection alone accounts to around 25-30 per cent. The various reports indicated that peripartum diseases, together with dystocia, retained foetal membrane, metritis and endometritis, have a depressing effect on reproductive performance (Curtis *et al.*, 1985; Erb *et al.*, 1985; Heuer *et al.*, 1999). In the past few

decades, the knowledge of the Pathophysiology for diagnosing clinical abnormalities e.g., metritis, endometritis, and subclinical endometritis (SE) has been increased significantly (Pothmann *et al.*, 2015). Most of the times, these postpartum reproductive disorders have same etiology and interlink with each other (Deori, 2015). Most of the bacteria are environmental contaminants and are cleared by the uterus without impairing fertility. The presence of various bacterial pathogens in the uterus of cows suffering from metritis has been attributed to various factors during and after calving.

Antimicrobial drugs are used either to control infection or prevent metritis progression. When dairy animal suffering from Clinical Metritis is infected with resistant bacteria, not only treatment of that animal becomes more difficult, but the antibiotic-resistant bacterium may spread to other animals at farm. The treatment failure with antibiotics results in prolonged illnesses with, more complications, more Veterinarians visits and use of stronger and more expensive drugs along with more deaths caused by bacterial infections. The inadequate treatment or use of ineffective antibiotics leads to chronic mild metritis and subsequent repeat breeding problems. The continuous introduction of new antibiotics and their indiscriminate use emphasizes the importance of *in vitro* testing for specific antibiotic susceptibility. Proper selection of antibiotic is essential to prevent the development of anti – microbial resistance and elimination of infection as fast as possible (Chandrakar *et al.*, 2002). Therefore isolation and identification of microbial agents involved in the Metritis and to know their antibiogram pattern is a prerequisite for treating infectious metritis in dairy herd. In view of this, a clinical study was investigated with the objective to determine common aerobic pathogens associated with postpartum Metritis in crossbred dairy cows with their antimicrobial drug resistance pattern.

Materials and Methods

Animals

The whole investigation was carried out on post-partum crossbred lactating dairy cow suffering from clinical metritis at Research cum Development Project (RCDP) on Cattle, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra State (INDIA).

Husbandry and management system

At Research cum Development Project (RCDP) on Cattle, Crossbred dairy cows were managed under loose housing system. All the cows were vaccinated twice in a year for Hemorrhagic Septicemia, Black Quarter and Foot and Mouth Disease. Twenty one days prior to vaccination deworming was carried out. They are provided with green grass and concentrate diet and are kept together in common shed but at peri-parturient period (advanced pregnancy and early lactating stage) they are maintained in separate byres.

Diagnosis of metritis

It was done on rectal examination. On palpation uterus was hard, swollen, thick walled and fluid filled with moderate tone. The characteristic longitudinal ridges, usually palpable, were absent. Purulent or muco-purulent exudates evacuated per vagina on massaging the uterus (Bhat and Bhattacharya, 2012).

Uterine sample collection

All the cows suffering from clinical symptoms of Metritis were explored for isolation of aerobic bacterial flora. Prior to collection of transcervical uterine sample, the perineal

region of the cow was washed and cleaned properly with fresh water and 1.0 per cent potassium permanganate solution. Left handed rectal palpation was performed to collect uterine sample by using sterile disposable Artificial Insemination sheath attached with a 10 ml syringe (adjusted behind the sheath) was introduced into the uterus through cervix guided by rectal palpation. The aspirate was poured in sterile test tubes transferred to laboratory.

Culture and identification of microorganisms

The aspirate was inoculated on nutrient broth and incubated for 1 hr at 37^0C. The inoculum was streaked on Blood agar and MacConkey agar plates using sterile bacteriological loop. The plates were incubated at 37°C for 24 h. Identification of isolate was done on basis of colony morphology, Grams' staining, catalase test, Oxidase test, Indole production, Citrate utilization, Methyl Red test, Voges Proskauer test (Cheesbrough, 1994).

Antimicrobial susceptibility testing

The antibiotic-susceptibility profile of isolate for 14 different antibiotics was prepared using the disk diffusion method on Mueller-Hinton agar as recommended by Bauer *et al.*, (1966). In brief, isolates was grown overnight on blood agar at 37°C, and the colonies was suspended in sterile saline water equivalent to a 0.5 McFarland standard.

The suspension (100 µl) was spread over the medium plate. Then, the antibiotic disk was transferred aseptically on to the surface of the inoculated medium, and was incubated further at antibiotics at 37°C, for a period of 24 h. The antibiotics and their concentrations used are as follows: Ampicillin (25 µg), Amoxyclav (30 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), Cefotaxime (10 µg), Cephalexin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Co-Trimoxazole (30 µg), Enrofloxacin (10 µg), Gentamicin (20 µg), Levofloxacin (5 µg), Penicillin G (10 units) and Tetracycline (30 µg).

Results and Discussion

Fourteen post-partum crossbred lactating cows suffering from metritis at RCDP on Cattle were investigated in the present study. Ten (71.43 %) post-partum crossbred lactating cows suffering from metritis samples were found positive bacteriologically, which were identified phenotypically based on colony morphology and biochemical characteristics. The remaining 04 (28.57%) showed no bacterial growth. None of the sample was found to be positive for mixed colonies having growth of different colony morphology.

E. coli was isolated and biochemically characterized in 4 samples, while 3 each samples was found positive for *Klebsiella* spp. and *Staphylococcus* spp.

Antibiotic sensitivity pattern revealed that highest resistance was observed in Ampicillin and Amoxycillin antibiotic (90.0%), followed by Co-trimoxazole and Enrofloxacin (70.0%), Tetraacycline (50.0%) and Ceftriaxone and Gentamicin (40.0%). While all the isolates were found sensitive to Chloramphenicol and Ciprofloxacin antibiotics. Multiple drug resistant strains were observed in 7 isolates.

In post-partum crossbred lactating cows postpartum uterine diseases are common disorders that pessimistically affect production and reproductive performance, resulting in a huge economic drainage to the dairy industry by withdrawing profitability and sustainability of dairy operations. Uterine infections cause infertility in acute cases and subfertility in chronic cases. Consequently, uterine infection in cows will reduce conception rate, increase

calving to conception interval and contribute for increased involuntary culling rate (Sheldon *et al.,* 2009).

In the present investigation, from 14 post-partum crossbred lactating cows suffering from Metritis associated aerobic bacteria flora, *E. coli* was predominantly isolated from 4 cases (28.57), while *Staphylococcus* spp. and *Klebsiella* spp. was isolated from 3 each cases (21.43% each). Our findings was in concurrent with Bhat and Bhattacharya (2012) who reported *E. coli*, *Staphylococcus* spp. and *Klebsiella* spp. has the dominant bacteria associated with Metritis in crossbred cows. Similar, findings were also reported by Abreham *et al.,* (2017) and Baishya *et al.,* (1998) who reported *E. coli* has predominant aerobic bacteria associated with uterine infections *i.e.* 42.1 per cent and 29.7 per cent, respectively. This report also agrees with their findings in this study regarding the dominant species in the second order, which was *Staphylococci* species. The pathogenic microorganisms associated with uterine infections were *Escherichia coli* (Lewis, 1997; Bondurant, 1999), and also included opportunistic pathogens such as *Staphylococcus spp* (Sheldon, 2004), and *Klebsiella spp* (Dini *et al.,* 2012). The bacteriological examination of the cervical swabs from the cows with puerperal metritis showing purulent discharge revealed the presence of *E. coli* (44%) (Kozdrowski *et al.,* 2004). According to Sheldon *et al.,* (2004) and Williams *et al.,* (2005) the bacteria isolated belonged to obligate uterine pathogens (*E. coli*) and opportunistic bacteria (*Staphylococcus spp* and *Klebsiella spp*).

In the present study, no pathogenic aerobic bacteria could be isolated in as many as 4 cows which were positive for post-partum metritis. This may not mean the absence of clinical metritis as a number of other anaerobic bacteria or viruses could be associated with metritis.

Antibiotic resistances of different bacterial isolates were tested in this study. The highest resistance was observed in Ampicillin and Amoxicillin antibiotic (90.0%), followed by Co-trimoxazole and Enrofloxacin (70.0%), Tetracycline (50.0%) and Ceftriaxone and Gentamicin (40.0%). While all the isolates were found sensitive to Chloramphenicol and Ciprofloxacin antibiotics. Moges *et al.,* (2013) reported similar antibiotic resistance pattern with uterine isolates sensitive to Chloramphenicol and Enrofloxacin. But our reports are not in agreement with their report of all the isolates were sensitive to amoxicillin/clavulanic acid and gentamicin antibiotics. This may be due to indiscriminate use of amoxicillin/clavulanic acid, ampicillin and gentamicin antibiotics for treatment of uterine infections. While, all the isolates were found sensitive to Ciprofloxacin antibiotic in our study was also not in agreement with their study of moderate resistance to Ciprofloxacin. In another report of Anjaneyulu *et al.,* (1999) Trimethoprim - sulfamethoxazole and Chloramphenicol are effective antibiotics against endometritis isolates, that support our finding of our study of sensitive to Chloramphenicol while contrast to Co-trimozxazole in which we have found moderate resistance (70.0%) (Table 1).

In present study, multiple drug resistant strains were observed in 7 isolates having resistance from 4 to 8 antibiotics. Antimicrobial resistance in bacteria is a growing Veterinary and medical health concern globally, has these bacteria are shared equally by animals and human beings also. Furthermore, indiscriminate use of antimicrobial in treating Metritis in this locality for last few decades is probably largely responsible for development of resistant bacteria against these antibiotics.

Table.1 Antibiotic sensitivity pattern of isolates from metritis cases

Sr. No	Tattoo Number of Cows	Antibiotic Sensitivity Pattern									Bacteria
		AMC	AMP	C	CP	Co	CTR	Ex	G	TE	
1	8IH34	R	R	S	S	R	R	R	S	R	*E. coli*
2	PT45	R	R	S	S	R	R	R	S	R	*E. coli*
3	F10	R	R	S	S	R	R	R	S	R	*E. coli*
4	4IH112	R	R	S	S	R	R	R	S	R	*E. coli*
5	PT79	R	R	S	S	R	S	R	S	R	*Staphylococcus* Spp.
6	8IH28	R	R	S	S	R	S	R	R	S	*Staphylococcus* Spp.
7	PT60	R	R	S	S	R	S	R	R	S	*Staphylococcus* Spp.
8	PT6	S	S	S	S	S	S	S	R	S	*Klebsiella* Spp.
9	PT72	R	R	S	S	S	S	S	R	S	*Klebsiella* Spp.
10	F1	R	R	S	S	S	S	S	S	S	*Klebsiella* Spp.

AMP - Ampicillin (25 µg),
C- Chloramphenicol (30 µg),
CO - Co-Trimoxazole (30 µg),
Ex -Enrofloxacin (10 µg),
TE -Tetracycline (30 µg).

AMC- Amoxyclav (30 µg),
CTR - Ceftriaxone (30 µg),
CP - Ciprofloxacin (5 µg),
G -Gentamicin (20 µg),

However, oxytetracycline is nowadays least used antimicrobial under field conditions. Earlier research findings at RCDP on Cattle, MPKV, Rahuri revealed that Enrofloxacin was the drug of choice for treatment of *E. coli* associated Metritis. But present findings indicated the development of resistance of *E. coli* to Enrofloxacin antibiotic. The paradigm shift in resistance pattern may be due to consistent use of Enrofloxacin for treatment of Metritis resulting into development of resistant mutants. The metritis caused by bacteria is treated with antibiotics. However, the efficacy of such therapeutic agents needs to be evaluated from time to time due to continuous emergence of drug resistant bacterial strains. The use of proper antibiotic sensitivity pattern is required as a necessity, in the context of varying factors concerning the frequency of different involved bacterial species, and their susceptibility to different antimicrobials. The antibiotic susceptibility testing could be useful to the practitioner in choosing the most efficient antibacterial. However, therapeutic trials using these antibiotics should have been carried out to find out the most effective drug.

Impacts

The present investigation demonstrates the presence of *E. coli*, *Staphylococcus* spp. and *Klebsiella* spp in high percentage in post-partum Metritis of crossbred cows.

The Chloramphenicol and Ciprofloxacin antibiotics are highly effective against treatment of Metritis associated bacteria. The present finding indicates the paradigm shift in resistance of *E. coli* to Enrofloxacin antibiotic that may be due to consistent use of Enrofloxacin for treatment of Metritis resulting into development of resistant mutants strains of *E. coli*.

Multiple drug resistance observed indicates the necessity to implement a systemic application of *in vitro* antibiotic susceptibility testing prior to the use of the antibiotics in both treatment of post-partum metritis.

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References

Abreham, S., Hailu, M., Worku, A., Tsegaye, S. (2017). Antibiotic Resistance of Aerobic Bacteria Isolated from Uteri of Slaughtered Cows in and around Addis Ababa, Ethiopia. J. Vet. Sci. Technol., 8: 406

Anjaneyulu, Y., Wilson, J., James, R. (1999). Antibiogram in bovine endometritis, a field studies. Indian Veterinary Journal. 76: 351-352.

Barrow, G. I., Feltham, R. K. A. (1993): Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edn, 140-43, Cambridge University Press, Cambridge.

Bauer, A.W., Kirby, W. M. M., Sherris, J. C., Turck, M. (1966): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path. 45: 493-496.

Bhat, F. A. and Bhattacharyya, H. K. (2012). Management of Metritis in Crossbred Cattle of Kashmir using Oxytetracycline, Cephalexin and Prostaglandin F2α. Indian J. Anim. Res., 46 (2): 187 - 189.

Baishya, S., Das, K., Rahman, H., Borgohain, B. (1998). Antibiogram of bacteria isolated from uterine discharge of repeated cattle. Indian Journal of Comparative Microbiology, Immunology and infectious Diseases. 19: 130-131.

Bondurant, R.H., 1999. Inflammation in the bovine female reproductive tract. *J. Anim. Sci*., 77: 101-110

Chandrakar, D., Tiwari, R.P., Awasthi, M.K. and Hirpurkar, S.D., (2002). Microbial profile, their antibiogram pattern in cervico – uterine contents and conception rate following treatment in repeat breeder crossbred cows. Indian J. Anim. Reprod., 23(2): 148- 150

Cheesbrough, M. (1994). Medical Laboratory Manual for Tropical Countries, 1st edn., Vol.II,

Butterworth-Heinemann, Oxford.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. (1985). Path-analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347-2360.

Deori S., Phookan A. (2015) Bovine Postpartum Metritis and its Therapeutics: A Review. Indian J Sci Tech 8(23): 1.

Dini, P., Amarloie, O.A., Moghadam, M.F., Moosakhani, F., Mottaghian, P., and Barin, A. (2012). The Comparison of Antagonistic Effects of Normal Vaginal *Lactobacilli* and Some Commonly used Antibiotics on Isolated Bacteria of Uterine Infections in Dairy Cows. Int. J. Anim. Vet. Adv., 4(6): 358-362

Erb, H. N., R. D. Smith, P. A. Oltenacu, C. L. Guard, R. B. Hillman, P. A. Powers, M. C. Smith, and M. E. White. (1985). Path model of reproductive disorders and performance, milk fever, mastitis, milk-yield, and culling in Holstein cows. J. Dairy Sci. 68: 3337-3349.

Heuer, C., Y. H. Schukken, and P. Dobbelaar. (1999). Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J. Dairy Sci. 82:295-304.

Kozdrowski, R., Twardon, J. and Blaszkowska, M. (2004). Bacterial flora obtained from uterine cervix after antibiotic and non antibiotic treatment for puerperal metritis in cows. *J. Animal*

and Veterinary Advances., 3(11): 785-787

Lewis, G.S. (1997). Uterine health and disorders. J. Dairy Sci., 80(5): 984-994.

Moges, N., Regassa, F., Yilma, T. and Unakal C. G. (2013). Isolation and Antimicrobial Susceptibility of Bacteria from Dairy Cows with Clinical Endometritis. Journal of Reproduction and Infertility. 4 (1): 04-08,

Pothmann H, Prunner I, Wagener K, Jaureguiberry M, de la Sota R, *et al.*, (2015) The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. Theriogenology 83(8): 1249-1253.

Sheldon, I.M. (2004). The postpartum uterus. Vet. Clin. North Am. Food Anim. Pract., 20: 569-591.

Sheldon, I. M., Price, S. B., Cronin, J., Gilbert, R. O. and Gadsby, J. E. (2009). Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reprod. Dom. Anim., 44 (3): 1-9.

Williams, E.J., Fisher, D.P., Pfeiffer, D.U., England, G.C., Noakes, D.E.W., Dobson H. and Sheldon, J.M. (2005). Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology, 63: 102-117.

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