



Oxytetracycline is more suitable antibiotic for clinical endometritis cows

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ABSTRACT

We identified the bacterial isolates from the uterus of clinical endometritis affected cows and performed *in vitro* antibiotic sensitivity test (ABST) against major uterine pathogen (*E. coli*). We subsequently evaluated the sensitive antibiotics in clinical endometritis affected cows through fertility parameters, blood acute phase proteins (APPs; haptoglobin and serum amyloid A), cytokines level (IL-1 β and TNF- α) and milk yield. We found 12.7% of *E. coli* among 63 bacterial isolates from the endometritis cows. ABST against *E. coli* revealed gentamicin as the most sensitive and oxytetracycline (OTC) as intermediately sensitive drug. Minimum inhibitory concentration (μ g/ml) of gentamicin and OTC against *E. coli* was 5.47 and 0.78, respectively. Gentamicin, OTC, and povidone-iodine (PI) were administered for 3 days through intrauterine route in endometritis cows (8 per group) for *in vivo* evaluation. The first service conception rate was higher in OTC treated cows with lesser days open, while interval between treatment and conception was nonsignificantly lower in PI-treated cows. The concentration of APPs and cytokines was significantly reduced in antibiotics treated cows but not in PI-treated groups. In conclusion, OTC was found to be more suitable than gentamicin for treatment of clinical endometritis and PI had differential effects on clinical endometritis cows.

Key words: Antibiotic sensitivity test, Clinical endometritis, *E. coli*, *In vivo* evaluation, Uterine pathogens

Among the uterine diseases, clinical endometritis is a major challenge to livestock farming due to its financial implications through culling of sub- or infertile animals. LeBlanc *et al.* (2002) found lower conception rate with longer days open in endometritis cow. Several other researchers also found worse picture of days open and pregnancy rate in clinical endometritis affected cows (Gilbert *et al.* 2005, Gautam *et al.* 2009). It has been estimated that 40–50% of high yielding dairy cows are affected by endometritis during postpartum period (LeBlanc *et al.* 2002, Gilbert *et al.* 2005). Therefore, prompt and effective treatment of postpartum endometritis is important for improving the reproductive efficiency in farm animals.

Several authors reported that routine infusion of various antibiotics may not be efficacious in endometritis (Farca *et al.* 1997) and in toxic puerperal metritis affected cows

(Drillich *et al.* 2001). In contrast, beneficial effects of intrauterine (i.u.) infusion of antibiotics was reported by various researchers in subclinical (Kasimanickam *et al.* 2005) and clinical endometritis cows (LeBlanc *et al.* 2002). There are several biological factors such as days in milk and presence of palpable corpus luteum at the time of treatment that are believed to affect the efficacy of treatment (LeBlanc *et al.* 2002, Feldman *et al.* 2005). Further, Kaczmarowski *et al.* (2004) found better efficacy in cows when treated with the i.u. antibiotic with PGF₂ α . In contrast, Galvao *et al.* (2009) found no influence of ceftiofur on prevalence of clinical and subclinical endometritis, though it reduced the prevalence of positive uterine culture in these cows. The efficacy of the therapy should be evaluated through innate immune molecules, endocrine hormones levels, and milk yield of the animals since the pharmacokinetic and pharmacodynamics behaviour of antibiotics are influenced by these factors. Accordingly, we isolated major uterine pathogen, assessed its antimicrobial susceptibility and subsequently evaluated the effectiveness of sensitive antibiotics through innate immune molecules in clinical endometritis affected cows.

MATERIALS AND METHODS

Uterine sample collection and bacterial isolation: Cows with abnormal vaginal discharges between 7 and 47 days after calving were examined per rectum as well as per

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vaginum and diagnosis was made according to Sheldon *et al.* (2006). Uterine swab samples were collected from cows (23) with purulent or mucopurulent uterine discharge and incubated in nutrient broth at 37°C for 24 h. Bacterial isolates were identified on the basis of morphological characteristics of the colony, gram stain, and biochemical profile. Hecktoen entric agar (orange or orange-yellow colonies) and Hichrome ID agar (colourless colonies) were used for confirmation of *E. coli*. All chemical used in the present microbiological study were procured from M/s Himedia, Mumbai (India).

Preparation of culture isolate and antimicrobial sensitivity test (ABST): Minimum three isolated colonies (triplicates) of same morphological type of *E. coli* isolates were used for ABST by Kirby Bauer disk diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Based on ABST and retrospective study findings, we selected antibiotics (gentamicin and oxytetracycline; OTC) for *in vivo* evaluation. Minimum inhibitory concentrations (MIC) of selected antibiotics were determined against *E. coli* by broth microdilution method (Basri and Fan 2005).

Experiment animals and dose selection: During routine examination of postpartum animals by veterinarian, the cows diagnosed with clinical endometritis were selected (24) for *in vivo* evaluation of antibiotics and supportive therapy. Animals treated with antibiotics in early postpartum due to metritis, mastitis or any other health problems within 15 days before actual diagnosis of endometritis were not included in this study. Once diagnosed, cows were randomly grouped into 3 groups of eight each. Gr I received an i.u. infusion of gentamicin (250 mg for 3 days), Gr II received an i.u. infusion of OTC (3 g for 3 days) and Gr 3 received an i.u. infusion of 1% antiseptic solution for 3 days [10 ml povidone-iodine (5% w/v) diluted with 50 ml sterile distilled water].

Evaluation of treatment outcome: All the treated animals in 3 groups were re-examined by rectal palpation between 18–20 days after treatment. The absence of abnormal uterine discharge and horn sizes during rectal examinations were considered as clinically cured. Daily milk yield was recorded 1 week before treatment, during treatment period of 3 days and 3 weeks after treatment. Blood samples were collected before and after treatment for estimation of acute phase proteins (APPs; Hp and SAA) and cytokines (IL-1 β and TNF- α). Treated animals were followed till 180 days postpartum period for assessment of fertility parameters.

Statistical analysis: Student's *t*-test was used to compare MIC of gentamicin and OTC in endometritis cows. Two way ANOVA was used to compare changes in milk yield and blood levels of APPs and cytokines before and after antimicrobial treatment of endometritis cow. The interval between treatment and conception and days open were analyzed using Kaplan-Meier survival analysis and median values were compared using log rank test. The conception rates of various groups were analyzed using Chi-square test (SPSS version 16; M/s SPPS Inc., Chicago, IL, USA). When

the P value < 0.05, the means were considered to be significantly different. Values of the different parameters were expressed as Mean $\pm$ SE. All the analyses were performed using SigmaPlot 11 (Systat Software Inc., USA) statistical software package.

RESULTS AND DISCUSSION

About 12.7% of the isolates were *E. coli*, the major uterine pathogen of the endometritis cows. In ABST, *E. coli* was more sensitive to gentamicin than OTC, while on the basis of MIC, the opposite was found to be true. However, *in vivo* evaluation through fertility parameters indicated more suitability of OTC than gentamicin for treatment of endometritis. Antimicrobial therapy reduced inflammation, but not in the povidone iodine-treated animals.

About 63 bacterial strains were isolated from the clinical endometritis cows. Of this, 22% (14/63) coliforms, 12.70% (8/63) *E. coli*, 19% (12/63) *Streptococcus* sp., 23.53% (15/63) *Staphylococcus* and 22% (14/63) *Bacillus* sp., were identified. As observed in this study, Westermann *et al.* (2010) also isolated 10.4% of *E. coli* from a uterine swab of clinical endometritis cows during 21–28 days postpartum period. In our study, *E. coli* was isolated together with other opportunistic bacteria as reported by other researchers (Liu *et al.* 2013). Antimicrobial sensitivity assay revealed that *E. coli* was most sensitive to gentamicin (100%), ceftriaxone (87.5%), ampicillin with sulbactam (87.5%) and chloramphenicol (87.5%), while cefoperazone and oxytetracycline were found to be intermediately sensitive (Fig. 1). Overall ABST revealed that 60% of the isolates were sensitive to all drugs, 30% were intermediate and 10% of isolates were resistant to all drugs. Several researchers from India (Udhayavel *et al.* 2013) and other countries (Moges *et al.* 2013) reported that *E. coli* was sensitive to gentamicin or chloramphenicol while Farca *et al.* (1997) found that aerobic bacteria isolated from uterine swabs were resistant to OTC. In contrast to ABST, the observed MIC (μ g/ml) of gentamicin and OTC against *E. coli* was found as 5.47 and 0.78, respectively indicated that OTC is more sensitive. Since the disc diffusion test results are qualitative rather than quantitative results of MIC, the observed lower MIC of OTC might be the reason for its better efficacy in clinical endometritis cows as observed in this study. In fact, Devriese and Dutta (1981) studied the correlation between various *in vitro* antibiotic testing with *in vivo* situation and reported that MIC relation is the only one important facet of a complex *in vivo* situation.

Retrospective evaluation of routine postpartum uterine infection therapy in our farm showed (112) that hormonal preparation (41%) and antiseptic solution were most commonly (36%) used than antibiotics (13%) or combination of above strategies (10%). Among various antibiotics, we found that gentamicin and OTC were most commonly used antibiotics. Accordingly, we selected sensitive as well as most commonly used antibiotics (gentamicin and OTC) and antiseptic solution (povidone

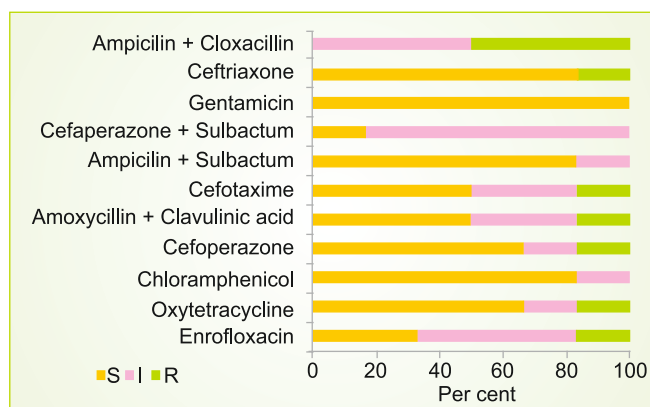


Fig. 1. Antimicrobial susceptibility pattern of *E. coli* (n=8) isolated from endometritic cows using disk diffusion method. S, sensitive; I, intermediate; R, resistant.

iodine; PI) for *in vivo* evaluation. Evaluation of treatment outcome through rectal examination after 18–20 days of treatment revealed lack of effectiveness in the gentamicin and PI-treated groups (2–4 cows had abnormal in uterine discharge and horn sizes) than OTC-treated cows (all the animals showed clear mucus with normal uterine size). In the present study, milk yield of endometritis affected cows before and after treatment remained unchanged (Fig. 2). Several researchers reported that there was no relationship between metritis or endometritis and milk yield (Fleischer *et al.* 2001, Dubuc *et al.* 2011). They suggested that improved dry matter intake, decline in severity of uterine infection and relative improvement in immune status during later postpartum compared to early postpartum may be the reason for no influence of endometritis on milk production.

Evaluation of antimicrobial therapy through fertility parameters indicated more efficacy of OTC than gentamicin in clinical endometritis cases (Table 1, Fig. 3). The first service conception rate was lesser while, days open was higher in gentamicin- than OTC-treated cows. On the

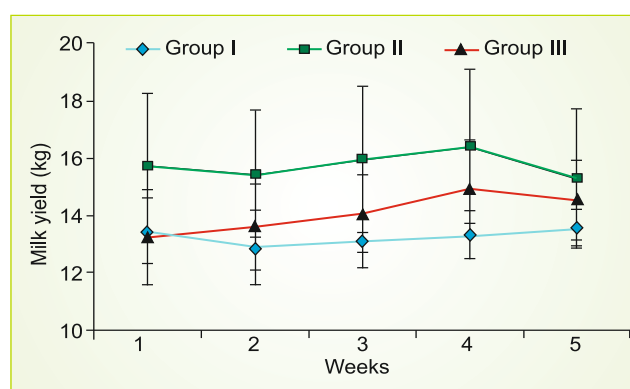


Fig. 2. Average milk yield of cows in different groups suffering from endometritis and treated with different antibiotics. 1, average milk yield of one week before antimicrobial treatment; 2, average milk yield during 3 days of antimicrobial treatment; 3, average weekly milk yield after antimicrobial treatment (days 4–10); 4, average weekly milk yield after antimicrobial treatment (days 11–17); 5, average weekly milk yield after antimicrobial treatment (days 18–24). Group I, gentamicin; Group II, oxytetracycline; and Group III, povidone iodine. Values are mean±SE of 8 animals.

Table 1. Fertility parameters of endometritic cows following antimicrobial treatment

Fertility parameter	Gentamicin	Oxytetracycline	Povidone iodine
Interval between treatment and conception	48±12.02	42.17±19.80	46
First service conception rate (%)	37.5	75	50
Days open	121±14.14	75±29.70	78

Values are median±SE of 8 animals. The chi square values (2.35; P=0.309) reveals nonsignificant difference between various treatment groups.

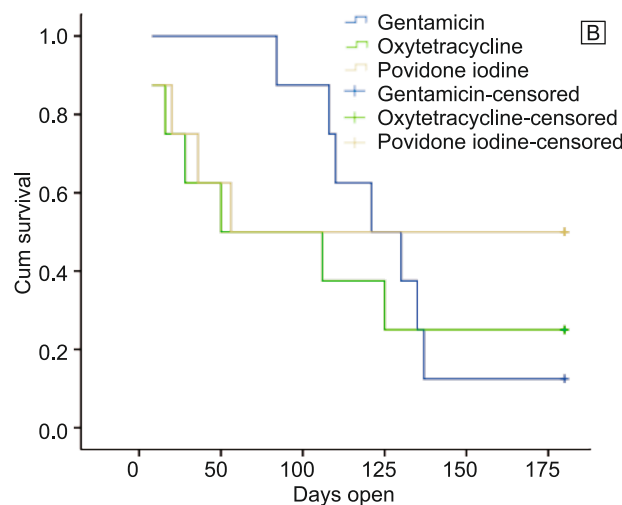
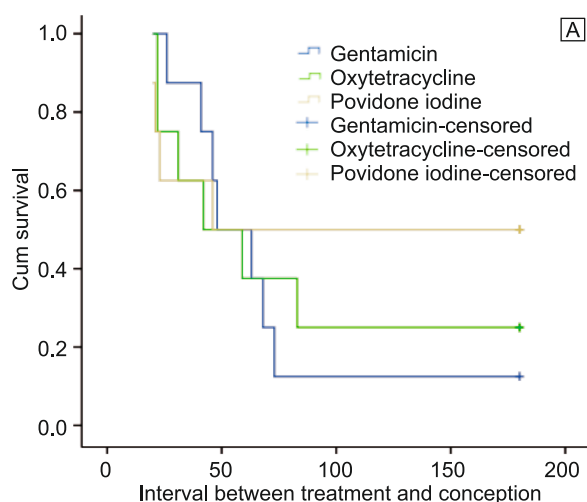


Fig. 3. Survival curve: chi squared values of log rank test for interval between treatment and conception (Fig. 3a; 0.760, P=0.68) and days open (Fig. 3b; 1.003, P=0.61) are different. Censored observation means that the animals were not conceived during follow-up period of 180 days postpartum.

Table 2. Changes in plasma bovine major acute phase proteins ($\mu\text{g/ml}$) and inflammatory cytokines concentrations (pg/ml) before and after antimicrobial treatment in endometritic cows

Treatment	Haptoglobin		Serum amyloid A		IL-1 β		TNF- β	
	Before	After	Before	After	Before	After	Before	After
Gentamicin	365.54 $\pm$ 16.44 ^a	128.46 $\pm$ 10.22 ^b	62.23 $\pm$ 4.61 ^a	35.92 $\pm$ 4.63 ^b	140.94 $\pm$ 6.15 ^a	78.65 $\pm$ 1.91 ^b	255.56 $\pm$ 18.36 ^a	109.59 $\pm$ 7.13 ^b
Oxytetracycline	321.06 $\pm$ 19.55 ^a	121.01 $\pm$ 7.42 ^b	58.34 $\pm$ 3.71 ^a	32.71 $\pm$ 4.71 ^b	137.77 $\pm$ 9.57 ^a	69.16 $\pm$ 4.49 ^b	244.01 $\pm$ 18.09 ^a	96.50 $\pm$ 6.33 ^b
Povidone iodine	296.02 $\pm$ 24.76	216.43 $\pm$ 26.17	54.06 $\pm$ 4.84	44.85 $\pm$ 5.55	163.59 $\pm$ 14.36	125.66 $\pm$ 17.08	224.04 $\pm$ 17.86	192.49 $\pm$ 13.95

Values are mean $\pm$ SE of 8 animals. Means with different superscripts in the same row differ significantly ($P < 0.05$).

contrary, OTC was not as sensitive as gentamicin in *in vitro* test. Pecsí (2007) also found better response in OTC-treated cows than other treatment groups (amoxicillin and gentamicin). Therefore, the observed better efficacy of intrauterine OTC might be due to its higher sensitivity against *E. coli* (based on MIC) and other uncultured or unknown organisms. It is well known that OTC is a broad spectrum antibiotic and indicated for rapidly growing uterine pathogens (Sheldon *et al.* 2004) and anaerobic organism (Konigsson *et al.* 2001). Therefore, intrauterine administration of OTC is considered as a useful therapy for endometritis (Malinowski *et al.* 2004). Further, the efficacy of OTC may also related to its non-antimicrobial effects such as anti-inflammatory effect or inhibition of matrix metalloproteinases and inducible nitric oxide synthase, which are known to play a role mucosal inflammation and immune response (Hoyt *et al.* 2006). PI-treated cows had lesser interval between treatment and conception and it could be due to irritant-mediated shortening of estrous cycle length or regression of CL. Since endometritis could be due to very less or over-stimulation of inflammatory pathways, optimum stimulation is warranted to check endometritis (Galvao *et al.* 2011). Antiseptic solution-mediated optimum stimulation of endometrium and subsequent release of PGF $_{2\alpha}$ might be the reason for beneficial effects observed in PI-treated cows (Chaudhery *et al.* 1987). The decreased level of APPs and inflammatory cytokines after the antibiotic treatment indicated the suppression of inflammatory processes in these animals (Table 2). It was reported that Hp concentration was reduced after antibiotic treatment in endometritis cows (Heidarpour *et al.* 2012). Overall it may be concluded that OTC was more suitable than gentamicin for treatment of clinical endometritis and PI had differential effect in endometritis cows.

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