

**Antimicrobial Susceptibility to some Therapeutic Agent used to  
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# Antimicrobial Susceptibility to some Therapeutic Agent used to Treat Uterine Infection in Cows

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## Abstract

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The aim of this study was to determine the in-vitro susceptibility of microorganisms isolated from infected cow's uteri, in association with the characters of the uterine discharges. Nine cows with pathological discharges were clinically diagnosed per rectum and vaginum. Swabs from the uterine lumen were aseptically collected for Bacteriological examination according to commonly accepted procedures. Susceptibility to 10 different antibiotics (used routinely by the veterinarians to treat uterine infections) was tested by the disk diffusion method and performed in Mueller–Hinton agar medium and covered evenly with the bacterial suspension. Characters of the discharges were scored from 0-3. The cultures were most susceptible to Gentamycin followed by Cephalexin. Kanamycin was found to be as a third choice followed by Amoxicillin, Neomycin and Sulphamethazole. Tetracycline was less effective in comparison to the above mentioned antibiotics, Streptomycin and Penicillin were found to be the least effective, with resistant results in 11.1% and 22.2% of the samples, respectively. These are followed by the antibiotic Cloxacillin had clear resistance in 55.5% of the Samples. Scores for the characters of the discharges were found between grades 2 and 3 in eight cows (88.8%) while the ninth cow had score one type discharge.

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**Keywords:** Antibiotics susceptibility, uterine infection, cows.

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## Introduction

Uterine infections are considered as a major problem affecting reproductivity and productivity in cows. They can have a negative impact on reproductive performance. This would likely have been more severe if the cows had not been participating in a routine health program in which uterine infections and other postpartum reproductive problems were detected and treated early. A high percentage of cows have bacteria present in their uteri during the first two weeks after calving. However, by about 50 days post-calving, the prevalence of uterine infections should decline to very low percentage (Noakes, Parkinson *et al.*, 2009) Uterine infections in cattle can be classified as puerperal metritis, clinical metritis, clinical endometritis and subclinical endometritis (Sheldon, Lewis *et al.*, 2006). Clinical endometritis affects from 5.0 to >30% in some herds (LeBlanc, Duffield *et al.*, 2002; McDougall, Macaulay *et al.*, 2007; Galvão, Frajblat *et al.*, 2009). Subclinical endometritis is the most prevalent of all uterine diseases; it affects from 11 to >70% in some herds (Kasimanickam, Duffield *et al.*, 2004; Gilbert, Shin *et al.*, 2005; Hammon, Evjen *et al.*, 2006; Barlund, Carruthers *et al.*, 2008; Galvão, Frajblat *et al.*, 2009), and Metritis affects 8 to more than 40% in some cattle farms (Goshen and Shpigel 2006; Hammon, Evjen *et al.*, 2006; Huzzey, Veira *et al.*, 2007; Galvão, Greco *et al.*, 2009).

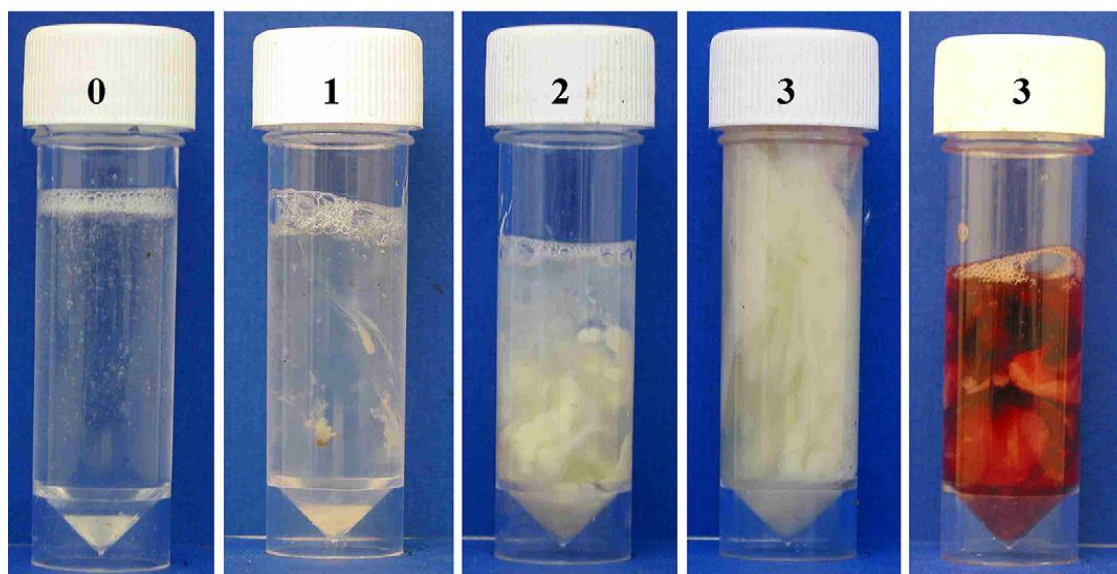
In general, usage of antibiotics in farm animals is quite heavy and widespread, and has been a typical practice of veterinarians in Libya. Consistently using drugs in farm animals raises concerns that this administering of antibiotics has caused an increase in the antibiotic-resistant bacteria that can be harmful to humans. The antibiotics and antiseptic agents used in the treatment of acute postpartum uterine infections induce residues in foods, spread of bacterial resistance and increase in financial costs and failures in defense mechanisms of the host (Lewis 1997; Otero and Nader-Macas 2006). The clinical microbiology laboratory important task is the performance of antimicrobial susceptibility testing of significant bacterial isolates, and the goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to

drugs of choice for particular infections (Reller, Weinstein *et al.*, 2009). The objective of the present study was to examine the associations between uterine bacterial infections with: The degrees of vaginal discharge, and the susceptibility of some antimicrobial drugs available in the Libyan markets.

## Materials and Methods

The present study was conducted on nine Holstein/Friesian cows maintained at Al-Gweaa Bovine Farm, about 60 kilometers east Tripoli, Libya. They had a history of repeat breeding (availed more than three services but failed to conceive). The cows had moderate body condition (score 2, according to Kellogg/University of Arkansas/Division of Agriculture, USA) aged between four and eight years. Throughout the experimental period, the cows were maintained as a group and were housed in semi-covered sheds under similar conditions of feeding and management. Three intramuscular injections of 150 microgram PGF2 alpha (Dalmazin, FATRO S.p.A.- Ozzano Emilia, Bologna, Italy), in two weeks intervals, were given intramuscularly for each of the nine cows after insuring that all of them had corpora lutea on their ovaries. Three days later after each injection, swabs from the lumen of the uterus were aseptically collected. At the same times, samples of uterine discharge were collected from the uterine lumen, with sterilized AI catheter wrapped with plastic sheath prepared especially for this purpose. The perineal area was washed with disinfectant and water. The sterilized covered catheter was inserted through the vulva, vagina and the cervix up to the body/horn of the uterus by holding the cervix through the rectum. The inner end of the catheter was pushed through the cover sheath to be open; and by using 50 ml syringe, aspiration of uterine content was collected then few drops from the discharge was taken by a sterile ready swab stick and embedded in its ready test tube media (Sterile Stuart Transport Swab; Pipe Star Ket/Hungary, and Firatmed Plastic Son. Tic. A.S/Turkey) and transferred directly to the laboratory of microbiology to be tested. The discharges were scored from 0-3 according to (Sheldon, Lewis *et al.*, 2006) (Figure:1).

## Endometritis scoring scheme



**Fig. 1:** Grading scheme for clinical uterine infections; uterine discharge character is graded as 0 (clear or translucent mucus), 1 (mucus containing flecks of white or off-white pus), 2(exudate containing, 50% white or off-white mucopurulent material), or 3(exudate containing, 50% purulent material, usually white or yellow but occasionally sanguineous). (Sheldon, Lewis *et al.*, 2006).

Bacteriological examination of the collected swabs was carried out in accordance with the standard microbiological techniques. Susceptibility to antibiotics was tested by the disc diffusion method on Mueller–Hinton agar medium. Ten different antibiotic discs were selected and placed over the surface of the agar plate. For this purpose, separate antibiotic discs (Antimicrobial Susceptibility TEST DISCS, ODOID, UK) containing Tetracycline (TE), Sulphamethazole (SXT), Gentamycin (GN), Penicillin G (PG), Amoxicillin (AML), Cloxacillin (OB), Streptomycin (S), Neomycin (N), Kanamycin (K), and Cephalexin (CL) per discs were employed. The anti-microgram pattern was determined according to the Kirby-Bauer procedure described by (Demissie 2011). The discs were placed 1.5 cm away from the edges of the plates and 3 cm away from each other by using discs dispenser. The plates

were then inverted and incubated aerobically at 37°C for 24 hours. The zones of inhibition of bacteria by the antibiotic discs were measured in millimeters using a caliper on the underside of the plates. The susceptibility of the bacteria was determined based on the break points recommended by the Clinical Laboratory Standards Institute (CLSI 2011).

### Results

Table 1 shows the susceptibility results. Most of the microorganisms, recovered from the tested samples, were susceptible to Gentamycin (77.8% with 4+ and 22.2% with 3+) which was followed by Cephalexin. Kanamycine was considered as third choice, as its susceptibility found to be with 3+ in 77.8% and 2+ in the other 22.2%.

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**Table 1:** The degrees of susceptibility to antibiotics routinely used in the Libyan markets.

Antibiotic	Susceptibility grades				
	++++	+++	++	+	R
Gentamycin	21 (77.8%)	6 (22.2%)	-	-	-
Cephalexin	3 (11.1%)	12 (44.4%)	9 (33.3%)	3 (11.1%)	-
Kanamycine	-	21 (77.8%)	6 (22.2%)	-	-
Amoxycillin	-	9 (33.3%)	18 (66.7%)	-	-
Neomycine	-	9 (33.3%)	15 (55.6%)	3 (11.1%)	-
Sulphamethazole	-	12 (44.4%)	9 (33.3%)	-	6 (22.2%)
Tetracycline	-	6 (22.2%)	6 (22.2%)	15(55.6%)	-
Streptomycine	-	3 (11.1%)	12 (44.4%)	9 (33.3%)	3 (11.1%)
Pencilline	-	-	3 (11.1%)	18 (66.7%)	6 (22.2%)
Cloxacillin	-	-	9 (33.3%)	3 (11.1%)	15 (55.6%)

The pathogens were susceptible to some other antibiotics (Amoxicillin, and Neomycin) at rates of only 33.3% with 3+, while the rest were with 2+ and 1+ Sulphamethazole was found to be effective in 21 samples (77.8%) with susceptibility between 3+ (44.4%) and 2+ (33.3%), but it was found to be resistant in the rest 22.2%. Tetracycline was less effective in comparison to the above mentioned antibiotics, while Streptomycin, Penicillin and Cloxacillin were the least effective (lowest susceptibilities were observed against them), with

resistant results at 11.1%, 22.2% and 55.6%, respectively. The scores for the characters of the uterine discharges were found between grades 2 and 3 in eight cows (88.8%). A fluctuation in the score was noticed between the successive samples (Table 2). The discharges were containing more than 50% whitish or yellowish mucopurulent or purulent materials; only one of the samples was sanguineous. The ninth cow had flecks of whitish pus within its mucoid vaginal discharge which is considered as score 1 type discharge.

**Table 2:** Scores of characters of uterine discharge, according to grades in Figure 1.

Cow number	Sample 1	Sample 2	Sample3
1	3	2	3
2	2	3	2
3	2	2	2
4	2	3	3
5	2	3	3
6	3	3	2
7	3	2	3
8	1	2	1
9	2	3	2

## Discussion

Uterine infections are considered as major problem that can affect the reproductive performances in cattle industry. Such infections would likely have been more severe effects on reproduction if the affected cows are not involved in a routine health program check, and were detected and treated early. To reduce the expected incidence of resistance development against pathogenic-specific antimicrobials, susceptibility testing is advised. The results of this study revealed that Gentamycin was the most effective antimicrobial against the infections of the uteri in the cows examined. This result is in agreement with (Takamtha, Phanaratkitti *et al.*, 2013) and also with (Malinowski, Lassa *et al.*, 2010) who found that *E. coli* is most susceptible to Gentamycin (88%) and some other antibiotics. Gentamycin is effective against many gram-negative bacteria, especially *Pseudomonas* species, as well as certain gram-positive bacteria, especially *Staphylococcus aureus*.

Cephalexin, in this study, came as a second most effective antibiotic followed by Kanamycin. This result is supported by (Pillar, Goby *et al.*, 2009) whom work was on evaluating the in vitro susceptibility of bovine mastitis pathogens to a combination of Kanamycin and Cephalexin which were commonly used in Europe for the treatment of mastitis in dairy cows. Amoxicillin and Neomycin showed moderate susceptibility in comparison to the above mentioned antibiotics, as still the result did not show any resistant reactions. (Fuquay, Harris *et al.*, 1975) did not recommend the use of neomycin sulfate boluses as a routine treatment to the cows, as their trials did not alter significantly the fertility of the infected cows.

Sulphamethazole work mainly against gram negative microorganisms (Moges, Regassa *et al.*, 2013). The susceptible resistance found in six samples (22.2%) in this study, could be due to excessive use of it in the field for general treatment and/or prophylactic of many diseases due to infections. Streptomycin and Penicillin were found to be the least effective antimicrobial within this study. In contrary (Smith, Donovan *et al.*, 1998) recorded good results in treating cows affected with uterine infections using procaine penicillin G.

The evaluation of the characters of uterine discharges reflect the severity of the infections in the uterus (Table 2). Eight of the nine infertile cows examined had uterine discharges characterised within scores 2 and 3, according to (Sheldon, Lewis *et al.*, 2006) scores. This result was similar to those results of work had been done in Taiwan where most of cows (82.8%) had vaginal discharges score 2 (Takamtha, Phanaratkitti *et al.*, 2013). Cows are more resistant to uterine infection during estrus, and as cows undergo more estrous cycles after parturition, the prevalence of endometritis diminishes. This has led to increased use of PGF2 $\alpha$  or its analogs, at usual luteolytic doses, for the management of endometritis, although there is little evidence that such use reduces the incidence of endometritis.

Within this study, it was clear that PGF2 $\alpha$  injection was not effective method to treat uterine infections. This is supported by (Hirsbrunner, Burkhardt *et al.*, 2006) as it has been demonstrated that the benefit of a prostaglandin therapy was not the consequence of luteolysis (Young, Anderson *et al.*, 1984), but the beneficial effect of PGF2 $\alpha$  administered post-partum is rather the result of myometrial contraction and there by accelerated uterine involution (Gustafsson 1984). (McDougall, de Boer *et al.*, 2013) reported that PGF2 $\alpha$  was found to be not statistically different from intrauterine antibiotic treatment in cows having corpus luteum in their ovaries. A general global rise in antibiotic resistance has been linked to an increased use of antibiotics (Fox 1997). In clinical veterinary practices antibiotics have long been used both prophylactically and as a treatment of endometritis (Kenney, Bergman *et al.*, 1976). In the present study, resistance to some commonly used antimicrobials was recorded. However, current antimicrobial use policies have to be taken into account to avoid further development of antibiotic resistance to reserve the strong antibiotics for the treatment of severe infections which have responded poorly to other classes of antimicrobials (Sulyok, Kreizinger *et al.*, 2014). Such information is useful for therapeutic treatment planning and antibiotic controls usage.

Combination of proper anti-microbial therapy and prostaglandins allows not only elimination of existing bacterial infections but also prevents

recurrence of the condition in the next cycle. The treatment of clinical endometritis remains a subject of considerable controversy in the literature. For more efficient diagnostic methods and a more efficient therapeutic approach, better understanding of the reproductive biology of normal versus abnormal uterine involution and immune mechanisms is needed (Lefebvre and Stock).

In conclusion, the results emphasize the necessity of periodic testing for antibiotic susceptibility in the state of Libya based on the *in vitro* examinations, which has been done in this work, Gentamycin could be the most effective drug for the therapy of uterine infections in Libya.

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### References

- Barlund CS, Carruthers TD, Waldner CL, Palmer CW (2008). "A comparison of diagnostic techniques for postpartum endometritis in dairy cattle." *Theriogenol.*, 69(6): 714-723.
- Demissie M (2011). "Isolation and identification of aerobic, septicemic bacteria from cattle in and around Sebata town and antimicrobial susceptibility testing." *Afr. J. Microbiol. Res.*, 5(1): 87-92.
- Fox J (1997). Antibiotic resistance on rise globally, AMER SOC MICROBIOLOGY 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171 USA.
- Fuquay JW, Harris RA, McGee WH, Beatty JF, Arnold BL (1975). "Routine postpartum treatment of dairy cattle with intrauterine neomycin sulfate boluses." *J. Dairy Sci.*, 58(9): 1367-1369.
- Galvão KN, Frajblat M, Brittin SB, Butler WR, Guard CL, Gilbert RO (2009). "Effect of prostaglandin F 2 $\alpha$  on subclinical endometritis and fertility in dairy cows." *J. Dairy Sci.*, 92(10): 4906-4913.
- Galvão KN, Greco LF, Vilela JM, Sá Filho MF, Santos JE (2009). "Effect of intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows." *Journal of dairy science* 92(4): 1532-1542.
- Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M (2005). "Prevalence of endometritis and its effects on reproductive performance of dairy cows." *Theriogenol.*, 64(9): 1879-1888.
- Goshen T, Shpigel NY (2006). "Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows." *Theriogenol.*, 66(9): 2210-2218.
- Gustafsson BK (1984). "Therapeutic strategies involving antimicrobial treatment of the uterus in large animals." *J. Am. Vet. Med. Assoc.*
- Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL (2006). "Neutrophil function and energy status in Holstein cows with uterine health disorders." *Vet. Immunol. Immunopathol.*, 113(1): 21-29.
- Hirsbrunner G, Burkhardt HW, Steiner A (2006). "Effects of a single administration of prostaglandin F2 $\alpha$ , or a combination of prostaglandin F2 $\alpha$  and prostaglandin E2, or placebo on fertility variables in dairy cows 3-5 weeks post partum, a randomized, double-blind clinical trial." *Reprod. Biol. Endocrinol.*, 4(1): 65.
- Huzzey JM, Veira DM, Weary DM, Von Keyserlingk MAG (2007). "Prepartum behavior and dry matter intake identify dairy cows at risk for metritis." *J. Dairy Sci.*, 90(7): 3220-3233.
- Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, Johnson WH (2004). "Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows." *Theriogenol.*, 62(1): 9-23.
- Kenney RM, Bergman RV, Cooper WL, Morse GW (1976). Minimal contamination techniques for breeding mares: technique and preliminary findings. Proceedings of the... annual convention.
- LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH (2002). "Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows." *J. Dairy Sci.*, 85(9): 2223-2236.
- Lefebvre RC, Stock AE (2012). "Therapeutic efficiency of antibiotics and prostaglandin F 2 $\alpha$  in postpartum dairy cows with clinical endometritis: An evidence-based evaluation." *Veterinary Clinics of North America: Food Anim. Pract.*, 28(1): 79-96.
- Lewis GS (1997). "Uterine health and disorders." *J. Dairy Sci.*, 80(5): 984-994.
- Malinowski E, Lassa H, Markiewicz H, Kaptur M, Nadolny M, Niewitecki WA, Zietara J (2010). "Sensitivity to antibiotics of *Arcanobacterium pyogenes* and *Escherichia coli* from the uteri of cows with metritis/endometritis." *The Vet. J.*, 187(2): 234-238.
- McDougall S, de Boer M, Compton C, LeBlanc SJ (2013). "Clinical trial of treatment programs for purulent vaginal discharge in lactating dairy cattle in New Zealand." *Theriogenol.*, 79(8): 1139-1145.
- McDougall S, Macaulay R, Compton C (2007). "Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle." *Anim. Reprod. Sci.*, 99(1): 9-23.
- Moges N, Regassa F, Yilma T, Unakal CG (2013). "Isolation and antimicrobial susceptibility of bacteria from dairy cows with clinical endometritis." *J. Reprod. Infertil.*, 4: 4-8.
- Noakes DE, Parkinson TJ, England GCW (2009). *Arthur's Veterinary Reproduction and Obstetrics*, Elsevier Health Sciences.

- Otero MAC, Nader-Macias MAE (2006). "Inhibition of *Staphylococcus aureus* by H<sub>2</sub>O<sub>2</sub>-producing *Lactobacillus gasseri* isolated from the vaginal tract of cattle." *Anim. Reprod. Sci.*, 96(1): 35-46.
- Pillar CM, Goby L, Draghi D, Grover P, Thornsberry C (2009). "Evaluating the in vitro susceptibility of bovine mastitis pathogens to a combination of kanamycin and cefalexin: Recommendations for a disk diffusion test." *J. dairy Sci.*, 92(12): 6217-6227.
- Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ (2009). "Antimicrobial susceptibility testing: a review of general principles and contemporary practices." *Clin. Infect. Dis.*, 49(11): 1749-1755.
- Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006). "Defining postpartum uterine disease in cattle." *Theriogenol.*, 65(8): 1516-1530.
- Smith BI, Donovan GA, Risco C, Littell R, Young C, Stanker LH, Elliott J (1998). "Comparison of various antibiotic treatments for cows diagnosed with toxic puerperal metritis." *J. dairy Sci.*, 81(6): 1555-1562.
- Sulyok KM, Kreizinger Z, Fekete L, Hrivnak V, Magyar T, Janosi S, Schweitzer N, Turcsanyi I, Makrai L, Erdalyi K (2014). "Antibiotic susceptibility profiles of *Mycoplasma bovis* strains isolated from cattle in Hungary, Central Europe." *BMC Vet. Res.*, 10(1): 256.
- Takamtha A, Phanaratkitti V, Adirekkiet O, Panyapornwitaya V, Boonyayatra S, Kraesusukol K (2013). "Prevalence of isolated bacteria from clinical endometritis uterine and antimicrobial susceptibility in postpartum dairy cows." *Chiang Mai Vet. J.*, 11(3): 237-245.
- Young IM, Anderson DB, Plenderleith RW (1984). "Increased conception rate in dairy cows after early post partum administration of prostaglandin F<sub>2</sub> alpha THAM." *Vet. Rec.*, 115(17): 429-431.