Journal of Veterinary Science & Technology

Abreham et al., J Vet Sci Technol 2017, 8:1
DOI: 10.4172/2157-7579.1000406

Research Article Open Access

Antibiotic Resistance of Aerobic Bacteria Isolated from Uteri of Slaughtered Cows in and around Addis Ababa, Ethiopia

Solomon Abreham[1]*, Merry Hailu[1], Ali Worku[1] and Solomon Tsegaye[2]

[1]*Veterinary Drugs and Feed Administration Control Authority, Addis Ababa, Ethiopia*
[2]*College of Agriculture, Woldia University, Woldia, Ethiopia*

Abstract

Uterine samples were collected from Addis Ababa abattoir with the objective of isolating aerobic bacteria and determine the antibiotic sensitivity profiles of the isolates. A total of 38 uteri of cows were collected and samples were processed for bacteriology and isolates identified by morphological, staining and biochemical tests. At least one species of bacteria was isolated from each uterine sample. The highest isolate found in this study was *Escherichia coli* (42.1%) followed by *Bacillus* species (17.1%), *Staphylococcus spp*. (15.7%), *Arcanobacterium pyogens* (7.9%) and *Streptococcus spp.* and *Proteus spp*. (3.9% each). Other isolates include *Corynebacterium, Rhodococcus equi, Klebsiela* and *Citrobacter*. Among all bacteria screened, sensitivities for selected drug were: chloramphenicol (98.6%), amikacin (90.8%), nitrofurantoin (82.9%) and trimethoprim-sulfamethoxasole (81.5%), tetracycline (71%) and polymixin-B (67.1%). Contrary to this, penicillin-G, methicillin and erythromycin showed highest resistance to the isolates identified respectively.

Keywords: Abattoir; Addis Ababa; Antibiotic sensitivity; Biochemical tests; Uterine bacteria

Introduction

Cattle production is the main component of livestock production in most sub-Saharan Africa farming systems [1]. Tropical African countries have been reported to have 147 million heads of cattle. However the current milk and meat production of these countries, including Ethiopia, is still low and relies on the import of livestock products from other sources [2].

Bovine uterine diseases is mainly caused by the contamination of uterus after parturition which contribute to a great economic losses due to longer calving intervals, lower conception rates and increased rate of culling. *E. coli* is the most relevant pathogenic bacteria involved in the puerperal uterine infection and persistence of uterine disease, it is resistant to many antibiotics and mostly have synergetic action with other uterine bacteria such as A. pyogenes [3].

Different part of genital organs infections in ruminant animals are often caused by opportunistic bacteria, especially *E. coli* species, majorly isolated from uterus of ewes, goats and cows. Fecal origin coliforms and non-specific bacteria are also opportunistic invaders of the reproductive tract. Genital infection resulting reproductive failure in ruminants can be caused by opportunist bacteria under stressful conditions [4].

After parturition, mostly three weeks later a diverse group of bacteria including *Bacillus* spp. *Trueperella pyogenes*, *Streptococcus uberis* and *Escherichia coli* can be frequently isolated from the cattle uterus. Many bacteria including those pathogens are known to cause bovine endometritis, but also other bacterial species invade cattle uterus after delivery. The uterine clearance is a unique process, during which a progression of bacteria and their interactions will result the occurrence of endometritis [5].

Much effort to improve the productivity of cattle by cross breeding local breeds with improved breeds has been implemented for long time in Ethiopia. However, the result of cross breeding was not successful related to several reproductive abnormalities. Due to less performance cows are culled and sent to abattoir for slaughter [6].

Antimicrobial agents (antibiotics) are used either to control infection or prevent disease progression. Antibiotic compounds like tetracyclines, sulfonamides, aminoglycosides, β-lactams and cephalosporins have been used together or in single for the treatment of postpartum metritis. Extensive use of antimicrobial agents in dairy cattle could result bacterial resistance, milk-residue effect and human health risk [7]. The antimicrobial susceptibilities of these isolates in tropical zones may vary; that limit the efficacy of the common antibiotic treatment protocols in use in these areas [4].

Naturally, specific and non-specific bovine uterine infections occur. Unlike the specific infections, the non-specific infections require a predisposing factor. Hence, different species of bacteria cause uterine infection under favorable conditions [8]. Even though, a variety of antibiotic have been used against uterine infections; the disease is gradually becoming refractory and incurable in Addis Ababa and its surroundings. Antibiotic resistance can result from different factors such as bacterial adaptations and faulty administrations. However, sensitivity tests on uterine bacterial isolates for different antibiotic preparations have rarely been conducted.

The objectives of this study were therefore:

To isolate the common aerobic bacteria from normal and infected uterus of cattle slaughtered in Addis Ababa Abattoir's Enterprise.

To determine Antibiotic susceptibility patterns of those aerobic bacterial isolates.

***Corresponding author:** Solomon Abreham, Veterinary Drugs and Feed Administration Control Authority, Addis Ababa, Ethiopia, Tel: +251335400706; E-mail: sollatta@gmail.com

Received November 28, 2016; **Accepted** December 12, 2016; **Published** January 02, 2017

Citation: 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. doi: 10.4172/2157-7579.1000406

Materials and Methods

Study area

The study was conducted from November 2004 to 2005. Uterine samples were obtained from Addis Ababa Abattoir's Enterprise, Addis Ababa. Bacteriological study was then conducted at Addis Ababa University, Faculty of Veterinary Medicine (FVM), Debrezeit, 47 km southeast of Addis Ababa. Addis Ababa was located at an altitude of 2408 meter above sea level. It has an average minimum and maximum temperature of 9.4°C and 32.2°C respectively. It receives an annual rainfall of 1200 mm.

Sampling methods

The study was conducted on 38 uterine samples with different physiological and pathological statuses as: apparently healthy, endometrities with pus, endometrities without pus and gravid uterus. The whole uterus was separated and opened aseptically with sterile scalpel blades and scissors and endometrial tissues of all apparently healthy and abnormal uteri were sampled from both right and left horns. Samples labeled and immediately transported, keeping the cold chain, to microbiology laboratory of FVM, Debrezeit.

Bacterial isolation and identification

Different laboratory procedures were adopted for bacterial isolation and identification according to different literatures [9-13].

The surface of samples was heated with hot scalpel blade and chopped with sterile blade on a sterile petridish. Chopped samples were first incubated in to brain-Heart-Infusion broth and incubated at 37°C overnight and then a loop full of the suspension was inoculated on 7% sheep-blood agar. After overnight incubation at 37°C, aerobically, representative colonies were isolated on another blood agar. Cellular morphology and Gram's reaction, from gram stained smears, and growth on MacConkey agar were then made from pure cultures and further biochemical tests were conducted.

The following biochemical tests were employed: Catalase test, using 3% H_2O_2, Oxidase test, using aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride, Motility test by SIM (Sulfide Indole Motility) medium and modified Hugh Liefson's medium was used for Oxidation-Fermentation test.

Further secondary biochemical tests were applied to identify the bacteria to species level. These tests include: Citrete utilization test, Voges-Proskauer test, Methyl Red test, Indole test, Urease test, Gas production test and also by inoculating on different medias like TSI (Triple Sugar Iron) agar, Manitol salt agar, Eosin- Methylene blue agar and Edward's media.

Antibiotic susceptibility tests

Agar disk diffusion (Kirby-Bauer) method was used as described in Quinn et al. [13] and Ikram [14] to determine the antibiotic susceptibility patterns of each bacterial isolates.

A suspension was made from an 18 hour pure culture colonies from nutrient agar in sterile distilled water using a sterile plastic loop. The inoculums density in the suspension was adjusted to 0.5 McFarland using a device called colorimeter, which detects turbidity by UV (Ultra Violet) light. The standardized bacteria suspension was inoculated on Mueller Hinton II agar plates, which has 4-5 mm thickness. For *Streptococci* and *Arcanobacterium pyogens*, the agar was supplemented with 5% sheep blood. The bacterial suspensions were inoculated on the agar plates with sterile cotton swabs. The plates were allowed to dry for at least 15 minutes. Then antibiotic discs were placed on the plates using sterile forceps.

The isolates were subjected to disc assay testes using, Penicillin-G (10 units), Tetracycline (30 µg), Erythromycin (5 µg), Amikacin (30 µg), Nitrofurantoin (300 µg), Methicilin (5 µg), Chloramphenicol (30 µg), polymixin-B (300 units), Trimethoprim-Sulfamethoxazole (1.25/23.75 µg). Regularly spaced antibiotics were placed on two different Mueller Hinton agar plates (4 discs on the first and 5 on the second plate) for each isolate using paper templates under these plates. The plates are then incubated at 37°C within 15 minutes after the discs were placed on the plates. Zone of inhibitions of the antibiotic discs were measured after 18-24 hours of incubation, using a transparent ruler to the nearest mm. The inhibition zone diameters were then interpreted as Resistance, Intermediate and Susceptible as described by Ikram [14].

Data analysis

Descriptive statistics were used to analyze the findings of this study. This includes: Bar-graphs, Tables and percentage descriptions.

Results

Gross uterine examination findings

A total of 38 uterine samples were collected. Gross examination on apparently healthy 21 (55.2%), endometritis 5 (13.1%), endometrities with pus 7 (18.4%) and gravid uterus 5 (13.1%) was done as indicated in Table 1.

Bacterial isolates

All uterine samples were positive for bacteria, 16 (42.1%) of which were mixed infections with two bacteria, 11 (28.9%) were mixed infection of three bacteria. *E. coli* was the most common isolated bacteria from 32 (84.2%), followed by *Bacillus* species from 13 (34.2%) uterus samples, Staphylococcus species from 12 (13.6%) and *Arcanobacterium pyogenes* from 6 (15.6%) uterus samples (Table 2).

Antimicrobial susceptibility profiles of bacterial isolates

As shown on Figure 1, isolates of all apparently healthy and abnormal uterine samples are commonly sensitive to chloramfenicol (98.6%), amikacine (90.8%), nitrofurantion (82.9%) and trimethoprim-sulfamethoxazole (81.5%); where as they were less sensitive to tetracycline (71%) and polymixin-B (67.1%). Relatively, many isolates show resistance for the rest of the antibiotics used on this study: penicillin-G (80.3%), methicillin (65.8%) and erythromycin (48.7%).

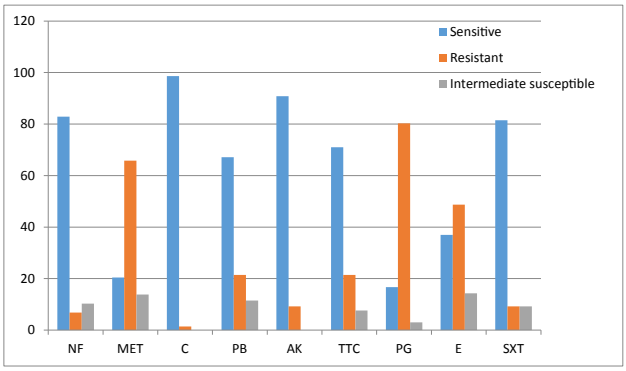
Resistance to one antibiotic was found in 5 (6.6%) isolates, for two antibiotics in 21 (27.6%) isolates and 47 isolates (61.8%) showed mixed resistance for at least three antibiotics. However, 3 isolates (3.9%) were found to be sensitive to all antibiotics in this study.

Antibiotic sensitivity of gram-negative isolates

As illustrated on Figure 2, all of the gram-negative isolates are sensitive to amikacin (100%) followed by chloramphenicol (97.4%),

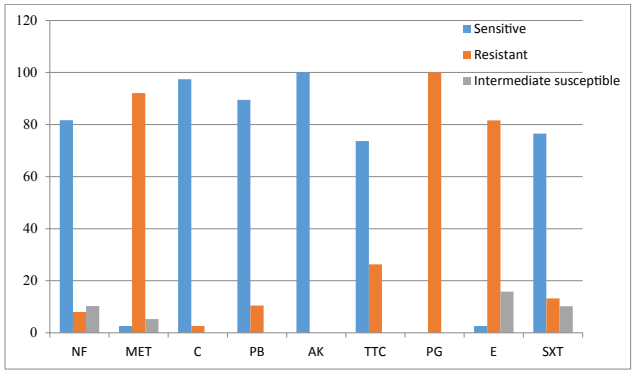
Types of Abnormality	Total examined	%
Apparently healthy	21	55.2
Endometritis	5	13.1
Endometritis with pus	7	18.4
Gravid uterus	5	13.1

Table 1: Different uterine status of cattle examined.



NF- Nitrifurantoin; MET- Methicillin; C- Chloramphenicol; PB- Polymixin-B; AK- Amikacin; TTC Tetracycline; PG- Penicillin; E- Erythromycin; SXT- Trimethoprim-Sulfamethoxazole.

Figure 1: Percentage sensitivity of bacterial isolates to different Antibiotics.



NF- Nitrifurantoin; MET- Methicillin; C- Chloramphenicol; PB- Polymixin-B; AK- Amikacin; TTC Tetracycline; PG- Penicillin; E- Erythromycin; SXT- Trimethoprim-Sulfamethoxazole

Figure 2: Sensitivity patterns of Gram -ve bacterial isolates.

polymixin-B (89.5%) and nitrofurantoin (81.65%) in that order. Meanwhile, they were resistant to penicillin-G (100%), methicillin (92.1%) and erythromycin (81.6%). Some resistance to other antibiotics was also seen on tetracycline (26.3%) and trimethoprim-sulfamethoxazole (13.2%).

Antibiotic sensitivity of gram-positive isolates

The sensitivity patterns of all gram-positive uterine isolates are illustrated in Figure 3. Among the antibiotics used in this study gram positive isolates were highly sensitive to Chloramphenicol (100%), Trimethoprim-sulfamethoxazole (86.8%), Nitrofurantoin (84.2%) and Amikacine (81.6%); and less sensitive to Erythromycin (73.7%), Tetracycline (68.4%) and Methicillin (55.3%). Complete resistance was seen on some isolates to, Penicillin-G (60.5%), Methicillin (39.5%) and Polymixin-B (34.2%). Resistance to Tetracycline, Amikacin and Erythromycin is observed to the extent of 23.7%, 18.4% and 15.8%, respectively.

Antibiotic resistance of uterine isolates

Antibiotic resistances of different bacterial isolates were tested in this study. *E. coli*, *Bacillus* species and *Corynebacterium* species 100% resisted Penicillin and different rates of resistance found as indicated in Table 3.

Discussion

Nonspecific infection of a bovine uterus relates to the endocrine state at time of infection. Even if massive invasion occurs, during estrus and parturition, infection is not established unless there is a serious problem. However, during diestrus and pregnancy, uterine infections are more likely to establish [15]. Common organisms in cattle, that can be isolated aerobically, are *Arcanobacterium* (Actinomyces) *pyogenes*, *E. coli, Staphylococci*, *Streptocci* and less frequently proteus species and others [15,16]. In the present study different types of bacterial isolates from endometrial tissue samples were identified. Moharana et al. [17] from India has also reported similar bacterial isolates from uterine mucus samples.

Among all the bacterial isolates *E. coli* was the most dominant bacterium followed by *Bacillus* Species and *Staphylococci*. This finding agrees with previous work done by Baishya et al. [18] in India, who reported *E. coli* was the most dominant uterine isolate (29.7%). The report also agrees with the findings in this study regarding the dominant species in the second order, which were *Bacillus* species, *Staphylococci* and *Streptococci* species.

Gram positive bacteria isolated in this particular study, there was multi-drug resistance to various levels. For *A. pyogenes* it was high, which was resistance to 8 of them. This organism shared higher resistance to Tetracycline, which was also seen on another similar study, on uterine isolates in Israel [19].

Likewise, significant levels of resistance were also found for one other important antibiotic (Tetracycline) in this study. Supporting the current study *E. coli* isolated from uterus was found to be resistant to Oxytetracycline and Sulfonamides [20]. Pejsak and Kolodziejcxyk [21] observed a similar resistance pattern against Teracycline on *E. coli* isolated from diseased lungs. According to Adegoke [22] resistance develops owing to the use of the drug as food additive and mass treatment in different outbreaks.

Penicillin-G is considered as an appropriate drug for systemic treatment of cows with endometritis. However, isolates of this study showed resistance to penicillin-G to a high level, which makes it ineffective for the treatment of uterine infections. Resistance to penicillin may be likely due to β-lactamase production. It is mentioned that, of veterinary isolates, 50-60% of Staphylococcus strains and 40-70% of *E. coli* strains are resistant to Penicillin–G due to similar effect [23]. Similarly, other studies on uterine isolates of cattle reported the resistance of these isolates against Penicillin-G [24,25].

Although Tetracycline has showed considerable resistance against isolates from this study, a study done by Cohen et al. [19], on *in-vitro* antibiotic sensitivity of uterine isolates from post parturient dairy cows, showed a high level of resistance of the isolates (*E. coli, A. pyogenes, Streptococci* and *Bacteroides* species) to tetracycline. It was suggested that, the use of the drug for treating post- parturient cows is inconsistent, in Israel. Resistances of uterine isolates against tetracycline were also mentioned by other authors [18,20]. Tetracycline resistance could result from mutation of plasmid mediated resistance [26]. But most commonly it is easily developed through indiscriminate drug usage.

Trimethoprim-sulfamethoxazole is the most recommended drug when compared to the other drugs tested in this study. It is better than Amikacin in that, its effect against anaerobic pathogens of uterus and resistant bacteria which could be isolated from uterus and development of resistance to this drug is not common to occur [23].

	No. of isolates (%)	Apparently healthy	Endometritis with pus	Endometritis without pus	Gravid uterus	Total Rate of isolation
E. coli	32 (42.1%)	21	2	4	3	84.2%
***Bacillus* species**	13 (17.1%)	7	3	3	-	34.2%
Staphylococcus aures	7 (9.2%)	2	3	1	1	18.45
- other staph.	5 (6.6%)	3	2	-	-	13.2%
A. pyogenes	6 (7.9%)	-	5	1	-	15.8%
Streptococci	3 (4%)	1	2	-	-	7.9%
***Corynebacterium* sp.**	2 (2.6%)	1	1	-	-	5.3%
Rhodococcus Equi	2 (2.6%)	1	-	-	1	5.3%
Proteus	3 (4%)	2	1	-	-	7.9%
Salmonella	1 (1.3%)	1	-	-	-	2.6%
Klebsiela	1 (1.3%)	1	-	-	-	2.6%
Citrobacter	1 (1.3%)	1	-	-	-	2.6%
Total	76 (100%)	41	19	9	6	

Table 2: Bacterial species isolated from different status of uteri from cows slaughtered.

Bacterial Isolate	Antibiotics applied								
	NF	**MET**	**C**	**PB**	**AK**	**TTC**	**PG**	**E**	**SXT**
E. coli	4 (2.4%)	31 (93.7%)	1 (3.1%)	2 (6.3%)	-	9 (28.2%)	32 (100%)	31 (93.7%)	9 (28.1%)
Bacillus Species	3 (23.1%)	10 (76.9%)	-	11 (84.6%)	-	3 (23.1%)	13 (100%)	5 (38.5%)	
Staphylococcus	-	1 (8.3%)	-	2 (16.7%)	-	5 (41.7)	6 (50%)	2 (16.7%)	2 (16.7%)
Arcanobacterium pyogenes	1 (16.7%)	3 (50%)	-	4 (66.7%)	4 (66.7)	2 (33.3%)	2 (33.3%)	(33.3%)	3 (50%)
Streptococcus	-	-	-	3 (100%)	3 (100%)	-	-	-	1 (33.3%)
Corynebacterium species	1 (50%)	2 (100%)	-	1 (50%)	-	-	2 (100%)	1 (50%)	-
Rhodococcus egui	1 (50%)	1 (50%)	-	-	-	-	1 (50%)	-	-
Other Enterobacteriacae	3 (50%)	6 (100%)	-	2 (33.3%)	-	1 (16.7%)	6 (100%)	6 (100%)	-

Tetracycline; PG- Penicillin; E- Erythromycin; SXT- Trimethoprim-Sulfamethoxazole.

Table 3: Antibiotic resistance (%) profiles of aerobic bacteria isolated.

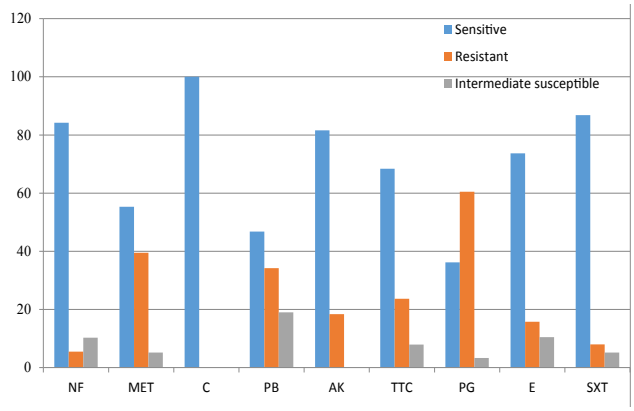


Figure 3: Sensitivity patterns of Gram +ve bacterial isolates.

The finding of this study, indicating the drugs as the most effective, agrees with studies by other authors. Prahland et al. [27] mentions Chloramphenicol and Nitrofurantoin being the most effective on uterine isolates. The study by Anjaneyulu et al. [24] shows Trimethoprim-sulfamethoxazole and Chloramphenicol are the most effective with levels of 69% and 62%, respectively. Uterine isolates in a study by Moharanan et al. [17] were highly sensitive to Chloramphenicol, whereas isolates of Rajangam et al. [25] shows highest sensitivity to Nitrofurantoin.

In conclusion, cattle uteri are free from bacteria due to several defensive factors. But all the sampled uteri harbor bacteria during isolation. *E. coli*, *Bacillus* species, *Staphilococcus, A. pyogenes* and *Streptococci*, which are environmental contaminants, were the dominant isolates from the uterus. Among these isolates *E. coli* and *A. pyogenes* found to be highly resistant. Tetracycline and penicillin-G are commonly available drugs on market in Ethiopia. However, the use of these drugs for uterine infections are not recommended, due to the resistance showed by the isolates and side effects, particularly by tetracycline after extensive use for many infectious diseases.

Conflict of Interest

No conflict of interest declared.

Acknowledgements

The authors would like to thank Faculty of Veterinary Medicine and Agriculture, Addis Ababa University and Addis Ababa Abattoir's Enterprise for their material and laboratory support.

References

1. Tekelye B, Kassali O (1988) Reproductive problems in cattle. International foundation for science. Joint seminar on Animal Reproduction for African countries, ILCA, Addis Ababa, Ethiopia, pp: 1-8.
2. Jahnke H (1982) Livestock production systems and livestock development in tropical Africa. Kielerwissen Chafts-VerlaVauk, Kiel, pp: 253.
3. Yang L, Wang Y, Peng Y, Min J, Hang S, et al. (2015) Genomic characterization and antimicrobial susceptibility of bovine intrauterine Escherichia coli and its relationship with postpartum uterine infections. J Integr Agr 15: 1-20.
4. Mshelia G, Bilal V, Maina1 V, Okon K, Mamza S, et al. (2014) Microbiological studies on genital infections in slaughtered ewes from tropical arid zone of Nigeria. Sokoto Journal of Veterinary Sciences. 12: 18-22.
5. Wagener K, Grunert T, Prunner I, Ehling-Schulz M, Drillich M (2014) Dynamics of uterine infections with Escherichia coli, Streptococcus uberis and Trueperella pyogenes in post-partum dairy cows and their association with clinical endometritis. Vet J 202: 527-532.
6. Singleton G, Dobson H (1995) A survey of the reasons for culling pregnant cows. Veterinary Record 136: 162-164.

7. El-Khadrawy H, Wahid M, Zaabal M, Emtenan M (2015) Strategies for diagnosis and treatment of uterine infection in bovines. Global Veterinaria 15: 98-105.
8. Arthur G, Noakes O, Person H (1989) Veterinary Reproduction and Obstetrics, 6th edn. Bailliere Tinelall, London, pp: 341-416.
9. Carter G (1984) Diagnostic procedures in veterinary bacteriology mycology, 4th edn. pp: 12-47.
10. Koneman E, Allen S, Dowell V, Janda W, Sommers H, et al. (1988) 3rd edn. J.B Lippincott Company, Philadelphia, pp: 311-390.
11. Baron E, Peterson L, Finegold S (1994) Bailey and Scott's Diagnostic Microbiology. 9th edn. Clanrinda company, USA, pp: 79-96.
12. Barrow G, Feltham R (1995) Cowan and steel's manual for identification of medical bacteria. 3rd edn. Cambrige University Press.
13. Quinn P, Carter M, Markey B, Carter G (1999) Clinical veterinary microbiology. Mosby International Limited, London, pp: 13-236.
14. Ikram M (2002) Diagnostic microbiology. In: Laboratory procedures for veterinary technicians. 4th edn. Mosby Publishing Company, pp: 105-115.
15. Hafez E, Jainudeen M (1993) Reproductive failure in females. Reproduction in Farm Animals. 6th edn. Lea & Febiger, Philadelphia, pp: 261-286.
16. Linda LS (2006) The Merck Veterinary Manual Online. 8th edn, Reference Reviews, pp: 40-49.
17. Moharanan H, Pradhan R, Patro D, Sahoo P, Nanda K, et al. (2000) Antibiotic sensitivity studies on repeat breeder crossbred sersey cows in coostal districts of Orissa. Indian Veterinary Journal 77: 809-810.
18. 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.
19. Cohen R, Ziv G, Winkler M, Saran A, Bernstein M, et al. (1997) Sensitivity to antibiotics of bacteria isolated from uterine secretion of dairy cows with retained fetal membranes and post parturient metrities and concentration of tetracycline in uterine secretions after intra uterine administration. Israel Journal of veterinary Medicine 52: 30.
20. Ziv G, Cohen R, Winkler M, Saran A (1996) Antimicrobial susceptibility of Escherichia coli and streptococcus species recovered from the uterus of dairy cows with post parturient metritis. Israel Journal of Veterinary Medicine 51: 63-66.
21. Pejsak Z, Kolodziejezyk P (2002) Incidence and drug sensitivity of bacteria isolated from diseased lungs in pigs. Medycyna Veterynaryjna 57: 480-485.
22. Adegoke G (1991) Antibiotic resistance in Staphylococci isolated from goats. Bulletin of Animal health. Production in Africa 29: 361-364.
23. Aiello S, Mays A (1998) Metritis in large animals, antibacterial agents. The Merck Veterinary Manual. 8th edn. Merck & Co Inc., White house station, USA, pp: 1016-1788.
24. Anjaneyulu Y, Wilson J, James R (1999) Antibiogram in bovine endometritis, a field studies. Indian Veterinary Journal 76: 351-352.
25. Rajangam R, Jeyaram N, Rajeswar J (2001) Treatment of repeated breeding crossbred cattle. Indian Journal of Animal Reproduction 22: 172 -173.
26. Haryh D, Zee Y (1999) Laboratory Diagnosis, Antimicrobial Drugs. Veterinary Microbiology, Blackwell Science Inc., pp: 15-50.
27. Prahland R, Nanda A, Arora A (1997) Bacteriology and therapeutics of placental retention in buffaloes (Bubalus bubalis). Indian veterinary Journal 74: 660-661.