ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA ISOLATED FROM THE GENITAL SYSTEM OF COWS IN AL-HILLA, IRAQ

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ABSTRACT. The aim of this study was to investigate the bacteria found in different parts of the genital system in cows and the susceptibility to different types of antibiotics. The genital systems of sixteen cows were collected from Al-Hilla, Iraq slaughterhouse. Isolation and identification of bacteria were made for each part of the genital system and antibiotic susceptibility tests was conducted to the isolated bacteria. The results of this study indicated that there were several types of bacteria present in the genital system sof cows. Different species of bacteria were isolated from the samples including, *Escherichia coli* (28.97)%, *Klebsiella* spp. (16.82)%, *Salmonella* spp. (14.95)%, *Proteus* spp. (13.08)%, *Staphylococcus aureus* (11.21)%, *Staphylococcus epidermidis* (8.41)% and *Streptococcus* spp. (6.54)%. *In vitro* susceptibility towards different types of antibiotic indicated high susceptibility of *Escherichia coli* to antibiotic group impenem and ciprofloxacin, while *Klebsiella* spp. was found to be most susceptible to ciprofloxacin and amikacin. Both *Escherichia coli* and *Klebsiella* spp. showed resistance to piperacillin and tetracycline. It was concluded that *Escherichia coli* was the most predominant bacteria in genital system of cows and were most susceptible to antibiotic impenem and ciprofloxacin.

Keywords: cow, genital system, *E. coli*, antibiotic susceptibility, ciprofloxacin

INTRODUCTION

Infection of uterus by different types of bacteria in cows affects on reproductive efficiency with decrease in milk yield and mainly affects on cost of treatment (Wang *et al*., 2013; Sheldon *et al*., 2006). Previous study by Al-jebori (2013) in Iraq reported that the percentage of pathological cases of uterus in cow consist about 29.4% distributed as either acute or subacute and also causes chronic inflammation, which represent as adenomyoma and fibroma. Endometritis, pyosalpinx, hemosalpinx, salpingitis, mucometra, pyometra, permetritis, parametritis, uterine abscess, these are the most pathological cases observed in genital systems in cows and buffalo cows (Azawi *et al*., 2008; Mansor and Majeed, 2005). Hameed *et al*. (2010) revealed that *Escherichia coli*, *Streptococcus* spp. and *Klebsiella* spp. were

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the most isolated bacteria from aborted fetuses of cows under stress due to other infection. Bacteria that cause inflammation of uterus have been considered as uterine pathogens, potential uterine pathogens or opportunistic contaminant bacteria which could affect ovarian activity (Sheldon *et al.,* 2002a; Williams *et al*., 2005; Williams *et al*., 2007). These bacteria include *Arcanobacterium pyogenes*, *Escherichia coli*, *Clostridium perfringens*, *Corynebacterium* spp., *Staphylococcus aureus*, *Streptococcus uberis*, *Proteus mirabilis*, *Pseudomonas aeroginosa*, *Klebsiella pneumonia* and *Bacillus* spp. (Dolezel *et al*., 2010; Udhayavel *et al*., 2013; Dini *et al*., 2012; Mshelia *et al*., 2012; Mshelia *et al*., 2014a; Šiugždaitė *et al.,* 2013 and Burfeind *et al*., 2014). Studies have also been carried out to investigate the susceptibility to antibiotics for bacteria responsible for infection of the genital system in domestic animals (Udhayavel *et al*., 2013; Mshelia *et al*., 2014b; Tel, 2011 and Silva *et al*., 2011). The aim of this study was to investigate the bacteria found in different parts of the genital system in cows and the susceptibility to different types of antibiotics.

MATERIALS AND METHODS

Collection of samples

The genital system of sixteen cows were collected from an Al-Hilla slaughterhouse in the period from October 2013 to April 2014. The collection consisted of 112 samples of different parts of the system including vagina, cervix, uterine body, uterine horns and oviducts. Each part was dissected using a surgical blade and the mucosae gently swabbed for bacteriological studies. Each swab was cultured immediately or stored in a transport medium until cultured.

Isolation and identification of bacteria

The culture media used for isolation and purification of bacteria included nutrient agar, blood agar, MacConkey agar, Mannitol salt agar, SS agar medium (for Salmonella) and Eosin methylene blue (EMBA) agar. Inoculated media were incubated aerobically at 37ºC for 24 hours.

All the isolates were stored in brain heart infusion broth with 15% glycerol at -20ºC until further use.

The bacteria isolates were identified by culture morphology and biochemical characteristics. For the culture characteristics, discrete colonies on the agar surface were observed. The shape, size, consistency and colour were studied. Gram-stained slides of the isolates were examined microscopically as a study of its cellular morphology. Biochemical tests were catalase, oxidase, IMVIC test (indol production, methyl red, vogas-proskauer and citrate utilization), TSI (triple sugar iron). Individually isolated colonies of the same morphology were inoculated on appropriate culture media prepared according to standard protocols as described by (Forbes *et al*., 2007).

Antibiotic susceptibility testing

Antimicrobial susceptibility tests of the isolates on antibiotics was determined by the disc diffusion technique on Muller Hinton agar using commercially available discs following CLSI guidelines (2010). Sterile swabs were used to inoculate the suspension by streaking on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay for 3-5 minutes. Sterile forceps were used to place the antimicrobial discs on the inoculated plates. The plate was incubated at 37°C for 18-24 hours within 30 minutes after applying the disc. The diameter of each zone of inhibition was measured in millimeters using a meter rule on the underside of the plate. The zone diameter of each isolate was compared with CLSI Published Limits and its chart was then used to interpret the zone sizes of inhibition. Results were recorded as susceptible, intermediate susceptible or resistant, based on the zone size of each antimicrobial disc used. The results were then interpreted according to CLSI documentation (CLSI, 2010).

RESULTS AND DISCUSSION

From the 112 samples collected, only 20 samples did not show any bacterial growth, while the other 92 samples showed growth of more than one bacteria. A total of 107 isolates were identified. 79 isolates (73.83%) were Gram-negative bacteria distributed as *Escherichia coli* (28.97)%, *Klebsiella* spp. (16.82)%, *Salmonella* spp. (14.95)% and *Proteus* spp. (13.08)%, while the remaining 28 isolates (26.16%) were Gram-positive bacteria identified as *Staphylococcus aureus* (11.21)%, *Staphylococcus epidermidis* (8.41)% and *Streptococcus* spp. (6.54)%. Table 1 shows the distribution of bacteria species in different parts of the genital system.

Out of the sixteen genital systems tested, only two cows were in pregnancy condition. The distribution of bacteria isolated from these samples were illustrated in Table 2. Pathological cases were found in the dissection of uterine horns and uterine body where inflammation, pus formation or degeneration of endometrium were observed.

E. coli and *Klebsiella* spp. isolates were subjected to antimicrobial sensitivity profile. Figure (1) showed that *E. coli* were highly sensitive to imipenem and ciprofloxacin, whereas *Klebsiella* spp. was highly sensitive to amikacin and ciprofloxacin. *E. coli* and *Klebsiella* spp. had different degrees of sensitivity to other antibiotics.

The results of this study indicated that there were several bacterial types present in a cow genital system which has no effect on the reproductive function. These observations are in accordance with many authors (Wang *et al.*, 2013; Mshelia *et al.*, 2014b; Otero *et al.*, 2000 and Al-Hilali and Al-Delemi, 2001) in cow and also in ewe (El-Arabi *et al.*, 2013 and Al-Zubaidi *et al.*, 2013).

The most predominant bacteria were *E. coli* and *Klebsiella* spp for which

Table 1. Bacteria distribution in different parts of the genital system of cows

Part	*Staphylococcus aureus*	*Streptococcus* spp.	*S. epidermid*	*E. Coli*	*Klebsiella* spp	*Proteus* spp	*Salmonella* spp.	**Total** (%)
Right Salpinx	4	-	-	3	2	2	3	14 (13.08)
Left Salpinx	2	1	-	7	-	3	1	14 (14.08)
R. Uterine horn	1	1	3	3	4	-	4	16 (14.95)
L. Uterine horn	2	2	1	4	2	3	3	17 (15.88)
Uterine body	1	1	2	-	3	1	2	10 (9.34)
Cervix	1	2	-	6	4	3	1	17 (15.88)
Vagina	1	-	3	8	3	2	2	19 (17.75)
Total (%)	12 (11.21)	7 (6.54)	9 (8.41)	31 (28.97)	18 (16.82)	14 (13.08)	16 (14.95)	107 (100)

Table 2. Distribution of bacteria in genital systems of pregnant cows

Part	*Staphylococcus aureus*	*Streptococcus* spp.	*S. epidermid*	*E. Coli*	*Klebsiella* spp	*Proteus* spp	*Salmonella* spp.	**Total** (%)
Right Salpinx	4	-	-	3	2	2	3	14 (13.08)
Left Salpinx	2	1	-	7	-	3	1	14 (14.08)
R. Uterine horn	1	1	3	3	4	-	4	16 (14.95)
L. Uterine horn	2	2	1	4	2	3	3	17 (15.88)
Uterine body	1	1	2	-	3	1	2	10 (9.34)
Cervix	1	2	-	6	4	3	1	17 (15.88)
Vagina	1	-	3	8	3	2	2	19 (17.75)
Total (%)	12 (11.21)	7 (6.54)	9 (8.41)	31 (28.97)	18 (16.82)	14 (13.08)	16 (14.95)	107 (100)

R.S. = Right Salpinx, L.S. = Left Salpinx, R.U.H. = Right Uterine horn, L.U.H. = Left Uterine horn, U.B. = Uterine body, C=Cervix, V=Vagina.

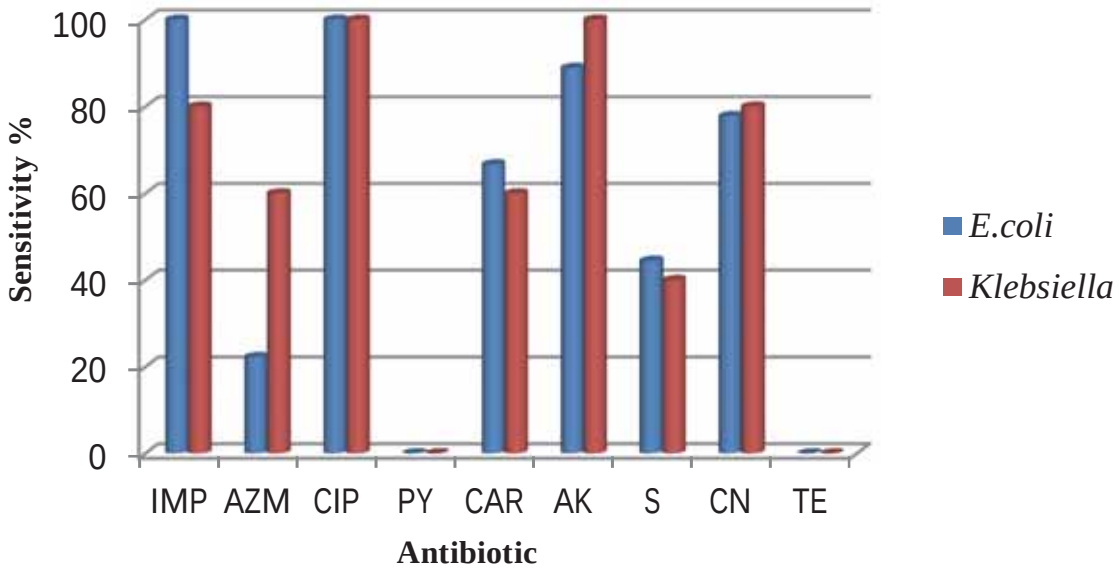

Figure 1. Antibiotics susceptibility patterns of most predominant bacteria (*E. coli* and *Klebsiella* spp.) isolated from the genital system of cows. IMP: imipenem, AZT : aztreonam, CIP: ciprofloxacin, PY: piperacillin, CAR: carbenicillin, AK: amikacin, S: streptomycin , CN: gentamicin, TE: tetracycline.

sensitivity test was made. Regarding *E. coli*, many authors reported it to be the predominant bacteria in cow, ewe and doe genital systems either as microflora or as a pathogen (Wang *et al*., 2013; Williams *et al*., 2005; Williams *et al*., 2007; Dolezel *et al*., 2010; Udhayavel *et al*., 2013; Dini *et al*., 2012; Mshelia *et al*., 2012; Mshelia *et al*., 2014a; Šiugždaitė *et al*., 2013; Otero *et al*., 2000; Sheldon *et al*., 2002b; Sheldon *et al*., 2009; Sheldon *et al*., 2010; Martins *et al*., 2009; Oliveira *et al*., 2013; Penna *et al*., 2013 and Mshelia *et al*., 2014b). Regarding *E. coli*, ciprofloxacin and imipeneme have been found to be the most sensitive among the battery of antibiotics used in the *in vitro* study. The sensitivity of *E. coli* to ciprofloxacin is in accordance to the finding by Mshelia *et al*., 2014a; Zinnah *et al*., 2008; Gani *et al*., 2008; Goncuoglu *et al*., 2010; Romanus *et al*., 2012 and Parul *et al*., 2014. However, Udhayavel *et al*. (2013) reported low sensitivity of *E. coli* to ciprofloxacin. None of the isolates were found to be sensitive to tetracycline. Moges *et al*. (2013) observed resistance of *E. coli to* tetracycline in reverse to *Klebsiella* spp. which is highly sensitive to tetracycline, whereas the sensitivity to gentamycin for *E. coli* and *Klebsiella* spp. related to this study. In the present study, *Klebsiella* spp. was found to be highly sensitive to

amikacin and ciprofloxacin. Udhayavel *et al*. (2013) demonestrated low sensitivity of *Klebsiella* spp. to ciprofloxacin. Rajeev *et al*. (2010) reported moderate sensitivity of *Klebsiella* spp. to amikacin.

REFERENCES

1. Al-Hilali, H.A & Al-Delemi, D.H. (2001). The uterine bacterial flora of normal reproductive tract, non-pregnant Iraqi cows. Vet., 11: 112-120.
2. Al-jebori, Kareem kadhim Radhi. (2013). Comparison study of pathophysiological conditions of genitalia in cows by rectal and pathological examination. Thesis Ms.C, College of Veterinary Med., Baghdad University, Iraq.
3. Al-Zubaidi, S. F., Hasson, S. O., Ajeel. (2013). Isolation and identification of microflora species at different levels of the ewe genital tract. Journal of Agriculture and Veterinary Science 6(3):54-57.
4. Azawi. O. I., A. J. Ali and E. H. Lazim. (2008). Pathological and anatomical abnormalities affecting buffalo cows reproductive tracts in Mosul. Iraqi Journal of Veterinary Sciences, Vol. 22, No. 2 (59-67).
5. Burfeind, O., Bruins, M., Bos, A., Sannmann, I., Voigtsberger, R. and Heuwieser, W. (2014). Diagnosis of acute puerperal metritis by electronic nose device analysis of vaginal discharge in dairy cows. Theriogenology Volume 82, Issue 1, Pages 64-70.
6. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). (2010). Performance standards for antimicrobial susceptibility testing : Seventeenth informational supplement.
7. 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. International Journal of Animal and Veterinary Advances 4(6): 358-362.
8. Dolezel, R., Palenik, T., Cech, S., Kohoutova, L., Vyskocil, M. (2010). Bacterial contamination of the uterus in cows with various clinical types of metritis and endometritis and use of hydrogen peroxide for intrauterine treatment. Veterinarni Medicina, 55 (10): 504–511.
9. El-Arabi, A. A., Taylor, D. J., Logue, D. N. and Benothman, M. (2013). Isolation and Identification of Bacterial Flora from Reproductive Tracts of Normal Ewes in Glasgow. J Vet Adv, 3(10): 275-280.
10. Forbes, B. A., Sahm, D. F., and Weissfeld, A. S. (2007). Bailey and Scotts' Diagnostic microbiology, 12th ed. Elsevier.
11. Gani, M.O., Amin, M.M., Alam, M. G. S., Kayesh, M. E. H., Karim, M.R., Samad, M. A. , Islam, M. R. (2008). Bacterial flora associated with repeat breeding and uterine infections in dairy cows. Bangladesh J Vet Med 6:79–86
12. Goncuoglu, M., Seda, F., Ormanci, B., Ayaz, N.D. and Erol, I. (2010). Antibiotic resistance of Escherichia coli O157:H7 isolated from cattle and sheep. Ann Microbiol 60:489–494.
13. Hameed H. A. Abdul, H. R. Abass, M. Y. Obeed. (2010). Study the incidence of abortion and retained placenta in pregnant cows with ephemeral fever and its relation with treatment and bacterial causes. AL-Qadisiya Journal of Veterinary Medicine Science, Vol. 9 No.2 (33-37).
14. Mansor A. R. and A. F. Majeed. (2005). Anatomical and pathological abnormalities of female genitalia of cows. (2005). Al-Anbar Journal of Agriculture Sciences, Vol. 3, No. 2 (141-151).
15. Martins, G., Figueira, L., Penna, B., Brandão, F., Varges, R., Vasconcelos, C. and Lilenbaum, W. (2009). Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progestin-impregnated intravaginal sponges. Small Ruminant Research. Volume 81, Issue 2 , Pages 182-184.
16. 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.
17. Mshelia, G. D., Abba, Y., Voltaire, Y. A. C., Akpojie, G., Mohammed, H., & Aondona, D. U. (2012). Comparative uterine bacteriology and pathology of camels (Camelus dromedarius) and cows in north-eastern Nigeria. Comp Clin Pathol DOI 10.1007/s00580-012-1549-8.
18. Mshelia, G. D., Bilal, V. T., Maina, V. A., Okon, K., Mamza, S. A., Peter, I. D. & Egwu, G. O. (2014a). Microbiological studies on genital infections in slaughtered ewes from tropical arid zone of Nigeria. Sokoto Journal of Veterinary Sciences, Volume 12 (Number 1).
19. Mshelia, G. D., Okpaje, G., Voltaire, Y. A. S. and Egwu, G. O. (2014b). Comparative studies on genital infections and antimicrobial susceptibility patterns of isolates from camels (Camelus dromedarius) and cows (Bos indicus) in Maiduguri, north-eastern Nigeria. SpringerPlus, 3:91.
20. Otero, C., Saavedra, L., Silva de Ruiz, C., Wilde, O., Holgado A. R. and Nader-MacõÂasb, M. E. (2000). Vaginal bacterial microflora modifications during the growth of healthy cows. Applied Microbiology, 31, 251-254.
21. Oliveira, J. K., Martins, G., Esteves, L. V., Penna, B., Hamond, C., Fonseca, J. F., Rodrigues, A. L., Brandão, F. Z. and Lilenbaum, W. (2013). Changes in the vaginal flora of goats following a short-term protocol of oestrus induction and synchronisation with intravaginal sponges as well as their antimicrobial sensitivity. Small Ruminant Research, volume 113, Issue 1 , Pages 162-166.

22. Parul, B. B., Sharma, B. and Jain, U. (2014). Virulence associated factors and antibiotic sensitivity pattern of Escherichia coli isolated fromcattle and soil, VeterinaryWorld 7(5): 369-372.
23. Penna, B., Libonati, H., Director, A., Sarzedas, A. C., Martins, G., Brandão, F. Z., Fonseca, J. and Lilenbaum, W. (2013). Progestin-impregnated intravaginal sponges for estrus induction and synchronization influences on goats vaginal flora and antimicrobial susceptibility. Animal Reproduction Science. volume 142, Issue 1 , Pages 71-74.
24. Rajeev, R., Gupta, M. K., Singh, S. and Kumar, S. (2010). Current trend of drug sensitivity in bovine mastitis. Veterinary World Vol.3, No.1, January.
25. Romanus, I. I., Chinyere, O.E., Amobi, N.E., Anthonia, O.E., Ngozi, A.F., Chidiebube, N.A. and Eze, A.T. (2012). Antimicrobial resistance of Escherichia coli isolated from animal and human clinical sample. Glo. Res. J. Microbiol. 2 (1):85-89.
26. Sheldon I.M., Noakes, D.E., Rycroft, A.N., Pfeiffer, D.U., Dobson, H. (2002a). Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction, 123:837–845.
27. Sheldon, I. M., Noakes, D. E., Rycroft, A. N., Dobson, H. (2002b). Effect of postpartum manual examination of the vagina on uterine bacterial contamination in cows. Veterinary Record 151, 531-534.
28. Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. (2006). Defining postpartum uterine disease in cattle. Theriogenology, 65:1516–1530.
29. Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G., & Schuberth, H-J. (2009). Defining Postpartum Uterine Disease and the Mechanisms of Infection and Immunity in the Female Reproductive Tract in Cattle. Biology of reproduction, 81, 1025–1032
30. Sheldon, I.M., Rycroft, A.N., Dogan, B., Craven, M., Bromfield, J.J., *et al*. (2010). Specific Strains of Escherichia coli Are Pathogenic for the Endometrium of Cattle and Cause Pelvic Inflammatory Disease in Cattle and Mice. PLoS ONE 5(2): e9192. doi:10.1371/journal.pone.0009192.
31. Silva, V.F., Damasceno, T.E.F., Souza, N.J.D., Franco, I. & Costa, M.M. (2011). Cervical-vaginal microbiota of crossbred sheep in Petrolina/PE, Brazil, and its susceptibility to antibiotics. Pesq. Vet. Bras. 31(7):586-590.
32. Šiugždaitė, J., Juodžentis, V., Petkevičius, S. (2013). Bacterial contamination of the uterus in different lactation cows on endometritis. Veterinarija ir zootechnika. T. 61 (83).
33. Tel, O. Y. (2011). Aerobic bacteria and their antibiotic susceptibility of sheep with vaginitis due to intravaginal sponges application. Etlik Vet Mikrobiyol Derg, 22, 7-10.
34. Udhayavel, S., Malmarugan, S., Palanisamy, K., and Rajeswar, J. (2013). Antibiogram pattern of bacteria causing endometritis in cows, Vet World 6(2): 100-102.
35. Wang, Y., Ametaj, B. N., Ambrose, D. J., & Gänzle, M. G. (2013). Characterisation of the bacterial microbiota of the vagina of dairy cows and isolation of pediocinproducing Pediococcus acidilactici. BMC Microbiology, 13:19.
36. Williams EJ, Fischer DP, Pfeiffer DU, England GCW, Noakes DE, Dobson H, Sheldon IM. (2005). Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology, 63:102–117.
37. Williams, E. J. , Fischer, D. P. , Noakes, D. E. , England, G.C.W., Rycroft, A., Dobson, H., Sheldon, I. M. (2007). The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68(4): 549–559.
38. Zinnah, M.A., Haque, M.H., Islam, M.T., Hossain, M.T., Bari, M.R., Babu, S.A.M., Rahman, M. T. and Islam, M.A. (2008). Drug sensitivity pattern of Escherichia coli isolated from samples of different biological and environmental sources. Bangl. J. Vet. Med. 6 (1): 13–18.

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