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Prevalence and antibiotic resistance pattern of certain types of bacterial flora in uterine ewe's samples

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Abstract

A study was carried out in abattoirs to identify certain bacterial flora in a number of samples collected from ewes' uteri with detection of phenotypic and genotypic antibiotic resistance patterns of the identified bacteria. The study was done in the South of Iraq during a period that started in February and ended in March 2015. Seventy-nine samples were collected randomly and aseptically, examined grossly, cultured using standard bacteriological techniques and examined for antibiotic resistance. Thirty-one isolates were reported belong to the following bacteria with resistance percentages accordingly: *Escherichia coli* 41. 94% (No: 13), *Klebsiella* spp. 29.03% (No: 9), *Enterobacter* spp. 16.13% (No: 5), *Pseudomonas aeruginosa* 6.45% (No: 2) and *Proteus* spp. 6.45% (No: 2). Results revealed that 100% (No: 31) of isolates were resistant to oxacillin while resistance to both ampicillin and tetracycline appeared in 93.64% (No: 30), 41.92% (No: 13) of isolates respectively, moreover there was for some extent resistance to ceftriaxone 9.68% (No: 3), while the isolates were highly susceptible to cefamandole and gentamicin. The isolates were also examined to determine the presence of bla_{SHV} genes by PCR assay which showed that 12.9% of isolates harbored this gene. This study contributes to a better knowledge about identified bacterial species inhabiting the uterus of ewes and exerting a significant and distinct antimicrobial resistance pattern.

Keywords: Flora; bla_{SHV} genes; Ewes; Uteri

1. Introduction

The normal uterus is a clean environment in contrast to the vagina, which contains numerous opportunistic secondary invaders that can invade uterus of ewes and behave in a different manner from normal vaginal flora to a pathogenic agents [1,2]. An effective control of postpartum contamination of the uterus provides the opportunity to improve both fertility and general health condition of ewes and other ruminant animals; however, the uterus of ruminants is usually contaminated with different microflora in the immediate postpartum period [3].

It has been reported that goats have many microflora in their genitalia and recorded that these microflora are usually harmless but certain predisposing factors such

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as trauma or another infection can drive them to a pathogenic state and disease causing agents [4].

There are also other pathogens which are harmless in normal situation but can be converted to a more pathogenic form when favorable conditions are available in the reproductive tract such bacteria as coliform bacteria and other non-specific bacteria under stressful conditions, may cause genital infection that usually leads to reproductive failure in dairy cows, sheep and goats [5,6,7]. Different types of bacteria have been isolated from the genitalia of the doe and these include (*Staphylococci*, *Streptococci*, *Actinomyces*, *Pseudomonas*, *Escherichia coli*, *Mycoplasma* and *Brucella*) species [8].

The importance of studying such microorganisms is related to diseases caused by them due to reduction of the immunity of the reproductive system [9]. Several studies mentioned that there were several types of bacteria found in the reproductive tract [10–13]. Several studies have been made in Iraq which focused on isolation of certain microorganisms behaving as an opportunistic bacteria in uteri of different animals [14–16].

Many researchers indicated that the reproductive system contains normal flora [17–21].

Beta-lactamases are enzymes that are major causes of bacterial resistance to the beta-lactam family of antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems. These enzymes catalyze the hydrolysis of the amide bond of four membered beta-lactam ring and render the antibiotic inactive against its original cellular target, the cell wall transpeptidase. On the basis of their primary structure, beta-lactamases are grouped into four classes A, B, C, and D enzymes. Enzymes of classes A, C, and D have serine at the active site, whereas the class B enzymes are zinc-metallo enzymes. Extended-spectrum beta-lactam antibiotics have widely been used for treatment of serious Gram-negative infections.

However, bacterial resistance has emerged due to production of extended-spectrum beta-lactamases (ESBLs). These enzymes are capable of hydrolyzing extended-spectrum beta-lactam antibiotics such as penicillins, cephalosporins along with a monobactam (aztreonam) but are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. ESBLs are derived from genes for the Narrow spectrum beta-lactamases (TEM-1, TEM-2, or SHV-1) by mutations that alter the amino acid configuration around the enzyme active site. They are typically encoded by plasmids that can be exchanged readily between bacterial species. These enzymes are most commonly produced by the members of the *Enterobacteriaceae*, especially *E. coli* and *Klebsiella*. To date, more than 350 different natural ESBL variants are known that have been classified into nine distinct structural and evolutionary families based upon their amino acid sequence comparisons such as TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA [22–25].

Modern biological techniques for detection of antibacterial resistance have been rarely applied to study of the distribution of resistance. This study aimed to identify certain flora resident in the uterus of ewes and study some phenotypic traits and occurrence rate of bla_{SHV} genes among the identified bacteria.

2. Materials and methods

2.1. Samples collection

A total of seventy-nine uteri were collected from ewes immediately after slaughtering, and instantly transported to the laboratory in sterile polythene bags. The uterine sample collection processes were compatible to standard techniques described [26,27]. The surface of the uterus was sterilized by shearing the uterine wall with a preheated surgical blade then the uterine wall was lanced with another sterile blade and a sterile swab stick was inserted and rolled over into the uterine lumen to collect bacteriological samples.

2.2. Isolation and identification

Samples were cultured on selective and differential MacConkey agar (Merck, Germany) and the isolates were identified based on their ability to ferment lactose. Cultural and morphological characteristics of colonies such as the shape, size, consistency and color and Gram's stain had been performed; moreover, the biochemical tests such as IMViC tests (indol production, Methylred, Voges-Proskauer and Citrate utilization), catalase, oxidase, phenyldeaminase and motility were performed by which different bacteria have been identified [28,29].

2.3. Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed using a disk diffusion method described by Bauer et al. (1966) [30] using Mueller-Hinton agar (Oxoid, U.K.). Six antibiotic disks containing Ampicillin (10 μg), Ceftriaxone (30 μg), Cefamandole (30 μg), Gentamycin (30 μg) Oxacillin (1 μg) and tetracycline (30 μg) have been used. The susceptibility breakpoints for all antimicrobials were recommended by Clinical and Laboratory Standards Institute CLSI [31].

2.4. Plasmidic DNA extraction and Polymerase Chain Reaction

Plasmidic DNA was extracted from bacterial cells using High-Speed Plasmid Mini Kit (Geneaid, South Korea) according to the manufacturer's instructions. Detection of antibiotic resistance-conferring genes was performed by amplifying the bla_{SHV} gene by PCR using the following primers pair: Forward: TCA GCG AAA AAC ACC TTG and Reverse: TCC CGC AGA TAA ATC ACC which were previously suggested by Ref. [32] with expected amplicon lengths of 471 bp. Amplification was performed in a Sure cycler 8800 (Agilent, USA), For PCR bla_{SHV} amplification cycle conditions included an initial denaturation cycle at high temperature 94 °C for 2 min, then 30 cycles (denaturation temperature 94 °C for 1 min, annealing temperature 58 °C for 1 min and elongation temperature 72 °C for 1 min) followed by final elongation temperature 72 °C for 7 min.

The concentration of reagents in PCR reaction mixture was 1X PCR Buffer, 2 mM of $MgCl_2$ (Invitrogen, USA), 0.4 μM of each forward and reverse primers, 0.2 mM of dNTP mix (Promega, USA), 0.04 U/μl of Taq polymerase (Invitrogen, USA) and 15 ng/μl of DNA template in a final volume of 25 μl.

2.5. Gel Documentation

The amplified PCR product was separated on a 1% agarose gel and visualized with a UV transilluminator after ethidium bromide (0.5 μg/ml) staining. Gels were electrophoresed in 1X TBE buffer at constant voltage of 75 volts for 90 min. The sizes of the DNA fragments were measured according to 100 bp DNA ladder (Geneaid, South Korea) The gel was visualized and photographed under UV light using a digital imaging system.

2.6. Statistical procedure

The statistical analysis was performed by using the Pearson Chi-square test and the analysis of variance (ANOVA). Data were analyzed using SPSS 17.0 (Chicago, IL, USA). Furthermore, the differences were significant at ($P < 0.05$).

3. Results

3.1. Isolates identification rates

The results revealed that 31 isolates were identified and distributed as following: *E. coli* was found to be the most common identified microflora, with occurrence of 13 (41.94%) followed by *Klebsiella* spp. 9 (29.03%), *Enterobacter* spp. with 5 (16.13%), *Pseudomonas aeruginosa* were 2 (6.45%) and *Proteus* spp. with 2 (6.45%) as shown in Table 1 and Fig. 1.

3.2. Antibiotic susceptibility profile

As shown in Table 2 and Fig. 2 susceptibility of identified bacteria by disk diffusion test reported less activity of oxacillin and ampicillin with 31 (100%) and 30 (96.77%) resistant to these antibiotics, respectively, followed by tetracycline 13 (41.94%). The resistance to ceftriaxone was recorded in 3 (9.68%) of isolates while the most active antibiotics against isolates were cefamandole and Gentamicin with 0 (0%) of resistance was reported.

3.3. The result of molecular study

The result of molecular study revealed that 12.9% of isolates were carried bla_{SHV} genes where there was a high rate of recovered genes detected in *E. coli* (6.45%) (No = 2) followed by *Klebsiella* spp. (3.225%) (No = 1) and *Enterobacter* spp. (3.225%) (No = 1) as shown in Fig. 3.

4. Discussion

The normal microbial flora of the uterus develops as the animal encounters and responds to a number of environmental and physiological conditions, moreover opportunistic secondary invaders from the vagina frequently attack the uterus during the peripartum period causing metritis, endometritis and subsequent reduction in the reproductive capacities [33,34].

Table 1
Identification rates of bacterial flora.

Identified bacteria	No. of isolates for each identified bacteria	No. of isolates	% of isolates
E. coli	Ec1, 2, 5, 8, 10, 11, 15, 17, 19, 24, 25, 28, 31	13	(41.94)
Klebsiella spp.	K4, 6, 12, 16, 18, 22, 26, 29, 30	9	(29.03)
Enterobacter spp.	E3, 9, 14, 21, 27	5	(16.13)
Pseudomonas aeruginosa	Ps7, 23	2	(6.45)
Proteus spp.	P13, 20	2	(6.45)
Total	31	31	100

Ec = *E. coli*; K = *Klebsiella*; E = *Enterobacter*; Ps = *Pseudomonas aeruginosa*; P = *Proteus*.

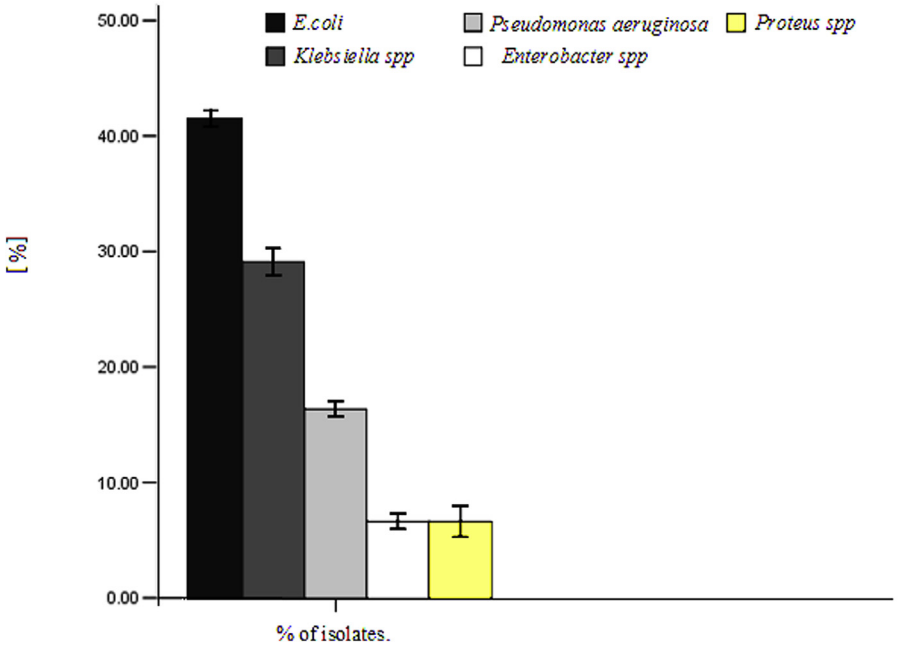

Fig. 1. Identification rates of bacterial flora isolates. All results are presented as means ± SD, statistically significant at the level ($P < 0.05$).

Table 2
Antibiotic susceptibility profile of identified bacterial flora.

Antibiotic	No. and % of susceptible	Identified bacteria with susceptible pattern	No. and % of intermediate	Identified bacteria with intermediate pattern	No. and % of resistant	Identified bacteria with resistant pattern
Ampicillin (AMP)	0 (0)	Null	1 (3.23)	Ps 21	30 (96.77)	All except Ps 21
Cefamandole (CFM)	31 (100)	All	0 (0)	Null	0 (0)	Null
Ceftriaxone (CRO)	28 (90.32)	All except Ec1, 10, K29	0 (0)	Null	3 (9.68)	Ec1, 10, K29
Gentamicin (CN)	28 (90.32)	All except Ec10, 25, K12	3 (9.68)	Ec10, 25,K12	0 (0)	Null
Oxacillin (OX)	0 (0)	Null	0 (0)	Null	31 (100)	All
Tetracycline (TET)	13 (41.94)	Ec2, 3, 15, 17, 19, 24, 28, 31, K12, 16, 22, 30, P20	5 (16.13)	Ec5, 15, 25, K22, 26	13 (41.94)	Ec1, 8, 10, 11, K4, 6, 29, E3, 9, 14, 21, 27, P13

Bacterial identification is thus an important part for providing remedial interventions that will restore fertility.

The Iraqi ewes were studied by the researches [26,35] who found that most of ewes' reproductive systems contain normal flora, while in the goat as reported by Refs. [36,37] non specific infections appear to play a role in causing infertility in does.

The result of this study confirmed prevalence of Gram negative bacterial species in the uterus of slaughtered sheep including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa*. and *Proteus* spp. which is also in accordance with previous studies [38]. A similar study was conducted in Al-Hillah city by Ref. [39] to isolate and identify bacterial flora from vagina in normal ewes (slaughtered and while live) where they found that *E. coli* was the predominant isolated bacteria followed by *Proteus mirabilis* and *Klebsiella pneumoniae*. Other studies have reported that *Bacillus* spp., *Corynebacterium* spp., *Escherichia* spp., *Staphylococcus* spp., and *Streptococcus* spp. are commonly isolated from the ewes' vagina. On the other hand [41] explained that staphylococcus was most predominant identified bacteria followed by bacillus, *E. coli*, and Pseudomonas [40], another study [42] showed that *E. coli*, *Arcanobacterium pyogenes*, *Staphylococci* and *Streptococci* spp. were the most dominant bacteria in the uteri of ewes [41]. Other

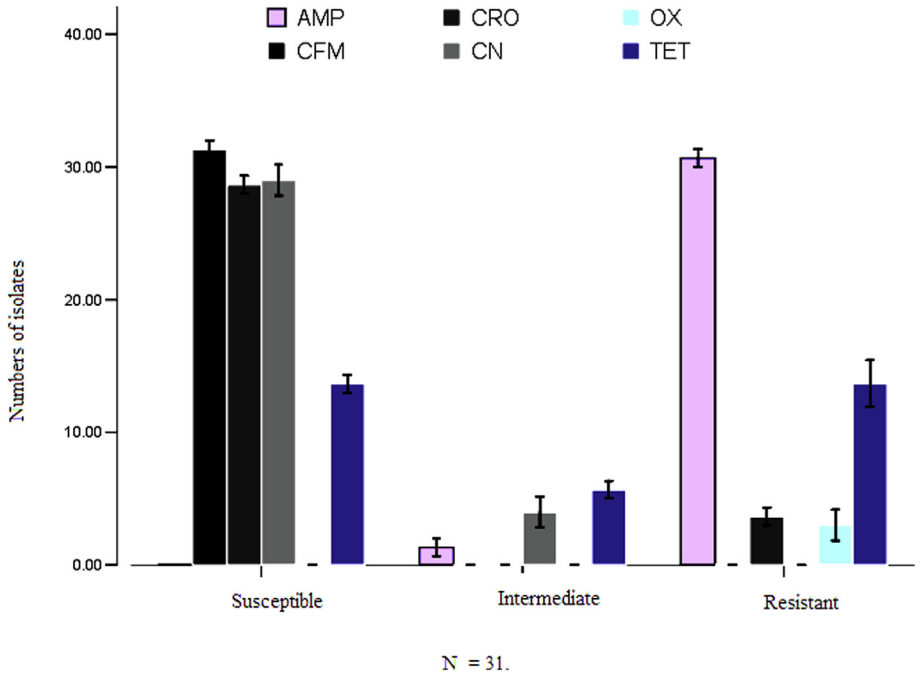

Fig. 2. Antibiotic susceptibility profile of identified bacterial flora. All results are presented as means ± SD, statistically significant at the level ($P < 0.05$).

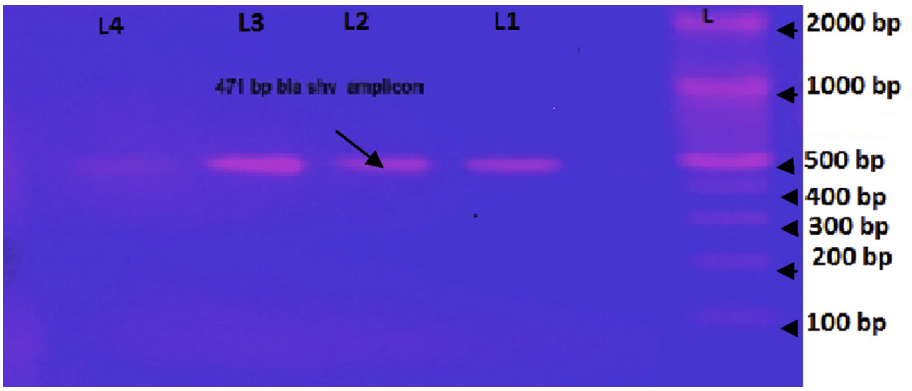

Fig. 3. The electrophoresis diagram of bla_{SHV} PCR amplicon 471 bp. DNA ladder L (100 bp). Lane 1 and 2 represent the bla_{SHV} genes from Ec1, 10; Lane 3 and 4 represent the bla_{SHV} genes from K29 and E9. The electrophoresis was performed at 75 volts for 90 min, agarose was stained with ethidium bromide.

reports have indicated that *E. coli* and *Staphylococcus aureus* are the most commonly isolated bacteria from slaughtered ewes in Nigeria [42].

Beta-lactam antimicrobial agents are considered first-line agents in treating bacterial infections however resistance to β-lactam antibiotics among Gram negative bacteria has proven to be tough challenge for researchers and clinicians. Considering the results of antibiotic resistance pattern to bacterial isolates, cefamandole and gentamicin exhibited very good activity, with 0% of resistance, where rates of resistance in the range of (9.68%) for Ceftriaxone and 43.75%, for tetracycline have been described. In general ampicillin and oxacillin had the less activity against isolates with resistance reached to 100% and 96.87%, respectively. Similar, observations were recorded earlier by others [43].

BlaSHV are considered common β-lactamases detected in clinical isolates of *E. coli* and *Klebsiella* spp. predisposing animals to genitourinary tract

infections [44]. In the present study, 12.9% bacterial flora harbored bla_{SHV} genes with high rate of occurrence in *E. coli* followed by *Klebsiella* spp. and *Enterobacter* spp.

In a study of a collection of *E. coli* isolates recovered from hospitalized animals in Ireland results showed that bla_{SHV} genes were always accompanied by bla_{TEM} and 4 of 5 isolates that were positive for both of these genes were resistant to ampicillin, cefpodoxime, ceftiofur, and cephalothin [45].

Antimicrobial use in production animals has been shown to lead to the emergence of resistant bacteria. The use of low doses of antibiotics by the modern food animal industry as growth-promoting substances in farm animals to promote animal growth and to prevent infections rather than cure infections is responsible for drug-resistant bacteria emerging on farms which reach the general population through human or animal carriers, and through the food consumers eat [46]. Also the misuse and overuse of broad-spectrum antibiotics, mainly cephalosporins, must be contributing to selection and spread of ESBL-producing Enterobacteriaceae in animals.

Gram-negative bacteria (GNB) that produce extended-spectrum β-lactamases (ESBLs) have become a common problem in veterinary medicine across the world. These enzymes have been identified in a wide range of Enterobacteriaceae, including *K. pneumoniae*, *Klebsiella oxytoca*, *E. coli*, *P. mirabilis*, *Enterobacter cloacae*, *Morganella morganii*, *Serratia* spp., *Shigella* spp., *Citrobacter* spp., and *Salmonella* species [47,48] around the world which strongly agreed with the results of this study which were supported by results of bacterial flora identification and antibiotic phenotypic and genotypic resistance patterns.

While acquisition of ESBLs confers resistance to penicillins, cephalosporins and monobactams, isolates remain susceptible to carbapenems, and *in vitro*, these enzymes are inhibited by beta-lactamase inhibitors, such as clavulanic acid, sulbactam, and tazobactam [49] this might be due to the fact that these genes encoding for these enzymes including bla_{SHV} genes carried on conjugative plasmid play role in acquisition of resistance by horizontal gene transfer.

ESBL-encoding genes are often carried on plasmids, which can easily be transferred between isolates, bearing additional resistance determinants for other classes of antimicrobial agents, mainly fluoroquinolones, aminoglycosides and sulfonamides, contributing to the multidrug-resistant phenotype [50].

Most reports of ESBL positive GNB isolated from pets come from Europe. In 1998, for the first time a resistant *E. coli* strain was isolated from a urine specimen from a dog with a recurrent urinary tract infection (UTI), in Madrid, Spain. This strain showed resistance to amoxicillin, cephalothin, cefotaxime, ceftazidime, and aztreonam and the ESBL enzyme was confirmed as SHV-12 variant [51].

In the United States, the largest threat from ESBLs has come from *Salmonella* spp. and *E. coli*. In 2007 [52] found *Salmonella enterica* harboring CTX-M, SHV, TEM and CMY-2 β-lactamases. From 1999 to 2003, 34,411 *Salmonella* spp. strains were isolated from cattle, birds, horses and dogs and the proportion of ceftiofur resistant isolates for each *Salmonella* serotype varied widely.

ESBLs (SHV-12-type) in Australia have been identified in *Enterobacter* spp. isolated from dogs with opportunistic infections (i.e., UTIs, post-surgery infection, osteomyelitis and multiple abscess) [53].

The first report of ESBL was published by Ref. [54], where *S. enterica* serovar Newport expressing TEM-1B and SHV-12 was isolated from affected animals during an outbreak of salmonellosis that led to a 3-month closure of one of the largest equine hospitals in the United States.

The first reports of ESBL-producing bacteria in poultry were performed in Europe. In this regard, in Spain, *E. coli* strains isolated from faecal samples of healthy and sick poultry were found to harbor *bla*CTX-M-14, *bla*CTX-M-9, or *bla*SHV-12 [55].

Standard microbiological procedures can take up to several days for culture, isolation and characterization and many comparative studies have shown that PCR-based methods have higher sensitivity [56,57], mostly due to variable levels of gene expression. Therefore, PCR and nucleotide sequence analysis [58], together with various PCR-based methods, remain the gold standard for extended-spectrum b-lactamase SHV-variants identification.

5. Conclusion

It was concluded that *E. coli*, *Enterobacter* spp. *Klebsiella* spp. *P. aeruginosa and Proteus* spp. were the most common gram negative members of the normal flora in uterine ewe's samples. These organisms were resistant to different antibiotics and there was high rate of resistance to bla_{SHV} genes carried by identified bacteria. Further investigations are needed to reveal antibiotic sensitivity pattern of the isolates

which was of significant interest and was alarming for the public health sector in Iraq.

References

[1] J. Manes, M.A. Fiorentino, G. Kaiser, F. Hozbor, R. Alberio, E. Sanchez, F. Paolicchi, Changes in the aerobic vaginal flora after treatment with different intravaginal devices in ewes, Small Rumin. Res. 94 (2010) 201–204.

[2] N.D. Sargison, F. Howie, R. Mearns, C.D. Penny, G. Foster, Shiga toxin-producing *Escherichia coli* as a perennial cause of abortion in a closed flock of Suffolk ewes, Vet. Rec. 160 (25) (2007) 875–876.

[3] B. Hafez, E.S.E. Hafez, Reproduction in Farm Animals, seventh ed., Lippincott Williams and Wilkins, USA, 2000, pp. 13–29.

[4] E. Atwa, F. Rady, Bacteria and fungi associated with abortion in sheep and goat in Menoufia Governorate, Assiut Vet. Med. J. 53 (2007) 326–349.

[5] W.E. Levinson, E. Jawetz, Medical Microbiology and Immunology, third ed., Prentice-Hall Int. Inc., Engle Wood Cliffs, New Jersey-USA, 1994, pp. 20–23.

[6] D.C. Hirsh, The genital tract as a microbial habitat, Rev. Vet. Microb. (1990) 243–244.

[7] A.A. Shallali, A.M. Hussein, M.M. Salih, E.A. Dafalla, A preliminary report on bacteria isolated from the female genital tract of sudanese sheep and goats, Sudan J. Vet. Res. 17 (1) (2001) 55–63.

[8] A.M. Ababneh, T. Degefa, Bacteriological findings and hormonal profiles in the post-partum balady goats, J. Rep. Domes. Anim. 4 (1) (2007) 12–16.

[9] S.Y. Al-Dahash, M.A. Fathalla, Veterinary Obstetrics, second ed., Book House for Printing and Publishing. Al-Mosul University, 2000, pp. 29–31.

[10] L. Elliott, K.J. McMahon, H.T. Gier, G.B. Marion, Uterus of the cow after parturition: bacterial content, Am. J. Vet. Res. 29 (1) (1968) 77–81.

[11] E. Studer, D.A. Morrow, Postpartum evaluation of reproductive potential: comparison of findings from genital tract examination per rectum, uterine culture and endometrial biopsy, J. Am. Vet. Med. Ass. 172 (4) (1978) 489–494.

[12] S. Messier, R. Higgins, Y. Couture, M. Morin, Comparison of swabbing and biopsy for studying the flora of the bovine uterus, Can. Vet. J. 25 (1984) 283–288.

[13] D.E. Noakes, L. Wallacce, G.R. Smith, Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms, Vet. Rec. 11 (128) (1991) 440–442.

[14] O.I. Azawi, J.B. Ali, D.S. Ali, Non-specific genital tract microflora of Iraqi local breed cows, Iraqi J. Vet. Sci. 8 (2) (1995) 261–265.

[15] H.A. Al-Hilali, D.H. Al-Delemi, The uterine bacterial flora of normal reproductive tract, non-pregnant Iraqi cows, Veterinarian 11 (1) (2001) 112–120.

[16] D.H.J. Al-Delimi, The uterine bacterial flora of reproductive tract of the Iraqi she-camels, Al-Qadisiya J. Vet. Med. Sci. 1 (2) (2002) 55–59.

[17] J.F.T. Griffin, P.J. Hartigan, W.R. Nunn, Non-specific uterine infection and bovine fertility I. Infection patterns and endometritis during the first seven weeks post-partum, Theriogenology 1 (3) (1974) 91–106.

[18] B.L. William, L.R. Senger, A.C.S. Ward, Relationships between days post partum, observed estrus and uterine microflora in commercial dairy cows, Theriogenology 30 (3) (1988) 555–561.

[19] A.M. Hussain, R.C.W. Daniel, D.O. Boyle, Postpartum uterine flora following normal and abnormal puerperium in cows, Theriogenology 34 (2) (1990) 291–302.

[20] M. Bekana, P. Jonsson, J. Ekman, H. Kindahi, Intra uterine bacterial findings in post-partum cows retained fetal membranes, Zentralbl. Veterinarmed. 41 (9) (1994) 663–670.

[21] E.B. Torres, T. Nakao, T. Hiramune, M. Moriyoshi, K. Kawata, K. Nakada, Streptococcus and uterine bacterial flora in dairy cows following clinical normal and abnormal puerperium, J. Reprod. Dev. 43 (1997) 157–163.

[22] J. Sharma, M. Sharma, P. Ray, Detection of TEM and SHV genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital from India, Indian J. Med. Res. 132 (2010) 332–336.

[23] U. Naseer, A. Sundsfjord, The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones, Microb. Drug Resist. 17 (2011) 83–97.

[24] M. Shahid, A. Singh, F. Sobia, M. Rashid, A. Malik, I. Shukla, et al., Bla (CTX-M), bla (TEM), and bla (SHV) in *Enterobacteriaceae* from North-Indian tertiary hospital: high occurrence of combination genes, Asian Pac. J. Trop. Med. 4 (2011) 101–105.

[25] F. Eftekhar, M. Rastegar, M. Golalipoor, N. Mansour Samaei, Detection mof extended spectrum beta-lactamases in urinary isolates of *Klebsiella pneumonia* in relation to BlaSHV, BlaTEM, BlaCTX-M gene carriage, Iran. J. Public Health 41 (2012) 127–132.

[26] D.M. Aziz, M.A.H. Al-Sultan, E.A.K. Al-Jawally, Uterine microflora in Awassi ewes, Iraqi J. Vet. Sci. 13 (1) (2000) 201–205.

[27] T.M. Al-Hamedawi, D.J. Khammas, A.S. Ai-Ubaidi, Effect of estrus synchronization on vaginal flora and subsequent fertility in ewes, Iraqi J. Vet. Sci. 16 (1) (2002) 73–79.

[28] Bergey's Manual of Systematic Bacteriology, Williams and Wilkins, Baltimore, 1994.

[29] J.J. Farmer, Enterobacteriaceae: introduction and identification, in: P.R. Murray, E.J. Baron, M.A. Phaler, F.C. Tenover, R.H. Yolken (Eds.), Manual of Clinical Microbiology, seventh ed., ASM Press, Washington, D.C., 1999.

[30] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Pathol. 45 (4) (1966) 493–496.

[31] Performance Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute Antimicrobial Susceptibility Testing Standards(CLSI) M02-a12, M07-a10, and M11-a8, twenty sixth ed., 2016.

[32] P. Lai, A. Kapil, B.K. Das, S. Sood, Occurrence of TEM and SHV gene in extended spectrum beta-lactamases (ESBLs) producing *Klebsiella* sp. isolated from a tertiary care hospital, Indian J. Med. Res. 125 (2) (2007) 173–178.

[33] J.D. Ambrose, S.R. Pattabiraman, R.A. Venkatesan, Types and incidence of aerobic bacteria in different puerperal conditions in bovines, Cheiron 15 (1986) 176–179.

[34] Yvonne Wang, B.N. Ametaj, D.J. Ambrose, M.G. Gänzle, Characterization of the bacterial microbiota of the vagina of dairy cows and isolation of pediocin-producing Pediococcus acidilactici, BMC Microbiol. 13 (2013) 19.

[35] D.H.J. Al-Delemi, The normal bacterial flora in the vaginal cavity of Iraqi cows, sheeps, goats and camels during the luteal phase, Al-Qadisiya J. Vet. Med. Sci. 4 (1) (2005) 23–29.

[36] S.J. Miller, C.K.S.V. Raja, Infertility in sheep and goats, in: C.R. Sane, S.N. Luktuke, B.R. Deshpande, A.S. Kaikini, D.P. Velhankar, V.B. Hukeri, S.B. Kodagali (Eds.), Reproduction in Farm Animals (Theriogenology), Varghese Publishing House, Bombay, 1982, pp. 371–382.

[37] G.H. Arthur, D.E. Noakes, H. Pearson, T.J. Parkinson, Veterinary Reproduction and Obstetrics, seventh ed., W.B. Saunders Company, 1996.

[38] G.D. Mshelia, V.T. Bilal, V.A. Maina, K. Okon, S.A. Mamza, I.D. Peter, G.O. Egwu, Microbiological studies on genital infections in slaughtered ewes from tropical arid zone of Nigeria, Sokoto J. Vet. Sci. 12 (1) (2014) 18–22.

[39] Z.B. Al-Hilli, H.H. Ajeel, Isolation and Identification of bacterial flora from vagina in normal Ewes (slaughtered and living Ewes), J. Pharm. Biol. Sci. 10 (6) (2015) 1–4.

[40] Swartz, et al., Characterization of the vaginal microbiota of ewes and cows reveals a unique microbiota with low levels of lactobacilli and near-neutral pH, Front. Vet. Sci. 1 (2014) 19.

[41] M.O. Gani, M.M. Amin, M.G.S. Alam, M.E.H. Kayesh, M.R. Karim, M.A. Samad, M.R. Islam, Bacterial flora associated with repeat breeding and uterine infections in Dairy cows, Bangl. J. Vet. Med. 6 (1) (2008) 79–86.

[42] V.S. Mavrogianni, G.S. Amiridis, D.A. Gougoulis, I.A. Fragkou, G.C. Fthenakis, Efficacy of difloxacin for the control of postpartum uterine infections of ewes, J. Vet. Pharmacol. Ther. 30 (2007) 583–585.

[43] G.D. Mshelia, G. Okpaje, Y.A.C. Voltaire, G.O. Egwu, Comparative studies on genital infections and antimicrobial susceptibility patterns of isolates from camels (Camelus dromedarius) and cows (Bos indicus) in Maiduguri, northeastern Nigeria, Springer Plus 3 (2014) 91.

[44] J.D. Buynak, Understanding the longevity of the β-lactam antibiotics and of antibiotic/β-lactamase inhibitor combinations, Biochem. Pharmacol. 71 (7) (2006) 930–940, 30.

[45] M. Karczmarczyk, C. Walsh, R. Slowey, N. Leonard, S. Fanning, Molecular characterization of multidrug-resistant Escherichia coli isolates from Irish cattle farms, Appl. Environ. Microbiol. 77 (20) (2011) 7121–7127.

[46] K. Todar, Bacterial Resistance to Antibiotics, 2013. Available: http://textbookofbacteriology.net/resantimicrobial.html. (Accessed 10 May 2013).

[47] C. Peña, M. Pujol, C. Ardanuy, A. Ricart, R. Pallares, J. Liñares, J. Ariza, F. Gudiol, Epidemiology and successful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases, Antimicrob. Agents Chemother. 42 (1) (Jan 1998) 53–58.

[48] Y. Chong, S. Shimoda, H. Yakushiji, Y. Ito, T. Miyamoto, T. Kamimura, N. Shimono, K. Akashi, Community spread of extended spectrum β-lactamase-producing *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis*: a long-term study in Japan, J. Med. Microbiol. (2013). Mar 28. [Epub ahead of print] PubMed PMID: 23538565.

[49] D.L. Paterson, R.A. Bonomo, Extended-spectrum beta-lactamases: a clinical update, Clin. Microbiol. Rev. 18 (4) (2005) 657–686.

[50] H.X. Jiang, D. Tang, Y.H. Liu, X. Zhang, Z.L. Zeng, L. Xu, P.M. Hawkey, Prevalence and characteristics of beta-lactamase and plasmid-mediated quinolone resistance genes in *Escherichia coli* isolated from farmed fish in China, J. Antimicrob. Chemother. 67 (10) (2012) 2350–2353.

[51] T. Teshager, L. Dominguez, M.A. Moreno, Y. Saénz, C. Torres, S. Cardeñosa, Isolation of a SHV-12 ß-lactamase-producing *Escherichia coli* strain from a dog with recurrent urinary tract infections, Antimicrob. Agents Chemother. 44 (2000) 3483–3484.

[52] J.G. Frye, P.J. Fedorka-Cray, Prevalence, distribution and characterisation of ceftiofur resistance in *Salmonella enteric* isolated from animals in the USA from 1999 to 2003, Int. J. Antimicrob. Agents 30 (2007) 134–142.

[53] H.E. Sidjabat, N.D. Hanson, E. Smith-Moland, J.M. Bell, J.S. Gibson, L.J. Filippich, D.J. Trott, Identification of plasmid-mediated extended-spectrum and AmpC beta-lactamases in *Enterobacter* spp. isolated from dogs, J. Med. Microbiol. 56 (3) (Mar 2007) 426–434.

[54] S.C. Rankin, J.M. Whichard, K. Joyce, L. Stephens, K. O'Shea, H. Aceto, D.S. Munro, C.E. Benson, Detection of a blaSHV extended-spectrum β-lactamase in *Salmonella enterica* serovar Newport MDR-AmpC, J. Clin. Microbiol. 43 (11) (2005) 5792–5793.

[55] L. Briñas, M.A. Moreno, M. Zarazaga, C. Porrero, Y. Sáenz, M. García, L. Dominguez, C. Torres, Detection of CMY-2, CTX-M-14, and SHV-12 beta-lactamases in *Escherichia coli* fecal sample isolates from healthy chickens, Antimicrob. Agents Chemother. 47 (2003) 2056–2058.

[56] B. Bedenic, J. Vranes, L.J. Mihaljevic, M. Tonkic, M. Sviben, V. Plecko, etal, Sensitivity and specificity of various b-lactam antibiotics and pheno typical methods for detection of TEM, SHV and CTX- M extended-spectrum b-lactamases, J. Chemother. 19 (2007) 127–139, http://dx.doi.org/10.1179/joc.19.2.127.

[57] K. Singh, K.A. Mangold, K. Wyant, D.M. Schora, B. Voss, K.L. Kaul, et al., Rectal screening for *Klebsiella pneumoniae* carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates, J. Clin. Microbiol. 50 (2012) 2596–2600, http://dx.doi.org/10.1128/JCM.00654-12.

[58] E. Stürenburg, I. Sobottka, H.-H. Feucht, D. Mack, R. Laufs, Comparison of BD Phoenix and VITEK2 automated antimicrobial susceptibility test systems for extended-spectrum b-lactamase detection in *Escherichia coli* and *Klebsiella* species clinical isolates, Diagn. Microbiol. Infect. Dis. 45 (2003) 29–34, http://dx.doi.org/10.1016/S0732-8893(02)00481-9.