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Bacterial and molecular studies on the causative agents of bovine endometritis with treatment trial

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Research

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Abstract
Bovine endometritis is a prevalent postpartum disorder affecting dairy cows. Antimicrobial-resistant bacterial infections represent a major concern in animal health. This work aimed to determine bacterial causes of endometritis in dairy cows, susceptibility of the isolates to different antibiotics, and molecular screen of antibiotic resistance genes in these isolates. Vaginal discharges were collected aseptically from 30 dairy cows suffering from postpartum endometritis using vaginal speculum. Bacteriological examination was accomplished for diagnosis of causative agents. All bacterial isolates were subjected to antibiotic sensitivity tests and molecular characterization of antibiotic resistance genes. Bacteriological examination revealed isolation of thirty strains of four different bacterial species including 13 *E. coli* (43.3%), 7 Klebsiella spp. (23.3%), 6 Streptococcus spp. (20%) and 4 *S. aureus* (13.3%). *E. coli* isolates displayed resistance to gentamicin (46.2%) followed by tetracycline and vancomycin (30.8%) each. All Klebsiella spp. isolates were sensitive to cefquinome. All Streptococcus spp. and *S. aureus* isolates were sensitive to cefquinome and sulfamethoxazole combined with trimethoprim. All the antibiotic-resistant *E. coli* and Klebsiella isolates encoded *Sul1, blaTEM, tetA, aadB, qnrS,* and *vanA* resistance genes. All the antibiotic-resistant Streptococcus spp. isolates harbored *Pbp1A, tetO, norA, Aac(6')* and *vanA* resistance genes. All the antibiotic-resistant *S. aureus* isolates encoded *blaz, tetK, norA, Aac(6')* and *vanA* resistance genes. About 83.3% of infected cows improved after the first course of the treatment, while 17.7% improved after the second course. Treatment was done according to antibiotic sensitivity test. Infected cases were treated with intramuscular injections of cefquinome, flunixin meglumine, and dinoprost in addition to intrauterine infusions of oxytetracycline. Follow-up was done to assess the outcome of treatment.

Keywords: *Bovine endometritis, E. coli, Klebsiella spp., S. aureus, Streptococcus spp., antibiotic susceptibility test, resistance genes*

Introduction

Bovine endometritis (BE) is among the principal diseases, that affects the reproduction capacity of cattle and decreases farm animal productivity **Adnane *et al.* (2017).** It results in decreased milk production, increased frequency of services per conception, and higher culling rates due to infertility, as well as, increases

diagnostic and therapeutic veterinary costs **Cheong *et al.* (2011)**. The global prevalence of BE is highly variable, ranging between 3.4% and 40% **Gilbert *et al*. (2005).**

Bovine endometritis is an inflammation of the endometrium without general manifestations, accompanied by chronic postpartum infection of the uterus with contagious pathogens. BE is classified based on vaginal mucus into mild mucopurulent and purulent. Also, it can be classified according to the severity of infection into acute, subclinical, and chronic BE **Parmar (2021)**. Clinical BE is characterized by purulent uterine discharge in the vagina after the 21st post-partum day. Metabolic disorders including milk fever and ketosis are related to an increased susceptibility to BE **Várhidi *et al.* (2024)**. BE is a complex disease that comprises pyometra, puerperal metritis, retained placenta and other non-specific uterine infections. Classical diagnosis of BE based on clinical manifestation and rectal examination **Raheel *et al.* (2020).** The ultrasonography examination is important for the proper diagnosis of BE. Bovine endometritis is usually a self-limiting infection with spontaneous improvement following subsequent estrous cycles **Yadav *et al.* (2017)**. The effective treatment promotes uterine defense mechanisms and inhibits bacterial infections **Parikh *et al.* (2022)**.

Bovine endometritis is a multi-factorial disease (bacteria, fungi, and viruses) making the disease difficult to diagnose, treat, and eradicate. However, the majority of BE outbreaks in dairy herds are due to bacterial agents **Zhao *et al.* (2011)**.

Bacterial infection is the principal reason of uterine inflammation occurring through or after artificial insemination, coitus, or parturition. Different pathogenic agents can produce animal genital tract infections namely, *Staphylococcus* spp., Streptococcus spp., *Escherichia coli*, Klebsiella spp., *Pseudomonas aeruginosa*, Clostridium spp., *Fusobacterium necrophorum*, Corynebacterium spp., and Bacillus spp. **Osawa (2021)**. Factors such as necrotized caruncles, blood, and cell debris represent a conducive environment for bacterial proliferation, leading to persistent infection and BE in 10 to 17% of postpartum animals **Umer *et al.* (2022)**.

Bacterial postpartum BE is a polymicrobial infection usually caused by two or more different pathogens. It is often a mixed aerobic and anaerobic flora. The resistance of bacteria to antibiotics increases and creates a therapeutic challenge in the management of cattle with postpartum BE **Appiah *et al.* (2020)**. The raised rates of antimicrobial-resistant infections represent a great problem in both human and animal health because of the emergence of numerous antibiotic-resistant isolates in domestic animals. A considerable part of multidrug-resistant bacterial isolates can encode integrons **Zhao *et al.* (2011)**, which can capture, integrate, and mobilize antibiotic-resistant gene cassettes. The bacteria possessing class 1 integron are a potential source for antibiotic resistance genes and possess a significant impact on the antimicrobial resistance of bacterial populations. Guidelines should encourage understanding bacterial resistance when prescribing antibiotics for treatment and prophylaxis of postpartum infections **Zhang *et al.* (2024)**.

This work aimed to determine bacterial causes of endometritis in dairy cows, study the susceptibility of these bacterial isolates to different antibiotics, and screen antimicrobial resistance genes in these isolates.

Materials and Methods

1. Animals:

This work was conducted at a private farm of Frisian dairy cows in Sohag governorate, Egypt from June to December 2024. The herd was composed of 200 cows with an average age of 4-5 years and weighing 300–350 kg.

Thirty dairy cows suffering from bovine endometritis two to six months after parturition were included in this study. This work was done following the requirements for the care and use of animals. The Medical Research Ethics Committee of the Faculty of Medicine, Sohag University approved the study under IRB Registration number; Soh-Med-24-10-12PD.

2. Clinical examination:

Clinical examination of each dairy cow included respiratory rate, pulse rate, body temperature, and rectal palpation according to **Hassan *et al.* (2023)**. All cows were repeated breeding of more than two natural services characterized

with regular oestrus cycles and with no signs of pregnancy.

3. Sampling:
Prior to sample collection, the external genitalia were washed with warm clean water and soap, disinfected with 0.1% povidone-iodine, and dried with a sterile towel. 5-10 milliliters of vaginal discharge were collected from the vaginal fornix using a sterilized vaginal speculum. Each sample was subjected to color and odor examination of vaginal discharge was performed according to color and odor scores **Williams *et al.* (2005), Sheldon *et al.* (2006)**:

- Grade 0, or normal (without BE), the discharge is clear or translucent with no odor.
- Grade 1, the mucoid discharge comprises flecks of white or off-white pus with mild odor (slightly foul smelling).
- Grade 2, the discharge comprises less than 50% white or off-white mucopurulent material with moderate odor (foul smelling).
- Grade 3, is purulent, usually with a white or yellow color, but sometimes it can include blood with strong foul odor.

4. Bacteriological examination:
The vaginal discharge aspirates were collected in sterile test tubes containing sterile Stuart media as transport media and incubated aerobically for 24 hrs at 37° C. A loopful from the enriched specimen was streaked onto McConkey agar, Blood agar, Baird-Parker agar, and Eosin methylene blue agar plates, then incubated aerobically for 24 hrs at 37°C according to **Collee *et al.* (1996)**. The growing surface colonies were identified by:-

- Microscopic examination: Identification of the suspected colonies according to morphology and Gram staining.
- Biochemical identification: Suspected colonies were subjected to triple sugar iron, indole, voges proskauer, methyl red, citrate, oxidase, urease, coagulase, and catalase tests.
- Slide agglutination test was done for serotyping of *E. coli* isolates **Orskov and Orskov (1984)**.

5. Antibiotic sensitivity test:
All bacterial isolates were examined for their antibiogram using the disc diffusion method against seven antibiotics (Oxoid) **Bauer (1996)**. The antibiotic discs were ampicillin (10 µg), cefquinome (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), tetracycline (50 µg), sulfamethoxazole and trimethoprim (25 µg), and vancomycin (30 µg). The interpretation of results was applied according to CLSI **He *et al.* (2024)**.

6. Molecular detection of antibiotic resistance genes:
6.1. DNA extraction and amplification:
DNA extraction from bacterial isolates was done by QIAamp DNA Mini kit (Qiagen, Germany, GmbH). In brief, two hundreds microliters of the sample suspension were incubated with twenty microliters proteinase K and two hundreds microliters lysis buffer, respectively at 56 °C for 10 min. Next, two hundreds microliters of 100% ethanol were added to the lysate. Washing and centrifugation of samples were done according to the manufacturer's instructions. Nucleic acid was eluted with one hundred microliters of the provided elution buffer. Primers used in this work were provided by Metabion (Germany). PCR amplification was conducted in an applied biosystem 2720 thermal cycler **Liu *et al*. (2009)**.
Target genes, primer sequence, and cyclic conditions were illustrated in table 1-3.
6.2. Analysis of PCR Products:
At room temperature, the products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer using gradients of 5V/cm. For gel analysis a gene ruler 100 bp ladder (Fermentas, thermo, Germany), gelpilot 100 bp and 100 bp plus ladders (Qiagen, Gmbh, Germany), and Genedirex 50 bp DNA ladder RTU, Cat. No. DM012-R500 were used to recognize the fragment sizes. The gel was photographed (Alpha Innotech, Biometra) and analyzed by computer software.

Table (1). Oligonucleotide primers sequences cyclic conditions for antibiotic resistance genes of *E. coli* and Klebsiella spp.

Target genes	Primers (5'-3')	Condition of amplification						Reference
Sul1	ATGGTGACGGTGTTCGG-CATTCTGA CTAGGCATGATCTAACCCTCGGTC T	432 bp	95˚C 5 min.	95˚C 1 min.	55˚C 1min.	72˚C 1 min.	72˚C 5 min.	**Toleman *et al.* (2006)**
blaTEM	TCAACATTTTCGTGTCGCCC AACTACGATACGGGAGGGCT	445 bp	95˚C 10 min	95˚C 2 min.	57˚C 35 sec.	72˚C 40 sec.	72˚C 7 min.	**Jiang *et al.* (2022)**
tetA	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	210 bp	5 min at 94 ° C	1 min at 94 °C	1 min at 57 °C	1 min at 72 °C	7 min at 72 °C	**Salvador-Membreve and Rivera (2021)**
aadB	CTAGCTGCGGCAGATGAGC CTCAGCCGCCTCTGGGCA	219 bp	95˚C 5 min.	94˚C 1 min.	62˚C 1min	72˚C 1 min.	72˚C 5 min.	**Doosti *et al.* (2016)**
qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417 bp	94˚C 5 min.	94˚C 30 sec.	48˚C 45 sec.	72˚C 45 sec.	72˚C 5 min.	**Robicsek *et al.* (2006)**
vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Patel *et al.* (1997)**

Table (2). Oligonucleotide primers sequences for antibiotic resistance genes of Streptococcus spp.

Target genes	Primers (5'-3')	Condition of amplification						Reference
tetO	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	519bp	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Olsvik *et al.* (1995)**
Pbp1A	AAGAACACTGGTTATGTA AGCATGCATTATGCAAAC	224bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**du Plessis *et al.* (1999)**
norA	TTCACCAAGCCATCAAAAAG CTTGCCTTTCTCCAGCAATA	620 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Pourmand *et al.* (2014)**
Aac (6')	GAAGTACGCAGAAGAGA ACATGGCAAGCTCTAGGA	491 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Duran *et al.* (2012)**
vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Patel *et al.* (1997)**

Table (3). Oligonucleotide primers sequences for antibiotic resistance genes of *S. aureus*

Target genes	Primers (5'-3')	Condition of amplification						Reference
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	94˚C 5 min	94˚C 30 sec.	54˚C 40 sec	72˚C 40 sec.	72˚C 10 min	**Duran *et al.* (2012)**
blaZ	TACAACTGTAATATCGGAGGG CATTACACTCTTGGCGGTTTC	833 bp	94˚C 5 min	94˚C 30 sec.	50˚C 40 se.	72˚C 50 sec.	72˚C 10 min	***Bagcigil et al.* (2012)**
norA	TTCACCAAGCCATCAAAAAG CTTGCCTTTCTCCAGCAATA	620 bp	94˚C 5 min	94˚C 30 sec.	55˚C 40 se.	72˚C 45 sec.	72˚C 10 min	**Pourmand *et al.* (2014)**
Aac(6')	GAAGTACGCAGAAGAGA ACATGGCAAGCTCTAGGA	491 bp	94˚C 5 min	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec	72˚C 10 min	**Duran *et al.* (2012)**
vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	94˚C 5 min	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec	72˚C 10 min	**Patel *et al.* (1997)**

7. Treatment of cows with BE: all infected cases received the following drugs:

- Cefquinome ® intramuscular injection 1cm / 25 kg bw for 3 days.
- Non-steroidal anti-inflammatory drug: Flunixin meglumine ® intramuscular injection 2cm /45kg for 3-5 days.
- Oxytetracycline intrauterine infusions 250–500 mg per uterine horn for 3-5 days.
- Dinoprost (Lutalyse®) intramuscular injection 25 mg per cow, single dose.

8. Follow up of the cases for evaluation of the outcome of treatment:

- Each cow was clinically reexamined ten days after therapy. Clinical cure was described as the absence of purulent vaginal discharge at the follow-up confirmed with bacteriological examination.
- Cows that did not show clinical improvement were subjected to a second course of the same drugs as previously administered. These cows were then evaluated after an additional ten days.
- All treated cases were naturally inseminated with fertile bulls.
- Diagnosis of pregnancy was done 60 days after the last treatment course by rectal palpation.

9. Statistical analysis:

Results were analyzed using GraphPad Prism 9.5.1 software (GraphPad Software Inc., San Diego, CA, USA). Where the Chi-square test and Kruskal-Wallis test were used to estimate whether a significant variation between them, a "P" value of <0.05 was assumed statistically to be significant **McHugh (2013).**

The correlation between phenotypic and genotypic resistance (Correlation coefficient r) and test agreement were measured using a statistical software program (MedCalc for Windows, version 22.0.18, Med- Calc Software, Mariakerke, Belgium).

Statistical significance was considered at $P<0.05$. Correlation coefficient (r), interpreted as follows: 0-0.19; very weak correlation, 0.2-0.39; weak correlation, 0.40-0.59; moderate correlation, 0.6-0.79; strong correlation and 0.8 -1; very strong correlation. Statistical significance was assumed at $P<0.05$. Inter-rater agreement was quantified by Weighted Kappa (K), interpreted as follows: < 0.20; poor, 0.21–0.40; fair, 0.41–0.60; moderate, 0.61–0.80; good, and 0.81–1.00; very good **Altman (1990).**

Results

The clinical examination of the thirty cows with repeat breeding revealed that the mean temperature, pulse rate, and respiratory rate were 38.9 ± 0.3°C, 110 ± 6.2 beats/minute, and 37.6 ± 2.3 breath/ minute respectively.

Odor and color examination of vaginal discharge revealed that there was mucoid discharge with a slightly foul odor in 5 cows (16.7%), mucopurulent discharge with a foul odor in 21 cows (70%), and purulent discharge with a strong foul odor in the rest 4 cows (13.3%) as shown in table (4), the identified bacterial isolates were 30 strains which included 13 *E. coli* (43.3%), 7 Klebsiella spp. (23.3%), 6 Streptococcus spp. (20%) and 4 *S. aureus* (13.3%). All samples were mixed infection with more than two organisms. There was a significant statistical difference between bacterial causes of BE (chi ^{2} = 8.000, P< 0.05) (Table 4).

Table (4). Bacterial spp. isolated from vaginal discharges of cows infected with endometritis (No: 30 samples)

Organism *	No.	%
E. coli	13	43.3%
Klebsiella spp.	7	23.3%
Streptococcus spp.	6	20%
S. aureus	4	13.3%

* Significant statistical difference between bacterial causes of endometritis (chi ^{2} = 8.000, P< 0.05).

Four *E. coli* serogroups were identified, O55 (38.5%), followed by O26 and O127 (23.1%) each, while the least was O103 (15.4%) as shown in table (5).

Table (5). Serotyping of *E. coli* strains

Serotype	No.	%
E. coli O55	5	38.5%
E. coli O 26	3	23.1%
E. coli l03	2	15.4%
E. coli O127	3	23.1%

Phenotypic antimicrobial sensitivity pattern of 13 *E. coli* isolates revealed resistance to gentamicin (46.2%) followed by tetracycline and vancomycin (30.8%) each, sulfamethoxazole and trimethoprim (23.1%), ampicillin and ciprofloxacin (15.4%) each, and cefquinome (7.7%) with high significant statistical difference between sensitive and resistant strains of *E. coli* (Chi ^{2} = 74.39, P< 0.001) as shown in table (6).

Phenotypic antimicrobial sensitivity pattern of the seven Klebsiella spp. isolates proved that all isolates were susceptible to cefquinome. There was resistance to tetracycline and vancomycin (42.9%) each, followed by sulfamethoxazole and trimethoprim and ampicillin (28.6%) each, ciprofloxacin and gentamicin (14.3%) each with high significant statistical difference between sensitive and resistant strains of Klebsiella (Chi ^{2} = 83.40, P< 0.001) as shown

in table (6).
Phenotypic antimicrobial sensitivity pattern of the six Streptococcus spp. isolates revealed that all isolates were susceptible to sulfamethoxazole and trimethoprim and cefquinome. There was resistance to ciprofloxacin (50%), followed by tetracycline and ampicillin (33.3%) each, and gentamicin and vancomycin (16.7%) each with high significant statistical difference between sensitive and resistant strains of Streptococcus spp. (Chi ^{2} = 121.3, P< 0.001) as shown in table (6).
The phenotypic antimicrobial sensitivity pattern of the four *S. aureus* isolates proved that all isolates were sensitive to sulfamethoxazole and trimethoprim and cefquinome. All isolates were resistant to vancomycin (100%), followed by ampicillin (75%), ciprofloxacin (50%), tetracycline gentamicin and (25%) each with a highly significant statistical difference between sensitive and resistant strains of *S. aureus* (Chi ^{2} = 359, P< 0.001) as noticed in table (6).

Table (6). Antimicrobial sensitivity profile of bacterial isolates

Isolates	Total No.	Sensitivity	Sulfamethoxazole and Trimethoprim	Tetra-cycline	Ampicillin	Cipro-floxacin	Gentamicin	Cefquinome	Vancomycin
E. coli	13	Sensitive	10 (76.9%)	9 (69.2%)	11 (84.6%)	11 (84.6%)	7 (53.8%)	12 (92.3%)	9 (69.2%)
		Resistant	3 (23.1%)	4 (30.8%)	2 (15.4%)	2 (15.4%)	6 (46.2%)	1 (7.7%)	4 (30.8%)
Klebsiella spp.	7	Sensitive	5 (71.4%)	4 (57.1%)	5 (71.4%)	6 (85.7%)	6 (85.7%)	7 (100%)	4 (57.1%)
		Resistant	2 (28.6%)	3 (42.9%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	0 (0%)	3 (42.9%)
Streptococcus spp.	6	Sensitive	6 (100.0%)	4 (66.7%)	4 (66.7%)	3 (50%)	5 (83.3%)	6 (100%)	5 (83.3%)
		Resistant	0 (0.0%)	2 (33.3%)	2 (33.3%)	3 (50%)	1 (16.7%)	0 (0%)	1 (16.7%)
S. aureus	4	Sensitive	4 (100.0%)	3 (75%)	1 (25%)	2 (50%)	3 (75%)	4 (100%)	0 (0%)
		Resistant	0 (0.0%)	1 (25%)	3 (75%)	2 (50%)	1 (25%)	0 (0%)	4 (100%)

% calculated according to the No. of isolates in each species.

Molecular characterization of *E. coli* isolates by PCR proved that all sulfonamide-resistant isolates carried the *Sul1* gene. All tetracycline-resistant isolates possess the *tetA* gene. All quinolone resistant isolates encode *qnrS* gene. Among three beta-lactam-resistant isolates, *blaTEM* gene was recognized in two isolates. Among six aminoglycosides-resistant isolates, *aadB* gene was determined in five isolates. Among four glycopeptides-resistant isolates, *vanA* gene existed in 2 isolates. Statistical analysis showed a very strong correlation and moderate inter-rater agreement between phenotypic and genotypic resistance of *E. coli* isolates (Table 7). 30.7% of *E. coli* isolates exhibit multi-drug-resistant profiles both phenotypic and genotypic to more than three antibiotics (Figure 1: A).
Molecular identification of Klebsiella spp. isolates by PCR proved that all sulfonamide-resistant isolates carried the *Sul1* gene. All tetracycline-resistant isolates possess the *tetA* gene. *aadB* gene was detected in the aminoglycosides-resistant isolate. *qnrS* gene was deter-

mined in the quinolone resistant isolate. Among two beta-lactam-resistant isolates, *blaTEM* gene presented in one isolate. Among three glycopeptides-resistant isolates, the *vanA* gene was recognized in 2 isolates. Statistical analysis showed a very strong correlation and good inter-rater agreement between phenotypic and genotypic resistance of Klebsiella spp. isolates (Table 7). 28.5% of Klebsiella spp. isolates exhibit multi-drug-resistant profiles both phenotypic and genotypic to more than three antibiotics (Figure 1: B).

Molecular identification of Streptococcus spp. isolates by PCR proved that all beta-lactam-resistant isolates carried the *Pbp1A* gene. *Aac (6')* gene was recognized in the aminoglycosides-resistant isolate. *vanA* gene was detected in the glycopeptides-resistant isolate. Among two tetracycline-resistant isolates *tetO* gene was detected in one isolate. Among three quinolone-resistant isolates, *norA* gene was detected in one isolate. Statistical analysis showed a very weak correlation and fair inter-rater agreement between phenotypic and genotypic resistance of Streptococcus spp. isolates (Table 7) 33.3% of Streptococcus spp. isolates exhibit multi-drug-resistant profiles both phenotypic and genotypic to more than two antibiotics (Figure 1: C).

Molecular identification of *S. aureus* isolates by PCR proved that *the tetK* gene was detected in tetracycline-resistant isolate. *The Aac(6')* gene was detected in aminoglycosides - resistant isolate. Among three beta-lactam-resistant isolates *blaz* gene was detected in one isolate. Among two quinolone-resistant isolates, *norA* gene was detected in one isolate. Among four glycopeptide-resistant isolates, the *vanA* gene was determined in 2 isolates. Statistical analysis showed a strong correlation and poor inter-rater agreement between phenotypic and genotypic resistance of *S. aureus* isolates (Table 7) 50% of *S. aureus* isolates exhibit multi-drug-resistant profiles both phenotypic and genotypic to more than two antibiotics (Figure 1: D).

The results of the present study revealed that 25 cows (83.3%) showed clinical and bacteriological improvement after receiving the first course of the treatment, while five cows (17.7%) improved after the second course of the treatment. All cows were diagnosed as pregnant by rectal palpation two months after the treatment.

Table (7). Correlation between phenotypic and genotypic resistance of bacterial isolates

Isolates	Resistance	Sulfon-amides	Beta-lactam	Tetra-cyclin e	Amino-glycosid e	Quinolones	Glyco-peptide s	Correlation coefficient (r)	Weighted Kappa (*K*)
E. coli	Phenotypic	3	3	4	6	2	4	0.8262.	0.53846
	Genotypic	3	2	4	5	2	2		
Klebsiella spp.	Phenotypic	2	2	3	1	1	3	0.8216.	0.625
	Genotypic	2	1	3	1	1	2		
Streptococcus spp.	Phenotypic	0	2	2	1	3	1	0.1336.	0.21053
	Genotypic	0	2	1	1	1	1		
S. aureus.	Phenotypic	0	3	1	1	2	4	0.7717	0.13793
	Genotypic	0	1	1	1	1	2		

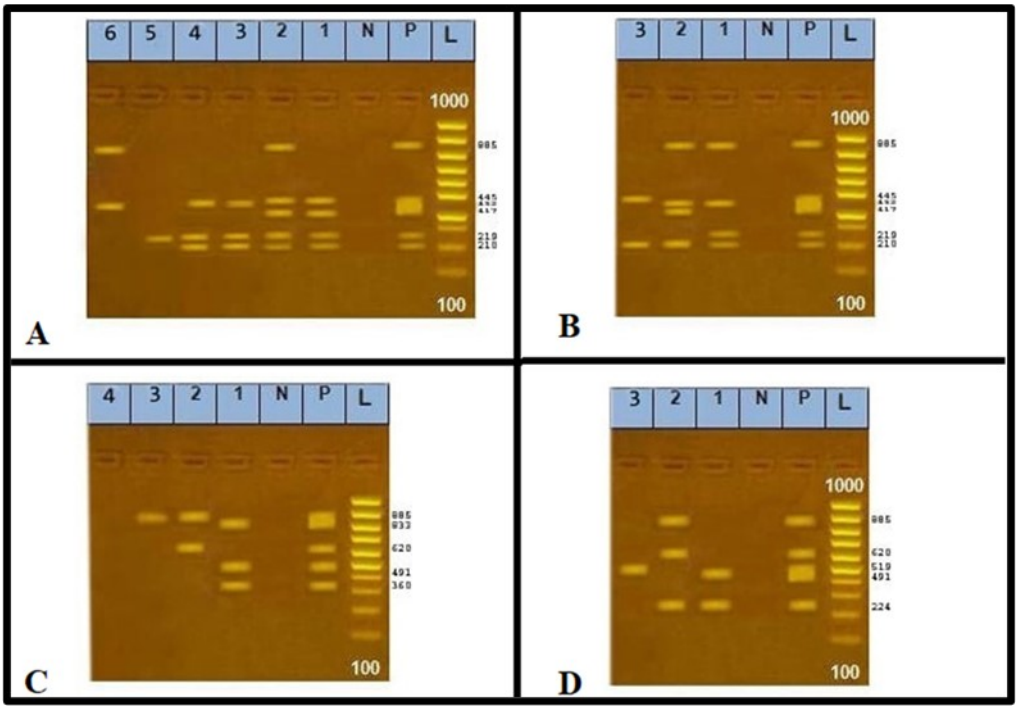

Figure (1): Gel electrophoresis and multiplex PCR profile for antibiotic resistance genes of bacterial isolates. Lane (L): DNA marker, lane (P): positive control, lane (N): negative control, (bp) base pair.
A: *E. coli* isolates.
Lane 1: positive for *tetA* gene (210 bp), *aadB* gene (219 bp), *qnrS* gene (417 bp), and *blaTEM* gene (445bp).
Lane 2: positive for *tetA* gene (210 bp), *aadB* gene (219 bp), *qnrS* gene (417 bp), *blaTEM* gene (445 bp), and *vanA* gene (885 bp).
Lane 3: positive for *tetA* gene (210 bp), *aadB* gene (219 bp), and *Sul1* gene (432 bp).
Lane 4: positive for *tetA* gene (210 bp), *aadB* gene (219 bp), and *Sul1* gene (432 bp).
Lane 5: positive for *aadB* gene (219 bp).
Lane 6: positive for *Sul1* gene (432 bp) and *vanA* gene (885 bp)
B: Klebsiella spp. isolates.
Lane 1: positive for *tetA* gene (210 bp), *aadB* gene (219 bp), *Sul1* gene (432 bp), and *vanA* gene (885 bp)
Lane 2: positive for *tetA* gene (210 bp), *qnrS* gene (417 bp), *Sul1* gene (432 bp), and *vanA* gene (885 bp)
Lane 3: positive for *tetA* gene (210 bp) and *blaTEM* gene (445 bp)
C: *S. aureus* isolates.
Lane 1: positive for *tetK* gene (360 bp), *aac(6')* gene (491 bp), and *blaz* gene (833 bp)
Lane 2: positive for *norA* gene (620 bp), and *vanA* gene (885 bp)
Lane 3: positive for *vanA* gene (885 bp)
D: Streptococcus spp. isolates.
Lane 1: positive for *pbp1A* gene (224 bp) and *aac(6')* gene (491 bp)
Lane 2: positive for *pbp1A* gene (224 bp), *norA* gene (620 bp), and *vanA* gene (885 bp)
Lane 3: positive for *tetO* gene (519 bp)

Discussion

Bovine endometritis is an important cause of infertility in dairy cattle, caused by uterine contamination, infection, and inflammation that occurs at the time of calving and persists until the time when the cow should be bred **Pascottini *et al.* (2023)**.
Noticeable mucopurulent (half mucus / half pus) or 100% purulent discharge at the vagina or vulva is a hallmark of clinical BE. The seriousness of the case is usually determined by assessing the vaginal discharge **Várhidi *et al.* (2024)**. In this study, clinical examination revealed that vital signs were slightly higher than normal. Odor and color examination of vaginal discharge revealed that there was mucoid discharge with a slightly foul odor in 5 cows (16.7%), mucopurulent discharge with a foul odor in 21 cows (70%), and purulent discharge with a strong foul odor in the rest 4 cows

(13.3%). Similarly, **Azawi *et al.* (2008)** examined vaginal discharge of cows with BE and found the picture of clear mucus, clear mucus with flakes of pus or mucopurulent discharge, in 23.7%, 30.7%, and 45.6% respectively.

Bacterial infection is considered the prime reason of BE. Many bacterial species can inhabit the uterus during the early postpartum period such as Staphylococcus spp., Streptococcus spp., Klebsiella spp., *Escherichia coli*, *Pseudomonas* spp. as well as *Prevotella melaninogenicus* **Dohmen *et al.* (2009**).

In this research, the identified bacterial isolates were 30 strains. *E. coli* was the most prevalent as 13 strains (43.3%), followed by 7 Klebsiella spp. (23.3%), 6 Streptococcus spp. (20%) and 4 *S. aureus* (13.3%). *E. coli* was reported to be in maximum frequency by **Bhat *et al.* (2014)**. Similarly, **Moges *et al.* (2013)** isolated Streptococcus spp. and *E. coli* (20.8% each), *S. aureus* (12.5%), Klebsiella spp. (8.3%) and *C. fetus* (4.2%) from cattle with BE. Also, **Iancu *et al.* (2023)** isolated *E. coli* (50%), Staphylococcus spp. (10.52%), Klebsiella spp. (19.47%), Streptococcus spp. and Pseudomonas spp. (10%) each from cattle infected with endometritis. On the contrary, *Staphylococcus* spp. was reported as the most frequently isolated bacteria from the female reproductive tract with repeat breeding **Çömlekcioğlu *et al.* (2024), Moges *et al.* (2013), and Neelam *et al.* (2018)**.

Proper microbiological diagnosis is necessary to provide adequate treatment of the infection. Antibiotic sensitivity tests ensure the ideal results of antibiotic use against bacterial infection. This can be achieved through correct selection based on antibiogram studies **Petrina *et al.* (2019)**.

In the current study, *E. coli* isolates showed resistance to gentamicin (46.2%), tetracycline and vancomycin (30.8%) each, sulfamethoxazole and trimethoprim (23.1%), ampicillin and ciprofloxacin (15.4%) cefquinome (7.7%). Similarly, **Moges *et al.* (2013)** found that *E. coli* isolates were highly resistant by (100%) to cefoxitin, polymixin and tetracycline. On other hand, these isolates revealed moderate resistance against oxacillin, gentamycin, and sulphamethoxazole as (40%). Also, **Zhao *et al.* (2011)** stated that *E. coli* exposed 100% resistance against sulfamethoxaz-ole-trimethoprim.

In this work, Klebsiella spp. isolates proved that all isolates were susceptible to cefquinome. There was resistance to tetracycline and vancomycin (42.9%) each, followed by, sulfamethoxazole and trimethoprim and ampicillin (28.6%) each, ciprofloxacin and gentamicin (14.3%) each. **Moges *et al.* (2013)** mentioned that Klebsiella spp. had high resistance only versus cefoxitin (100%) and was sensitive to sulphamethoxazole, polymixin, tetracycline, oxacillin, gentamycin, and vancomycin.

In this research, all Streptococcus spp. isolates were susceptible to sulfamethoxazole and trimethoprim and cefquinome. There was resistance to ciprofloxacin (50%), followed by tetracycline and ampicillin (33.3%) each, and gentamicin and vancomycin (16.7%) each. **Malinowski *et al.* (2010)** found that *Streptococcus* spp. were almost highly sensitive to amoxicillin/clavulanic acid, ampicillin and norfloxacin by 94.6%, 92.3%, and 92% respectively. Also the microorganism was susceptible to cephapirine, cefoperazone, rifaximine, and penicillin as 88%, 86.5%, 85.7%, and 84.9% respectively.

In this study, all *S. aureus* isolates were sensitive to cefquinome as well as the combination of sulfamethoxazole and trimethoprim and, while they were resistant to vancomycin (100%), followed by ampicillin (75%), ciprofloxacin (50%), tetracycline gentamicin and (25%) each. **Gani *et al.* (2008)** reported that *S. aureus* isolates were resistant to oxacillin, ampicillin, and vancomycin. They found that ciprofloxacin is one of the most effective antimicrobial agents against staphylococcal infections in dairy cows. **Zhao *et al.* (2014)** stated that *S. aureus* isolates obtained from cows with BE were resistant to penicillin (79.5 %), ampicillin (71.7 %), erythromycin (56.7 %), and tetracycline (52 %).

In this study, the multiplex polymerase chain reaction was assumed as substantial tool for the recognition of antibiotic resistance genes. All the antibiotic-resistant Klebsiella and *E. coli* isolates were analyzed for the presence of *Sul1, blaTEM, tetA, aadB, qnrS,* and *vanA* genes. All the antibiotic-resistant Streptococ-

cus spp. isolates were assessed for the existence of *Pbp1A, tetO, norA, Aac(6')* and *vanA* genes. On the other side, all the antibiotic-resistant *S. aureus* isolates were exposed for the detection of *blaz, tetK, norA, Aac (6')* and *vanA* genes.

Trobos *et al.* (2009) found that the *sul1* gene was associated to other resistance genes on large conjugative plasmids as well as those in class 1 integrons. In this study, the *Sul1* gene was determined in all resistant Klebsiella spp. and *E. coli* isolates. Similarly **Shin *et al.* (2015)** mentioned that *dfr* and *Sul1* genes were highly predominant in association to integron1 of *E. coli* and *Klebsiella pneumonia*.

Aminoglycosides, including amikacin and tobramycin, are effective drugs against resistant bacteria. A common resistance mechanism against aminoglycosides is the production of aminoglycoside-modifying enzymes **Shaw *et al.* (1993)**. In this study, *aadB* gene was detected in 83.3% of Klebsiella and *E. coli*-resistant isolates. Aac(6′) gene was recognized in *Streptococcus* spp. and *S. aureus* isolates. **Hassan *et al.* (2023)** detected *aadB* gene in all *E. coli* resistant isolates. Also, **Zajac *et al.* (2019)** reported *aadB* in 93.8% of the examined *E. coli* isolates.

Hooper (2001) mentioned that the resistance to quinolones can be occurred via various mechanisms such as point mutations in the quinolone resistance-recognizing region of the topoisomerase enzymes; type II and IV. The reduction in susceptibility to quinolones was developed due to the existence of *qnrS*, *aac(6') -Ib-cr,* 35 *qepA* and *41 oqxAB* genes **Hansen *et al.* (2007)**. Our results revealed that *qnrS* gene was determined in all Klebsiella and *E. coli* resistant isolates, while, *norA* gene was detected in 66.6% and 50% of *Streptococcus* spp. and *S. aureus* quinolone-resistant isolates. On the contrary, **Wang *et al.* (2008)** reported that the prevalence rates of *qnrS* among ciprofloxacin-resistant Klebsiella and *E. coli* isolates were 11.9% and 7.5%, respectively.

Ogutu *et al.* (2015) described that the ESBL resistance in gram-negative bacteria is attributed to the presence of blaSHV, blaTEM, and blaCTX-M genes. *E. coli* isolates that obtained from domestic livestock and harboring class 1 and/or class 2 integrons can confirm the animals' role as a potential source for spreading of resistance genes**.**

Regarding *blaTEM* gene, it was detected in Klebsiella spp. and *E. coli* isolates resistant to beta-lactam with a prevalence of 50% and 66.6% respectively. *Pbp1A* gene was recognized in all Streptococcus spp. isolates resistant to beta-lactam while the *blaz* gene was detected in 33.3% of *S. aureus* isolates resistant to beta-lactam. **Ojdana *et al.* (2014)** reported that 16.7% of *E. coli* and 100% of *K. pneumoniae* harbored *bla*TEM-1 gene encoding synthesis of TEM-1 enzyme with performance of broad-spectrum beta-lactamase.

Sengeløv *et al.* (2003) mentioned that the commensal isolates of *E. coli* in animals which have tetracycline efflux pump type were commonly related to existence of *tetA* gene. In this study, the *tetA* gene was recognized in all *E. coli* and Klebsiella spp. tetracycline-resistant isolates. *tetO* was found in 50% of Streptococcus spp. and *tetK* was recovered from *S. aureus* isolate resistant to tetracycline. **Shin *et al.* (2015)** detected tetA gene in 46.5% of *E. coli* isolates recovered from animals. **Giovanetti *et al.* (2003)** detected tetO gene in 73% of *Streptococcus* spp. resistant to tetracycline. A study concluded that the incidence of *S. aureus* isolates that carried *tetK* gene was 97%, **Ho *et al.* (2012)**.

Resistance to glycopeptides is frequently due to 2 types of gene clusters, appointed as *vanA* and *vanB*, that encode linked enzymes and award resistance by the same mechanism **Arthur and Quintiliani (2001)**.

Our finding regarding *the vanA* gene, it was detected in 50%, 66.6%, 100%, and 50% of *E. coli,* Klebsiella spp., Streptococcus spp., and *S. aureus* glycopeptides-resistant isolates respectively. **Hoque *et al.* (2018)** detected vanA gene in 37.5% of *S. aureus* isolates. **He *et al.* (2020)** detected vanA gene in 84.1% of *E.coli* isolates. **Silva *et al.* (2021)** reported that the most commonly used antibiotics for the treatment of BE were ampicillin, amoxicillin, ceftiofur, cephapirin, tetracycline, benzylpenicillin procaine, and sulfonamides often combined with trimethoprim.

Treating the infected cows was conducted in this study with cefquinome ® intramuscular injection, Flunixin meglumine ® intramuscular injection, Oxytetracycline intrauterine infu-

sions, and dinoprost (Lutalyse®) intramuscular injection. Cefquinome was selected because it was the most sensitive antibiotic against bacterial strains isolated from diseased cases. Prostaglandin induces oestrus which helps the getting rid of bacteria and inflammatory outputs **Poth-Szebenyi *et al.* (2021)**. Intrauterine infusion of tetracyclines represents an effective therapy in the prophylaxis and therapy of postpartum BE **Mileva *et al.* (2022)**. Oxytetracycline is a broad-spectrum antimicrobial used for the remedy and control of infections induced by or linked to oxytetracycline-susceptible, fast growing bacteria. Its antimicrobial efficacy versus many infections induced by both Gram- negative and Gram- positive bacteria is well-documented **Manimaran *et al.* (2019)**.

Follow-up of the treated cases revealed that 83.3% of infected cows showed clinical and bacteriological improvement after receiving the first course of the treatment, while 17.7% improved after the second course. All cows were diagnosed as pregnant two months after the treatment. **Barański *et al.* (2022)** reported a significant decline in BE prevalence after treatment with cefquinome, PGF2α, and intrauterine cephapirin infusion.

Conclusion

Bovine endometritis (BE) is one of the most common postpartum disorders affecting dairy cows. Bacterial infection is the prevalent reason of BE. *E. coli,* Klebsiella spp., *S. aureus* and Streptococcus spp. are the primary causative agents of BE. Antibiotic susceptibility test determines appropriate antibiotics for the control of BE. Molecular surveillance is essential for the detection of bacterial resistance genes. Treatment with antibiotics, such as cefquinome, could limit the infection. Oxytetracycline intrauterine infusions enhance cure rates in infected animals.

Recommendations

After conducting this study, animal care providers should consider the following:

Educating veterinarians on hygienic labor measures and recognizing high-risk cattle.

Application of hygienic and sanitation measures for farm animals to prevent peripartum infection.

Emphasizing disease prohibition rather than therapy is valuable to the cattle's health and the farm's economy.

Improvement of clinical laboratory techniques for detecting BE using specific and sensitive procedures.

Prompt treatment of cows suffering from BE to improve animal productivity.

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