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Original article

PHENOTYPIC CHARACTERISATION AND DETECTION OF ANTIBIOTIC RESISTANCE GENES OF *TRUEPERELLA PYOGENES* ASSOCIATED WITH BOVINE ENDOMETRITIS

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Summary

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Endometritis is considered an essential factor affecting both the longevity and profitability of a dairy herd. It causes enormous economic losses and is usually categorised as clinical and subclinical endometritis. *Trueperella pyogenes* can cause numerous infections in the organs and tissues of different farm animals. *T. pyogenes* is a clinically significant bacterium that causes severe endometritis, particularly in postpartum dairy cows. A total of 110 uterine swab samples from cows (75 cows with subclinical endometritis and 35 cows with clinical endometritis) were used in different veterinary clinics at Kaliobia Governorate, Egypt, for isolation of *T. pyogenes* strains, phenotypic identification and genotypic characterisation of antibiotic resistance genes. *T. pyogenes* was recovered from 30 examined uterine samples (27.3%). The isolates displayed greater resistance to tetracycline than to oxacillin, ampicillin, streptomycin, co-trimoxazole and doxycycline; nevertheless, they were sensitive to gentamicin, cephapirin, ciprofloxacin, norfloxacin, amoxicillin/clavulanic acid and cefotaxime. PCR showed that all five studied *T. pyogenes* strains had the *bla* gene and four strains carried *aad*A1 & *tet*(W) genes. *T. pyogenes* was resistant to multiple antimicrobial drugs and had antibiotic resistance genes; hence, it is a significant bacterium causing bovine endometritis.

Key words: antimicrobial profile, antibiotic resistance genes, endometritis, *Trueperella pyogenes*

INTRODUCTION

The postpartum interval is a crucial time in the dairy cow's life. Early intervention in managing critical health disorders during this period is fundamental to achieving effective and sustainable dairy herd management (Adnane *et al*., 2017).

Several factors contribute to the development of uterine disorders in dairy cows, including endometritis, metritis, and retained placenta. These include immune suppression, metabolic disorders, complications during calving such as dystocia, as well as reproductive issues like stillbirth, abortion, and caesarean section (Várhidi *et al.,* 2024).

Endometritis is categorised into clinical and subclinical types. Clinical endometritis is diagnosed when purulent discharge is observed in the vagina from day 21 postpartum onward, or mucopurulent discharge appearing after day 26. Subclinical endometritis, also called cytological endometritis, is marked by inflammation of the uterine lining without any visible discharge and is identified by an elevated count of polymorphonuclear leukocytes in cytological samples from the uterus (Sheldon *et al*., 2006).

Endometritis is a serious and severe disease that impairs the reproductive performance of livestock and affects a herd's profitability (Fesseha, 2020). Clinical and subclinical endometritis in dairy cows result in economic losses primarily through impaired reproductive performance and increased veterinary costs. Subclinical cases are associated with lower pregnancy rates, more artificial inseminations, and prolonged calving intervals. Clinical endometritis, especially when linked to *Trueperella pyogenes* (*T. pyogenes*), further reduces milk production, body condition score, and conception at first artificial insemination, while increasing the number of services per pregnancy and days open (Paiano *et al.,* 2021; 2023).

T. pyogenes is a Gram-positive bacterium with an irregular rod shape. It is facultatively anaerobic, non-motile, non-spore-forming, and lacks a capsule. *T. pyogenes* is distinguished by its robust proteolytic activity, fermentation metabolism and induces opportunistic pyogenic infections of economic significance in dairy cows (Rezanejad *et al*., 2019; Rzewuska *et al*., 2019; Liu *et al*., 2024). It also produces the haemolytic exotoxin pyolysin (PLO) that destroys red blood cells from mammals and participates in β-hemolysis on blood agar (Tamai *et al*., 2018; Zhang *et al*., 2021). It is the most prominent bacterium engaged in the pathogenicity of bovine clinical endometritis, metritis, and abortion due to its ability to remain in the uterus, wide resistance to antibiotics and synergy with Gram-negative bacteria (Maarouf *et al*., 2013; Tamai *et al*., 2018; Rezanejad *et al*., 2019; Liu *et al*., 2024).

T. pyogenes is thought of as a troublesome bacterium as it has cell-associated virulence factors responsible for promoting adherence, including fimbriae, extracellular matrix-binding proteins and neuraminidases, capability to build biofilms, where all of them play a role in bovine clinical endometritis and metritis (Flemming *et al*., 2016; Sharma *et al*., 2019; Zhang *et al*., 2021). The ability of *T. pyogenes* to form biofilms enhances their resistance to antimicrobial drugs, improves their adhesion properties, and permits improved defense against the cows' immunological response (Jost & Billington, 2005; Kasimanickam *et al*., 2016). The biofilm production is managed by the PloS/PloR two-component regulatory system, where PloR enhances the expression of biofilm (Zhao *et al*., 2017). Moreover, *T. pyogenes* presence in the endometrium is linked to tissue destruction due to the pyolysin's cytolytic action against the endometrial stromal cells, that are especially vulnerable to this cholesterol-dependent toxin (Amos *et al*., 2014; Griffin *et al*., 2018; Rzewuska *et al*., 2019).

Conventional treatment of bovine uterine diseases typically involves the use of intrauterine or systemic antibiotics, such as ceftiofur, ampicillin, and tetracyclines. However, the frequent and sometimes excessive use of these antibiotics in dairy herds has contributed to the emergence of antimicrobial resistance, which can reduce therapeutic effectiveness and pose a significant risk to public health. Consequently, there has been a growing interest in non-conventional therapies, including herbal extracts, essential oils, probiotics, bacteriophages, vaccines, nanoparticles, biomaterials, and ozone, as promising alternatives for the management of uterine infections in dairy cows (Paiano & Baruselli, 2022).

Even though *T. pyogenes* has long been known from earlier research, barely is known about its molecular and antimicrobial resistance-based characteristics in endometritis cases (Rezanejad *et al*., 2019; Liu *et al*., 2024). In clinical endometritis and metritis cases, the primary therapeutic option is the administration of broad-spectrum antibiotics; tetracyclines, fluoroquinolones, beta lactams, and macrolides are some of the preferred drugs in these situations (Haimerl *et al*., 2017; Tamai *et al*., 2018; Petkova & Rusenova, 2022). However, misuse of antibiotics in animal husbandry as growth inhibitors, preventative measures, and/or treatments has caused various bacterial species, including *T. pyogenes*, to build antimicrobial resistance (Santos *et al*., 2010; Tamai *et al*., 2018). Additionally, the failure of antibacterial therapy in cows with endometritis and metritis has been linked to the pathogens' ability to produce biofilms (Ozturk *et al*., 2016; Petkova & Rusenova, 2022). The treatment of endometritis and metritis is challenging due to the intrinsic and acquired resistance of *T. pyogenes* to a wide spectrum of antimicrobials, including aminoglycosides, tetracyclines, macrolides, and β-lactams, encoded by existence of antimicrobial resistance genes such as aminoglycosides resistance genes *aadA1* & *aadA2*; *bla* β-lactam resistance gene and *tet(W)* tetracyclines resistance gene (Santos *et al*., 2010; Zhang *et al*., 2014; 2017; Rezanejad *et al*., 2019; Liu *et al*., 2024).

Following the identification of the causal agent, antimicrobial susceptibility testing and molecular determination of antibiotic resistance genes are required in order to select the best course of treatment. Therefore, our research was set to detect the prevalence and phenotypic traits of *T. pyogenes* strains responsible for subclinical and clinical endometritis cases at Kaliobia governorate, Egypt, to identify their antimicrobial profile and to perform genotypic identification of some antibiotic resistance genes in certain isolates.

MATERIALS AND METHODS

Ethical approval

The Bioethics Committee has approved the proposal entitled "Phenotypic characteristics, antimicrobial profile and antibiotic resistance genes of *Trueperella pyogenes* associated with bovine endometritis" as meeting the requirements of the Faculty of Veterinary Medicine, Benha University research ethics, Egypt under approval number BUFVTM 17-03-25.

Specimens collection

A total of 110 uterine swab samples were collected from dairy cows presented to various veterinary clinics in Kaliobia Governorate, Egypt, between September 2021 and February 2025. The sampled cows included 75 animals (93–98 days in milk) suffering from repeat breeding and diagnosed with subclinical endometritis, and 35 animals (24–33 days in milk) diagnosed with clinical endometritis. None of the cows had received antibiotics or anti-inflammatory drugs prior to the study. All cows underwent a comprehensive clinical examination, including vaginal inspection and rectal palpation. Subclinical endometritis was diagnosed based on endometrial cytology using the low-volume uterine lavage technique, as described by Gilbert *et al*. (2005). Cows were considered to have subclinical endometritis when the proportion of polymorphonuclear neutrophils (PMNs) in the cytological sample exceeded 5%, a diagnostic threshold widely accepted in the literature (Wagener *et al*., 2017). Clinical endometritis was diagnosed based on the presence of purulent or mucopurulent vaginal discharge, following the criteria established by Sheldon *et al*. (2006). Following a clinical diagnosis, uterine swab samples were obtained from animals by a special catheter of Noakes *et al*. (1989) modified by Maarouf *et al*. (2013). Each swab was transferred directly to screw-capped tubes containing nutrient broth beside the flame and transferred as quickly as possible to the laboratory for isolation, phenotypic detection of *T. pyogenes* strains and genotypic identification of some antibiotic resistance genes in certain isolated strains.

Power analysis

To evaluate the statistical adequacy of the sample size, a *post-hoc* power analysis was conducted comparing the prevalence of *T. pyogenes* between cows with clinical (68.6%) and subclinical (8%) endometritis (n=35 vs. n=75). The analysis demonstrated a power of approximately 99.9%, indicating that the sample size was sufficient to detect the observed difference. Furthermore, a Chi-square test showed a statistically significant association between the clinical status and *T. pyogenes* isolation ($\chi^2 = 44.14$, $P<0.0001$), supporting the robustness of the findings.

Isolation and phenotypic characterisation of T. pyogenes

After inoculating 1 mL of each screw-capped tube in 9.0 mL of brain heart infusion (BHI) broth, the tubes were incubated for 24 hours at 37 °C in a microaerophilic environment (5% CO_2) to initial enrichment for *T. pyogenes* isolation. A loopful of each of enrichment cultures was streaked onto BHI agar (Himedia) enriched with 5% fresh defibrinated sheep blood and Baird Parker agar (Oxoid); consequently, incubation was made under the same conditions for 24 to 72 h. Suspected colonies were gathered and maintained on semisolid slope nutrient medium for more thorough morphological and biochemical determination, including by catalase, oxidase, urease, CAMP tests (*S. aureus* serving as indicator), indole, Voges-Proskauer, nitrate reduction, methyl red, gelatin hydrolysis, esculin hydrolysis, and fermentation of glucose, lactose, maltose, sucrose and mannitol, according to Malinowski *et al.* (2011) and Tamai *et al*. (2018).

Antimicrobial susceptibility testing for T. pyogenes isolates

The antimicrobial resistance (AMR) profiles of the isolated *T. pyogenes* strains were determined using the disc diffusion method, following the Clinical and Laboratory Standards Institute guidelines

(CLSI, 2018). Twelve standardised antimicrobial discs (Oxoid) were employed: amoxicillin/clavulanic acid (AMC/30); co-trimoxazole (COT/25); ampicillin (AM/10); cefotaxime (CTX/30); cephapirin (CEPR/30); doxycycline (DO/30); ciprofloxacin (CIP/5); gentamicin (GEN/10); oxacillin (Ox/10); norfloxacin (NOR/10); tetracycline (TE/30) and streptomycin (S/10). The test was performed on Mueller–Hinton agar (Oxoid) plates supplemented with 5% defibrinated sheep blood. Inoculated plates were incubated at 37 °C in a 5% CO_2 atmosphere for 24–48 h before interpretation of inhibition zones. Due to the lack of specific CLSI interpretive criteria for *T. pyogenes*, inhibition zones were interpreted based on CLSI breakpoints established for closely related Gram-positive organisms, as supported by Rzewuska *et al*. (2019). The interpretation of inhibition zone diameters for *T. pyogenes* isolates is summarised in Table 1.

Biofilm formation test

All *T. pyogenes* isolates were tested for phenotypic virulence activities, biofilm formation by Congo Red (CR) agar method following Hassan *et al*. (2011) using CRA medium (brain heart infusion broth (Oxoid, UK) 37 g/L, sucrose 50 g/L, agar No. 1 (Oxoid, UK) 10 g/L and Congo red dye (Oxoid, UK) 8 g/L). Colonies of black colour with a dry crystalline consistency showed strong biofilm formation; a darkening of the colonies with the absence of a dry crystalline colonial morphology revealed an indeterminate result and remained pink or pale colonies indicated negative result.

PCR detection of antibiotic resistance genes

Five random *T. pyogenes* isolates that exhibited antibiotic resistance by the disk diffusion method and strong phenotypic biofilm formation were examined for three antibiotic resistance genes: β-lactam (*bla*), streptomycin (*aad*A1) and tetracycline *tet*(W) genes by conventional PCR, following QIA amp® DNA Mini Kit instructions (Qiagen, Germany, GmbH; Cat. no. 51304), Emerald Amp GT PCR master-mix (Takara, Japan) with Code No. RR310A and 1.0% agarose gel electrophoreses (Sambrook *et al*., 1989) using primer sequences, target genes, amplicons sizes and cycling conditions shown in Table 2. A positive control DNA was obtained from a *T. pyogenes* field isolate confirmed in the Reference laboratory for veterinary quality control on poultry production (RLQP), Dokki, Giza, Egypt. Conversely, the negative control was a PCR mixture without the DNA template.

Statistical analysis

Data entry, coding, and analysis were conducted using the Statistical Package for the Social Sciences (SPSS), v. 23 (IBM Corp., Armonk, NY, USA, 2015). The data in this study included both quantitative and qualitative variables. Qualitative data were expressed as frequencies (n) and percentages (%), while parametric quantitative data were presented as means ± standard deviation (SD). Pearson's chi-square (χ^2) test was used to assess the significance of association between categorical variables. One-way analysis of variance (ANOVA) was used to compare parametric data across more than two groups. The level of statistical significance was set at 95% ($P<0.05$), while a P-value < 0.01 was considered highly significant. When multiple comparisons were performed, the Bonferroni correction was applied to adjust the significance threshold by dividing the original alpha value (0.05) by the number of comparisons, in

Table 1. Interpretation of inhibition zone diameters for the 30 *T. pyogenes* isolates

Antimicrobial agents		Disc concentrations	Zone of inhibition (mm)					
			n	Sensitive	n	Intermediate	n	Resistant
Tetracycline	TE	30 µg	1	25	2	18.00±1.41	27	9.41±2.39
Oxacillin	Ox	10 µg	1	15	3	11.67±0.58	26	6.54±1.94
Ampicillin	AM	10 µg	3	22.67±2.52	4	15.00±0.81	23	8.69±2.60
Streptomycin	S	10 µg	3	19.33±1.53	5	12.80±0.84	22	7.32±2.19
Co-Trimoxazole	COT	1.25/23.75 µg	4	21.00±1.83	5	12.80±1.64	21	6.62±1.94
Doxycycline	DO	30 µg	7	21.86±2.27	8	14.50±0.93	15	9.27±1.98
Gentamicin	GEN	10 µg	22	19.45±2.24	5	13.40±0.54	3	9.67±0.58
Cephapirin	CEPR	30 µg	21	22.29±2.19	6	16.00±0.89	3	12.00±1.00
Ciprofloxacin	CIP	5 µg	20	23.90±1.62	4	18.50±1.29	6	12.67±1.63
Norfloxacin	NOR	10 µg	20	21.45±2.48	7	14.29±1.11	3	9.67±0.58
Amoxicillin/clavulanic acid	AMC	30µg	17	22.88±1.41	4	18.00±0.81	9	12.44±1.94
Cefotaxime	CTX	30 µg	16	25.75±1.69	5	18.80±2.59	9	11.22±1.39
Test of Significance P-value				F=11.939 <0.0001		F=8.336 <0.0001		F=12.109 <0.0001

n: number of isolated strains.

Table 2. Oligonucleotide primer sequences and PCR conditions

Target genes		Primer sequences (5'-3')	Amplicon size (bp)	References
bla	F R	CAGTCTAGCCACTTCGCCAAT TGACTGCACGGATGGAGATGG	808	Alibi *et al.* (2017)
*aad*A1	F R	CGGTGACCGTAAGGCTTGAT ATGTCATTGCGCTGCCATTC	193	Gulaydin *et al.*(2024)
tet(W)	F R	GACAACGAGAACGGACACTATG CGCAATAGCCAGCAATGAACGC	1843	Gulaydin *et al.*(2024)

PCR conditions

Target genes	Primary denaturation	Amplification (35 cycles)			Final extension
		Secondary denaturation	Annealing	Extension	
bla	94˚C; 5 min	94˚C; 30 s	55˚C; 40 s.	72˚C; 50 s.	72˚C; 10 min
*aad*A1	94˚C; 5 min	94˚C; 30 s	60˚C; 30 s.	72˚C; 30 s.	72˚C; 7 min
tet(W)	94˚C; 5 min	94˚C; 30 s	63˚C; 1 min	72˚C; 2 min	72˚C; 12 min

order to minimise the risk of Type I error (false positives).

RESULTS

Bacteriological examination of 110 uterine swab samples from cows diagnosed with endometritis revealed that *T. pyogenes* was isolated in 30 cases (27.3%). Among these, 6 out of 75 repeat-breeding cows with subclinical endometritis (8%) and 24 out of 35 cows with clinical endometritis (68.6%) tested positive for *T. pyogenes*. The difference in prevalence between the two groups was statistically significant (χ^2 = 44.141, P<0.001).

Phenotypic identification of T. pyogenes

Every recovered isolate grew well and displayed pinpoint, smooth, convex, slightly translucent, dry, circular colonies with β-hemolysis zone on BHI agar enriched with 5% sheep blood and small, dark grey, opaque colonies on Baird Parker agar. The microscopic examination, demonstrated Gram-positive, non-capsulated, non-sporulated, coccobacilli to short-chain bacilli appearing in pairs or single arrangements. All 30 isolates had the characteristic biochemical reactions of *T. pyogenes* – positive results for gelatin hydrolysis and CAMP tests with haemolysis enhanced by *S. aureus,* but negative results in oxidase, indole, catalase, urease, nitrate reduction, Voges-Proskauer, methyl red and esculin tests. The isolates fermented glucose, sucrose, lactose and maltose into acid without gas formation but were negative for mannitol. All isolates were non-motile on semisolid agar.

Antimicrobial susceptibility testing

The results of the antimicrobial sensitivity testing of the 30 isolated *T. pyogenes* exhibited that the isolated strains were highly resistant to tetracycline (90.0%) followed by oxacillin (86.7%), ampicillin (76.7%), streptomycin (73.3%), co-trimoxazole (70.0%) and doxycycline (50.0%) (Table 3). Meanwhile, they were sensitive to gentamicin (73.3%); cephapirin

Table 3. The antimicrobial susceptibility testing for 30 isolated *T. pyogenes*

Antimicrobial agents		Disc concentrations	Sensitive n	Sensitive %	Intermediate n	Intermediate %	Resistant n	Resistant %	AA
Tetracycline	TE	30 µg	1	3.3	2	6.7	27	90.0	R
Oxacillin	Ox	10 µg	1	3.3	3	10.0	26	86.7	R
Ampicillin	AM	10 µg	3	10.0	4	13.3	23	76.7	R
Streptomycin	S	10 µg	3	10.0	5	16.7	22	73.3	R
Co-Trimoxazole	COT	1.25/23.75 µg	4	13.3	5	16.7	21	70.0	R
Doxycycline	DO	30 µg	7	23.2	8	26.7	15	50.0	R
Gentamicin	GEN	10 µg	22	73.3	5	16.7	3	10.0	S
Cephapirin	CEPR	30 µg	21	70.0	6	20.0	3	10.0	S
Ciprofloxacin	CIP	5 µg	20	66.7	4	13.3	6	20.0	S
Norfloxacin	NOR	10 µg	20	66.7	7	23.3	3	10.0	S
Amoxicillin/ clavulanic acid	AMC	30 µg	17	56.7	4	13.3	9	30.0	S
Cefotaxime	CTX	30 µg	16	53.3	5	16.7	9	30.0	S

n: number of isolated strains; % Percent relative to the total number of T. pyogenes strains (n=30)
AA: antibiogram activity

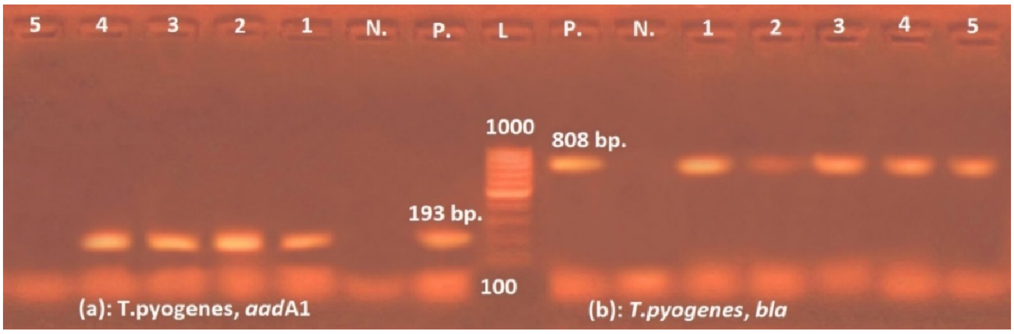

Fig. 1. A) PCR screening for streptomycin (*aad*A1) gene: (L) DNA ladder 100-1000 bp., P: positive control (*T. pyogenes* from RLQP at 193 bp.), N: negative control, lanes 1-4: positive amplification of *aad*A1gene in *T. pyogenes* at 193 bp.; lane 5: Negative *T. pyogenes* at 193 bp.; **B)** PCR screening for β-lactam (*bla*) gene: (L) DNA ladder 100-1000 bp., P: positive control (*T. pyogenes* from RLQP at 808 bp.), N: negative control; lanes 1-5: positive *T. pyogenes* at 808 bp.

(70.0%); ciprofloxacin and norfloxacin (66.7% for each); amoxicillin/clavulanic acid (56.7%) and cefotaxime (53.3%). A highly significant difference in susceptibility patterns was detected among the tested antimicrobials (χ^2=132.011, P<0.001).

CR assay results for the 30 isolated *T. pyogenes* demonstrated that all isolates were CR positive. Among them, 26 isolates (86.7%) were grown as blackish red colonies (strong biofilm formation) and four isolates (13.3%) from mild endometritis produced red colonies (moderate biofilm formation).

Genotyping identification results of antibiotic resistance genes revealed that the β-lactam (*bla*) gene was present in the five studied *T. pyogenes* isolates giving 808 bp products and four strains exhibited streptomycin (*aad*A1), tetracycline *tet*(W) genes giving 193bp and 1843 bp products, respectively (Fig. 1 and Fig. 2).

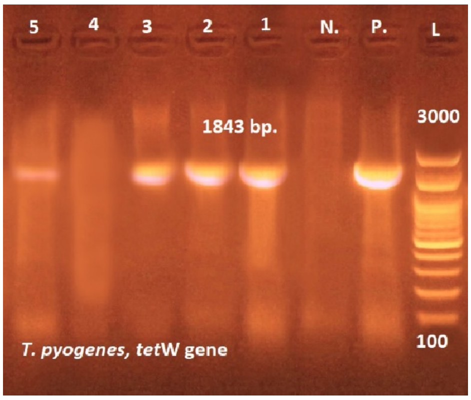

Fig. 2. PCR screening for tetracycline *tet*(W) gene. L: DNA ladder 100-1000 bp., P: positive control (*T. pyogenes* from RLQP at 1843 bp.), N: negative control, lanes 1-3 & 5: positive amplification of *tet(W)* gene in *T. pyogenes* at 1843 bp., lane 4: Negative *T. pyogenes* at 1843 bp.

DISCUSSION

The pathogenic bacterium *T. pyogenes* is especially closely linked to endometritis in cows (Liu *et al*., 2024). Our research was conducted to characterise *T. pyogenes* as a significant cause of subclinical and clinical endometritis in cows. Isolation, identification, screening of antibiotic sensitivity profiles, detection of some antibiotic resistance genes were performed during this study.

The obtained bacteriological results cleared that only six *T. pyogenes* isolates were recovered from 75 uterine swabs of repeat breeder cows with subclinical endometritis; meanwhile, 24 *T. pyogenes* strains were isolated from 35 cows with clinical endometritis. This may be due to the destructive effects of *T. pyogenes* on the environment of the uterus and its synergistic effect with other pathogens as *E. coli*, *S. aureus* and anaerobic Gram-negative bacteria. The same findings were reported by other reserachers (Maarouf *et al*., 2013; Drillich & Wagener, 2018; Wang *et al*., 2018; Umer *et al*., 2022; Mekibib *et al*., 2024; Liu *et al*., 2024).

Phenotypical properties ─ Gram reaction; β-hemolysis, CAMP and biochemical profile of isolated *T. pyogenes* were consistent with those from previous reports (Malinowski *et al*., 2011; Brodzki *et al*., 2014; Tamai *et al*., 2018; Rezanejad *et al*., 2019; Paiano *et al*., 2022).

Development of bacterial resistance to antibiotics is a worldwide issue. Antibiotic resistance is mostly caused by overuse of antimicrobial medicines, inadequate treatment, poor medication selection, and transfer of antibiotic resistance genes between bacteria like *T. pyogenes* (Rowe-Magnus *et al*., 2002). Antibiotics are frequently used to treat clinical endometritis without information on the susceptibility of the bacterial pathogens causing the uterine infections. The antimicrobial profiles of the 30 isolated *T. pyogenes* showed that the isolates were highly resistant to tetracycline followed by oxacillin, ampicillin, streptomycin, co-trimoxazole and doxycycline; yet were sensitive to gentamicin, cephapirin, ciprofloxacin, cefotaxime, amoxicillin/clavulanic acid and norfloxacin. Almost the same results were noted by Liu *et al*., (2009; 2024), Santos *et al*. (2010), Zhang *et al*. (2017), Tamai *et al*. (2018). Additionally, our results demonstrated that phenotypic antimicrobial resistance was widely spread among isolated *T. pyogenes* strains in line with earlier data (Tamai *et al*., 2018; Rezanejad *et al*., 2019; Liu *et al*., 2024).

Biofilm production was recognised as a key mechanism detecting the antimicrobial resistance of *T. pyogenes*, its virulence and inducing chronification of uterine infections (Markey *et al*., 2013; Hall & Mah, 2017). The current study reported that all recovered *T. pyogenes* isolated strains were phenotypically CR positive and formed biofilm as previously found (Rzewuska *et al*., 2019; Zhang *et al*., 2021; Petkova & Rusenova, 2022).

The current investigation assured that all 30 identified strains of *T. pyogenes* were phenotypically resistant to antimicrobial drugs. These results are concerning because the antimicrobials being studied (mainly β-lactam, sulfonamide, tetracyclines, and streptomycin) are still generally thought to be the most effective therapies for endometritis and other uterine infections in cows (Mileva *et al.*, 2020; 2022).

A few genotypic investigations on *T. pyogenes* resistance were published in Egyptian literature, with an emphasis on the existence of genes linked to the synthesis of β-lactamase, and genes conferring resistance against tetracyclines and streptomycin. PCR results showed that all five studied *T. pyogenes* strains had the β-lactam (*bla*) gene, and four strains carried streptomycin (*aad*A1) and tetracycline *tet*(W) genes. These findings are in line with several previous studies that reported the presence of antimicrobial resistance genes in *T. pyogenes* isolates from bovine endometritis. Specifically, the detection of the *bla* gene in our isolates is in line with-data of Santos *et al.* (2010), Zhang *et al.* (2014), Tamai *et al.* (2018) and Rezanejad *et al.* (2019) who confirmed the prevalence of this β-lactam resistance gene in *T. pyogenes*. Similarly, the *aad*A1 gene, associated with aminoglycoside resistance, was also identified in our study and has been previously reported (Santos *et al.*, 2010; Tamai *et al.*, 2018; Rezanejad *et al.*, 2019; Gulaydin *et al.*, 2024). In addition, we detected the *tet*(W) gene, which confers tetracycline resistance similar to other research findings (Billington & Jost, 2006; Liu *et al.*, 2009; Zhang *et al.*, 2017; Tamai *et al.*, 2018; Rezanejad *et al.*, 2019; Yapicier *et al.*, 2022).

CONCLUSIONS

T. pyogenes is a significant bacterium implicated in bovine clinical endometritis with antimicrobial resistance to the majority of antibiotics. The ability to form biofilm and the presence of antibiotic resistance genes in the isolated strains of *T. pyogenes* contributed their pathogenicity in these cases.

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