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Phenotypic characterization of bacterial isolates from the genital tract of dairy cows with endometritis

Hussien, A.M. Abdul Alime[1], Ahmed, A.A. Maarouf[2], Hayat Fayed[3], Ashraf, A. Abd El Tawab[1]

[1]*Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*
[2]*Department of Bacteriology, Immunology & Mycology, Animal Health Research Institute "Benha branch" (ARC), Egypt.*
[3]*Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*

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ABSTRACT

The present study aimed to investigate the prevalence and phenotypic characteristics of uterine pathogens in 110 pluriparous cows (75 cows with repeat breeding associated with subclinical endometritis and 35 cows with clinical endometritis) from various veterinary clinics in Qalyubia Governorate, Egypt, during the period from September 2021 to February 2025. Uterine swab samples were collected and subjected to bacteriological culture and phenotypic characterization, and antimicrobial resistance profiles. The bacteriological examination revealed that bacterial pathogens were isolated from 97 of the positive uterine swabs (88.2%), with 26 (26.8%) being single pure cultures and 71 (73.2%) mixed cultures. Among these, 64 out of 75 samples (85.3%) and 33 out of 35 samples (94.3%) were positive for bacterial pathogens from subclinical and clinical endometritis swabs, respectively. Antimicrobial resistance profiles showed that *Staphylococcus aureus* (*S. aureus*) was resistant to oxacillin, tetracycline, ampicillin, and streptomycin, but sensitive to norfloxacin, gentamicin, ciprofloxacin, amoxicillin/clavulanic acid, cephapirin, and cefotaxime. *Escherichia coli* (*E. coli*) was resistant to oxacillin, ampicillin, tetracycline, cefotaxime, cephapirin, and streptomycin, but sensitive to norfloxacin, gentamicin, ciprofloxacin, and co-trimoxazole. *Trueperella pyogenes* (*T. pyogenes*) was resistant to tetracycline, oxacillin, ampicillin, streptomycin, co-trimoxazole, and doxycycline, but sensitive to gentamicin, cephapirin, ciprofloxacin, norfloxacin, amoxicillin/clavulanic acid, and cefotaxime. In conclusion, *S. aureus*, *E. coli*, and *T. pyogenes* were the predominant multidrug-resistant (MDR) pathogens associated with subclinical and clinical endometritis in dairy cows.

1. INTRODUCTION

Pathogenic bacterial infections of the female reproductive tract can be either subclinical or clinical and pose a significant concern in dairy cattle production systems (Bakht et al., 2024; Várhidi et al., 2024). These infections, affecting the ovaries, vulva, vagina, cervix, and uterus, are closely linked to infertility (Neel et al., 2018; Rosales and Ametaj, 2021), uterine infections are of significant concern and are classified into five categories: clinical and subclinical endometritis, pyometra, clinical metritis, and puerperal metritis (Bakht et al., 2024). *Escherichia coli* (*E. coli*) typically initiates inflammation and, along with endotoxins (lipopolysaccharides), acts synergistically with *Trueperella pyogenes* (*T. pyogenes*) and *Fusobacterium necrophorum*, which are recognized as major uterine pathogens linked to metritis, endometritis, and purulent vaginal discharge (Rzewuska et al., 2019; Mekibib et al., 2024). These pathogens alter the pH of cervical, vaginal, and uterine mucus, damaging the uterine mucosa, disrupting implantation, and leading to endometritis, pregnancy failure, and infertility in cattle (Lima, 2020). Meanwhile, coagulase-positive *Staphylococcus aureus* (*S. aureus*) and other pathogens are considered potential causative agents and contaminants in bovine genital tract infections, particularly subclinical and clinical endometritis (Bakht et al., 2024).

Antimicrobial agents are commonly used to treat bovine genital tract infections, particularly endometritis, especially in developing countries (Neel et al., 2018; Mekibib et al., 2024). However, their indiscriminate use has contributed to the emergence of antimicrobial resistance (AMR) among causative bacteria, resulting in poor treatment outcomes (Bakht et al., 2024; Mekibib et al., 2024). Therefore, performing an antibiogram is essential for selecting the most effective antimicrobial therapy.

Current understanding of bacterial genital tract infections, particularly endometritis, and the antimicrobial susceptibility of their causative agents remains limited in Egypt. This study aimed to determine the prevalence and phenotypic characteristics of uterine pathogens in pluriparous cows with subclinical and clinical endometritis from 2021-2025 in Qalyubia Governorate, Egypt.

2. MATERIAL AND METHODS

Ethical approval

The Bioethics Committee has approved the proposal entitled "Phenotypic characteristics of some bacteria isolated from the female genital tract of dairy cows affected with endometritis" to meet the requirements of research ethics of the Faculty of Veterinary Medicine, Benha University, Egypt, under approval number (BUFVTM 05-07-25).

2.1. Animals

This study was conducted on 110 pluriparous cows (75 with repeat breeding and subclinical endometritis, and 35 with clinical endometritis) from various veterinary clinics in Qalyubia Governorate, Egypt, between September 2021 and February 2025.

* Correspondence to: hayat.fayed@fvtm.bu.edu.eg

2.2. Uterine sample collection

Uterine samples were collected using a modified catheter technique (Noakes et al., 1989; Maarouf et al., 2013). Uterine samples were collected using a sterilized guarded swab catheter. Each cow underwent a clinical vaginal and rectal examination, and the perineal area and vulva were thoroughly cleaned and disinfected. The metal guard tube containing the swab was inserted into the vagina and guided to the uterine horn. The swab was then exposed and pressed firmly against the endometrium approximately 5 cm from the horn bifurcation for one minute. Finally, the swab was retracted into the tube to prevent contamination and removed from the uterus. Each swab was immediately placed in nutrient broth (Oxoid) within sealed tubes, transferred under sterile conditions, and quickly transported to the laboratory for bacterial isolation and phenotypic identification.

2.3. Isolation and identification of bacterial pathogens from uterine swabs

Bacterial pathogens were isolated and identified from uterine swabs using standard protocols for each species. *T. pyogenes* was identified following the method of Malinowski et al. (2011) and Tamai et al. (2018). *Enterobacteriaceae* isolates were identified according to ISO 16649-2 (2001), Quinn et al. (2011), and Markey et al. (2013). *Pseudomonas* species were identified based on Quinn et al. (2011) and Markey et al. (2013). *Bacillus cereus* isolation was performed according to the methods described by Rhodehamel and Harmon (2001) and Quinn et al. (2011). *Enterococci* were identified using the methods of Facklam and Collins (1989), Domig et al. (2003), and Markey et al. (2013). *Streptococcus* species were identified according to Markey et al. (2013) and Public Health England (2014), while *Staphylococcus* species were identified following Arora (2003) and Markey et al. (2013).

2.4. In Vitro antimicrobial sensitivity testing of S. aureus, E. coli, and T. pyogenes isolates

In vitro antimicrobial sensitivity testing was performed on *S. aureus, E. coli, and T. pyogenes* isolates using 12 antimicrobial discs (Oxoid): amoxicillin/clavulanic acid (AMC/30), ampicillin (AM/10), cefotaxime (CTX/30), cephapirin (CEPR/30), ciprofloxacin (CIP/5), co-trimoxazole (COT/25), doxycycline (DO/30), gentamicin (GEN/10), oxacillin (Ox/10), norfloxacin (NOR/10), streptomycin (S/10), and tetracycline (TE/30). The tests were conducted on Mueller-Hinton agar (Oxoid) plates following the CLSI (2018) disc diffusion method.

3. RESULTS

3.1. Prevalence of bacterial pathogens isolated from examined uterine swabs

Bacteriological examination of uterine swabs revealed that 97 out of 110 samples (88.2%) were positive for bacterial pathogens, including 26 (26.8%) single and 71 (73.2%) mixed cultures, as shown in Table 1. Among cows with subclinical endometritis, 64 out of 75 samples (85.3%) were positive, with 23 (35.9%) being single and 41 (64.1%) being mixed. In clinical endometritis cases, 33 out of 35 samples (94.3%) were positive, with 3 (9.1%) single and 30 (90.9%) mixed cultures.

3.2. Percentage of bacterial pathogens isolated from examined uterine samples

Bacteriological examination of uterine swabs (Table 2) revealed a total of 221 bacterial isolates recovered from 97 positive samples out of 110 cows affected with subclinical and clinical endometritis.

3.3. Phenotypic identification of isolated Trueperella pyogenes

Microscopically, *T. pyogenes* appeared as Gram-positive, non-capsulated, non-sporulated coccobacilli or short-chain bacilli, typically arranged singly or in pairs. On brain heart infusion agar (Hi-media) with 5% sheep blood, colonies were pinpoint, smooth, convex, slightly translucent, dry, and circular with a β-hemolysis zone. On Baird-Parker agar (Oxoid CM0275), they formed small, dark grey, opaque colonies. Biochemically, all 30 isolates were positive for gelatin hydrolysis and CAMP tests (with enhanced hemolysis near *S. aureus*), and negative for oxidase, catalase, urease, nitrate reduction, indole, Voges-Proskauer, methyl red, and esculin tests. They fermented glucose, sucrose, lactose, and maltose into acid without gas production, but did not ferment mannitol. All isolates were non-motile on semisolid agar.

3.4. Phenotypic identification of isolated Escherichia coli

The isolated *Escherichia coli* exhibited pink colonies on MacConkey agar (Oxoid), indicative of lactose fermentation, and showed a characteristic metallic green sheen on Eosin methylene blue (EMB) agar (Oxoid). On selective media, it produced yellow-green colonies on Brilliant Green agar, yellow colonies on Xylose Lysine Deoxycholate (XLD) agar (LAB M), and blue colonies on Tryptone Bile X-glucuronide (TBX) agar (Oxoid), consistent with β-glucuronidase activity. Biochemical testing revealed that the isolate was oxidase-negative, indole-positive, Voges-Proskauer (VP)-negative, citrate-negative, and urease-negative. The Triple sugar iron (TSI) test showed an acid slant and butt (yellow/yellow) without gas or hydrogen sulfide production. These findings confirm the identification of *E. coli* as a lactose-fermenting, Gram-negative bacillus lacking H_2S production.

3.5. Phenotypic identification of isolated Pseudomonas species

All 19 *Pseudomonas* isolates were identified as *P. aeruginosa*, appearing as Gram-negative, non-sporulated, motile rods (100% motility), medium-sized, straight or slightly curved with rounded ends. Colonies were large, flat, spreading, and irregular with a greenish-blue pigment and characteristic fruity odor on nutrient agar (Oxoid). On MacConkey agar (Oxoid No. CM115), they formed large, pale, non-lactose fermenting colonies with a superimposed green-blue pigment. Colonies on Pseudomonas agar (Hi-media) and Cetrimide agar (Oxoid) were blue-green, and on blood agar (LAB28) showed irregular colonies with β-hemolysis and distinct pyocyanin pigment. Biochemically, all isolates were positive for oxidase, catalase, citrate, urease, lysine decarboxylase, and fermented glucose and mannitol, but negative for H_2S production, indole, methyl red, Voges-Proskauer, sucrose, and lactose fermentation.

3.6. Phenotypic identification of isolated Bacillus cereus

Microscopically, *Bacillus cereus* isolates appeared as Gram-positive, short rod-shaped, spore-forming bacteria. On Polymyxin-Pyruvate-Egg Yolk-Mannitol-Bromothymol blue agar (PEMBA), colonies were blue to turquoise with a surrounding blue zone of egg yolk precipitation on a greenish-yellow background. On blood agar (LAB28), colonies were large, greyish, circular, smooth, glistening, and surrounded by a clear β-hemolysis zone.

Biochemically, all six isolates were positive for catalase, citrate utilization, nitrate reduction, Voges-Proskauer, starch hydrolysis, and gelatin liquefaction. They were negative for

oxidase, indole, and urease tests. All isolates showed 100% motility on semisolid agar.

3.7. Phenotypic identification of isolated Enterococcus faecalis

All eight *Enterococcus* isolates were identified as *E. faecalis*. On bile esculin agar (Oxoid), they formed pinpoint colonies with a black precipitate, indicating bile tolerance and esculin hydrolysis. On blood agar (LAB28), colonies appeared white, grey, or greenish (α-hemolysis), round, and smooth. Microscopically, the isolates were Gram-positive cocci, typically in pairs or short chains.

Biochemically, all were positive for Voges-Proskauer and nitrate reduction tests, and grew in 6.5% NaCl, at pH 9.6, and at both 10°C and 45°C. They were negative for oxidase, catalase, H_2S production, urease, indole, and citrate utilization, and were non-motile.

3.8. Phenotypic identification of isolated Streptococcus species

All 11 *Streptococcus* isolates appeared microscopically as Gram-positive cocci in short chains. On blood agar (LAB28), colonies were minute, translucent, and showed either α- or β-hemolysis. Biochemically, all isolates were catalase-negative and aesculin-positive. They fermented glucose, sucrose, lactose, and maltose into acid without gas production, but did not ferment mannitol. All isolates were non-motile on semisolid agar.

3.9. Phenotypic identification of isolated Staphylococcus species

The isolated *Staphylococcus* species included *S. aureus* and *S. epidermidis*. *S. aureus* formed yellow, convex colonies on 7% salted nutrient agar (Oxoid), black shiny colonies with yellow halos on Baird-Parker agar (Oxoid CM0275) [due to tellurite reduction], and smaller clear zones indicating proteolytic activity. On Mannitol salt agar (BBLTM 211407), *S. aureus* produced yellow colonies with mannitol fermentation and halo formation; on 7% salted milk agar (Oxoid), yellow colonies caused a color change due to lipase activity. Blood agar (LAB28) colonies were smooth, round, shiny, with alpha or beta hemolysis.

In contrast, *S. epidermidis* showed white, raised convex colonies on nutrient agar (Oxoid); black colonies on Baird-Parker agar (Oxoid CM0275); white colonies without mannitol fermentation on Mannitol salt agar (BBLTM 211407); white colonies on salted milk agar (Oxoid); and smooth, round, shiny, non-hemolytic colonies on blood agar (LAB28).

3.10. In Vitro antimicrobial susceptibility of 33 Staphylococcus aureus isolates

The in vitro antimicrobial sensitivity testing of the isolated *Staphylococcus aureus* strains demonstrated high resistance to oxacillin (87.9%), followed by tetracycline (81.8%), ampicillin (72.7%), and streptomycin (60.6%). The isolates showed intermediate sensitivity to co-trimoxazole (57.6%) and doxycycline (51.5%). Conversely, they were largely sensitive to norfloxacin (84.8%), gentamicin (78.8%), ciprofloxacin (72.7%), amoxicillin/clavulanic acid (69.7%), cephapirin (60.6%), and cefotaxime (57.6%).

3.11. In Vitro antimicrobial susceptibility of 54 Escherichia coli isolates

The in vitro antimicrobial sensitivity testing of 54 *Escherichia coli* isolates revealed high resistance to oxacillin (85.2%), followed by ampicillin (81.5%), tetracycline (79.6%), cefotaxime (74.1%), cephapirin (70.4%), and streptomycin (59.3%). The isolates exhibited intermediate sensitivity to doxycycline (63.0%) and amoxicillin/clavulanic acid (57.4%), while showing sensitivity to norfloxacin (77.8%), gentamicin (68.5%), ciprofloxacin (66.7%), and co-trimoxazole (61.1%).

3.12. In Vitro antimicrobial susceptibility of 30 Trueperella pyogenes isolates

The in vitro antimicrobial sensitivity testing of 30 *Trueperella pyogenes* isolates revealed high resistance to tetracycline (90.0%), followed by oxacillin (86.7%), ampicillin (76.7%), streptomycin (73.3%), co-trimoxazole (70.0%), and doxycycline (50.0%). Conversely, the isolates were sensitive to gentamicin (73.3%), cephapirin (70.0%), ciprofloxacin (66.7%), norfloxacin (66.7%), amoxicillin/clavulanic acid (56.7%), and cefotaxime (53.3%).

Table (1) Total number and percentage of positive uterine swabs for bacterial pathogen isolation

Swabs samples	Number of examined samples	Negative swabs		Positive swabs		Prevalence of single and mixed cultures			
						Single		Mixed	
		No.	%[1]	No.	%[1]	No.	%[2]	No.	%[2]
Repeat breeder cows with subclinical endometritis	75	11	14.7	64	85.3	23	35.9	41	64.1
Cows with clinical endometritis	35	2	5.7	33	94.3	3	9.1	30	90.9
Total	110	13	11.8	97	88.2	26	26.8	71	73.2

%1 Percentage relative to the total number of samples in each group (75, 35, and 110, respectively). %2 Percentage relative to the number of positive samples in each group.

Table (2) Prevalence of bacterial isolates from examined uterine samples

Sample types	Repeat breeder cows with subclinical endometritis				Cows with clinical endometritis				Total isolates from 97 Positive samples	
Bacterial Isolate	Total isolates from 64 Positive samples		S.	M.	Total isolates from 33 Positive samples		S.	M.		
	No.	%[1]	No.	No.	No.	%[1]	No.	No.	No.	%[2]
Escherichia coli	35	26.9	11	24	19	20.9	0	19	54	24.4
Trueperella pyogenes	6	4.6	0	6	24	26.4	3	21	30	13.6
Staphylococcus aureus	22	16.9	9	13	11	12.1	0	11	33	14.9
Staphylococcus epidermidis	9	6.9	0	9	2	2.2	0	2	11	5.0
Klebsiella pneumoniae	4	3.1	0	4	3	3.3	0	3	7	3.2
Klebsiella oxytoca	4	3.1	0	4	6	6.6	0	6	10	4.5
Proteus mirabilis	8	6.2	1	7	5	5.5	0	5	13	5.9
Proteus vulgaris	7	5.4	0	7	3	3.3	0	3	10	4.5
Pseudomonas aeruginosa	13	10.0	2	11	6	6.6	0	6	19	8.6
Streptococcus spp.	6	4.6	0	6	5	5.5	0	5	11	5.0
Enterobacter cloacae	4	3.1	0	4	2	2.2	0	2	6	2.7
Enterococcus faecalis	6	4.6	0	6	2	2.2	0	2	8	3.6
Salmonellae	0	0	0	0	1	1.1	0	1	1	0.5
Citrobacter freundii	2	1.5	0	2	0	0.0	0	0	2	0.9
Bacillus cereus	4	3.1	0	4	2	2.2	0	2	6	2.7
Total	130	100.0	23	107	91	100.0	3	88	221	100.0.

S: Single bacterial isolate M: Mixed bacterial isolates %[1] Percentage relative to total isolates within each sample group (130 for subclinical, 91 for clinical). %[2] Percentage relative to total isolates from all positive samples (221).

4. DISCUSSION

There is a growing scientific interest in understanding the microbiota of the genital tract of dairy cows. Research in this area is fundamental to identifying the most prevalent pathogens responsible for uterine diseases, particularly subclinical and clinical endometritis, which are recognized as the primary causes of bovine infertility. Among the various risk factors, pathogenic bacterial infections have been highlighted as the most prominent contributors to the development of endometritis. Furthermore, mixed infections involving more than one pathogenic bacterium are considered the principal etiological determinant of this disease (Paiano et al., 2022; Zhang et al., 2024).

In this study, 97 uterine swabs (88.2%) yielded positive bacterial cultures, of which 26 (26.8%) were single isolates and 71 (73.2%) were mixed. Among repeat breeder cows with subclinical endometritis, 64 samples (85.3%) were culture-positive, including 23 single (35.9%) and 41 mixed isolates (64.1%). In clinically affected cows, 33 out of 35 samples (94.3%) were positive, with 3 single (9.1%) and 30 mixed cultures (90.9%). These findings are consistent with those reported by Umer et al. (2022), and Mekibib et al. (2024). The high proportion of mixed cultures indicates the polymicrobial nature of uterine infections, where interactions between pathogens intensify inflammation and hinder uterine clearance. This likely explains the higher culture-positivity in clinically affected cows, in agreement with previous studies that highlighted the role of co-infections in the pathogenesis of bovine endometritis.

Bacteriological examination of 110 uterine swab samples from cows with subclinical and clinical endometritis revealed 97 culture-positive samples, from which a total of 221 bacterial isolates were recovered (Table 2). *Escherichia coli* was the most frequently isolated pathogen (54 isolates; 24.4%), followed by *Staphylococcus aureus* (33; 14.9%), *Trueperella pyogenes* (30; 13.6%), *Pseudomonas aeruginosa* (19; 8.6%), and *Proteus mirabilis* (13; 5.9%). Other isolates included *Staphylococcus epidermidis* and *Streptococcus spp.* (11 each; 5.0%), *Klebsiella oxytoca* and *Proteus vulgaris* (10 each; 4.5%), *Enterococcus faecalis* (8; 3.6%), *Klebsiella pneumoniae* (7; 3.2%), *Bacillus cereus* and *Enterobacter cloacae* (6 each; 2.7%), *Citrobacter freundii* (2; 0.9%), and *Salmonella spp.* (1; 0.5%). These findings align with previous reports by Maarouf et al. (2013), Lima (2020), Rosales and Ametaj (2021), Paiano et al. (2022), and Várhidi et al. (2024). The predominance of *E. coli* confirms its well-recognized role as a primary uterine pathogen, often acting as an initial colonizer that facilitates secondary infections. The frequent detection of *S. aureus* and *T. pyogenes* further supports their significance as opportunistic pathogens associated with persistent endometritis. The presence of mixed bacterial isolates in the uteri of cows with subclinical and clinical endometritis may contribute to a more prolonged and severe disease course (Adiguzel et al., 2021).

From 64 culture-positive uterine swabs collected from repeat breeder cows with subclinical endometritis, a total of 130 bacterial isolates were identified. *Escherichia coli* was the predominant isolate (35; 26.9%), followed by *Staphylococcus aureus* (22; 16.9%), *Pseudomonas aeruginosa* (13; 10.0%), *Staphylococcus epidermidis* (9; 6.9%), *Proteus mirabilis* (8; 6.2%), *Proteus vulgaris* (7; 5.4%), and *Enterococcus faecalis*, *Streptococcus* spp., and *Trueperella pyogenes* (6 each; 4.6%). Other isolates included *Bacillus cereus*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae* (4 each; 3.1%), and *Citrobacter freundii* (2; 1.5%). *Salmonella* spp. were not detected. These findings are consistent with those recorded by Bedewy and Rahaway (2019), and Pascottini et al. (2020). The predominance of *E. coli* in subclinical cases suggests its ability to persist in the uterine environment without producing overt clinical signs, possibly by modulating the local immune response. The frequent recovery of opportunistic pathogens such as *S. aureus* and *P. aeruginosa* further indicates that compromised uterine defenses in repeat breeder cows may favor secondary colonization and establishment of mixed infections.

Similarly, in cows diagnosed with clinical endometritis, a total of 91 bacterial isolates were obtained from 33 culture-positive uterine swabs. *Trueperella pyogenes* was the most frequently isolated organism (24; 26.4%), followed by *Escherichia coli* (19; 20.9%), *Staphylococcus aureus* (11; 12.1%), *Klebsiella oxytoca* and *Pseudomonas aeruginosa* (6 each; 6.6%), *Proteus mirabilis* and *Streptococcus* spp. (5 each; 5.5%), *Klebsiella pneumoniae* and *Proteus vulgaris* (3 each; 3.3%), and *Bacillus cereus*, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Staphylococcus epidermidis* (2 each; 2.2%). In addition, a single isolate of *Salmonella* spp. (1.1%) was detected. These findings are in agreement with those mentioned by Maarouf et al. (2013), Bakht et al. (2024), Mekibib et al. (2024), and Zhang et al. (2024). The high prevalence of *T. pyogenes* among clinically affected cows highlights its central role in the pathogenesis of uterine infections, as it produces potent virulence factors such as pyolysin that damage endometrial tissue and exacerbate inflammation. The frequent isolation of *E. coli* and *S. aureus* further suggests that these pathogens often act synergistically with *T. pyogenes*, contributing to the severity and chronicity of clinical endometritis. The detection of opportunistic bacteria such as *Klebsiella spp.* and *Pseudomonas spp.* may also reflect compromised uterine defense mechanisms, which create favorable conditions for secondary infections.

The preliminary identification of bacterial pathogens isolated from uterine swabs was based on cell morphology, colony characteristics on various culture media, and biochemical profiles. These characteristics were consistent with those previously described by Quinn et al. (2011), Adiguzel et al. (2021), Paiano et al. (2022), Bakht et al. (2024), and Zhang et al. (2024).

Regarding the antimicrobial susceptibility of the isolated *Staphylococcus aureus*, *Escherichia coli*, and *Trueperella pyogenes*, in vitro testing revealed distinct resistance patterns. *S. aureus* isolates showed high resistance to oxacillin, followed by tetracycline, ampicillin, and streptomycin. Intermediate sensitivity was observed to co-trimoxazole and doxycycline, while high sensitivity was noted to norfloxacin, gentamicin, ciprofloxacin, amoxicillin/clavulanic acid, cephapirin, and cefotaxime. These findings are consistent with Bakht et al. (2024). The observed resistance patterns likely reflect the widespread and repeated use of these antibiotics in dairy farms, which can promote the selection of resistant strains. The high susceptibility to fluoroquinolones and cephalosporins suggests that these antimicrobials remain effective options for treating infections caused by *S. aureus*, while careful antibiotic stewardship is essential to prevent further development of resistance.

Similarly, *E. coli* isolates exhibited high resistance to oxacillin, followed by ampicillin, tetracycline, cefotaxime, cephapirin, and streptomycin. In contrast, they were highly sensitive to norfloxacin, followed by gentamicin, ciprofloxacin, and co-trimoxazole. These results align with those declared by Maarouf et al. (2013), Raheel et al. (2020), and Zhang et al. (2024). In the case of *T. pyogenes*, isolates

demonstrated high resistance to tetracycline, oxacillin, ampicillin, streptomycin, co-trimoxazole, and doxycycline. However, they were sensitive to gentamicin, cephapirin, ciprofloxacin, norfloxacin, amoxicillin/clavulanic acid, and cefotaxime. Comparable findings were noted by Zhang et al. (2017), Tamai et al. (2018), and Liu et al. (2024). Furthermore, the results revealed phenotypic resistance to at least two different classes of antimicrobials among the isolated *S. aureus*, *E. coli*, and *T. pyogenes* strains, indicating a multi-drug resistant (MDR) profile. These findings are consistent with previous reports by Bakht et al. (2024), Mekibib et al. (2024), and Zhang et al. (2024). Therefore, antibiogram profiling is essential for selecting the most appropriate and effective antimicrobial agents for the treatment and control of uterine infections.

5. CONCLUSIONS

In conclusion, the present study demonstrated that intrauterine pathogenic bacteria—particularly *Escherichia coli*, *Trueperella pyogenes*, and *Staphylococcus aureus*—which exhibited multidrug resistance (MDR), are the primary causative agents of subclinical and clinical endometritis. Other infections may be associated with poor hygienic conditions. Based on the antimicrobial susceptibility results, gentamicin, norfloxacin, ciprofloxacin, and amoxicillin/clavulanic acid are recommended for effective control and treatment.

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