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ISOLATION AND STUDY OF THE MOLECULAR CHARACTERISTICS OF MOST GRAM-NEGATIVE BACTERIA FROM CASES OF GENITAL TRACT INFECTIONS IN COWS

FRAH NOOH [1] AND BALSAM Y. RASHEED [2]

[1] Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq
[2] Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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ABSTRACT

Bacterial infections of the genital tract are among the most important problems facing livestock herds, leading to infertility, abortion, and poor meat production. Our research aimed to isolate and molecularly study the most crucial Gram-negative microorganisms responsible for genital tract infections in cows, investigating their susceptibility to the most significant antibiotics, and identifying the most important virulence factors. *Escherichia coli* had the highest isolation rate of 44%, followed by *Proteus mirabilis* at 7.4%, and the lowest percentage was for *Klebsiella* spp., *Serratia* marcescens, and *Enterobacter* spp., at 1.5%. Through the study, *E. coli* was the most isolated in cases of metritis, endometritis, and vaginitis. It was also isolated at a high rate in cases of retained placenta and dystocia, which are considered predisposing factors for reproductive system infections at 33.3% and 20%, respectively. *E. coli* has shown absolute resistance to the antibiotics Imipenem, Tetracycline, and Cefixime. At the same time, *Proteus mirabilis* showed absolute resistance to Azithromycin, Tetracycline, and Nitrofurantoin. The *UidA* gene was used to confirm the *E. Coli* isolates, and virulence factors were detected by PCR using specific primers for the *stx1* and *stx2* genes. All the isolates of *Proteus mirabilis* contained the virulence genes *zapA* and *ureC*.

Keywords: Genital tract infection*, E.coli, Proteus* spp*,* PCR.

INTRODUCTION

Reproductive efficiency in farm animals and cattle breeding fields plays a crucial role in determining the production rate from an economic perspective. Reproductive capacity and animal efficiency are affected by pathogenic infections resulting from the presence of pathogens, some of which are non-specific (Patel *et al.,* 2019). The reproductive efficiency of cows is significantly affected by problems that occur in the genital tract, which negatively affect it, such as repeated reproduction, retained fetal membranes, abortion, uterine secretions, mastitis, vaginal and uterine prolapse, stillbirth, and dystocia (Hussain *et al.,* 2024). The most common genital tract infections and diseases are salpingitis, oophoritis, uteritis, endometritis, cervicitis, and vaginitis. Among the diseases affecting modern dairy cows, metritis and endometritis are the most studied and economically important

Corresponding author: BALSAM Y. RASHEED
Email address: balsamyr20@uomosul.edu.iq
Present address: Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

(Channo *et al.,* 2022). Damage to the uterus occurs as a result of stillbirth, retained placenta, cesarean section, twin births, or metabolic disorders such as milk fever and ketosis; damage that occurs as a result of gastric displacement; also, to an imbalance in the immune system due to weak white blood cell function, all of which led to damage to the genital tract and thus expose it to bacterial infections (Safdarian *et al.,* 2023). The source of infection, the subsequent birth, the animal's diet, and postpartum problems are all linked to the presence and variety of bacteria in the uterus (Adnane & Chapwanya, 2022). Microbes present in the vagina and contamination through feces are a source of colonization of the uterine lining, causing infections of the genital tract. *Escherichia coli*, *Proteus* spp., *Gardnerella vaginalis*, *Trueperella pyogenes*, and Gram-negative bacteria are among the most important bacteria that cause uterine disorders and infections (Ong *et al.,* 2021). *Escherichia coli* is naturally present in the microbiome, where it contributes to maintaining the balance of organisms., is also present in the large intestine of infants for hours after birth (Murphy *et al.,* 2021). These bacteria live symbiotically, causing no harm, producing vitamins, maintaining immune balance, and creating a barrier against pathogenic bacteria that would otherwise seize the opportunity to cause infection (Aleksandr-owicz *et al.,* 2021). Many researchers have confirmed that it is the most important and widely studied on a global scale (Puvača & Frutos, 2021). There are various types of *Escherichia coli*, including one that is pathogenic within the intestine, another that causes infections outside the intestine (Akhardanesh *et al.,* 2016), and a third type that is symbiotic and does not cause harm to the host (Osinska *et al.,* 2023). Certain isolates of *E. coli* cause intestinal or extraintestinal diseases in both humans and animals, such as hemolytic uremic syndrome, hemorrhagic colitis, mild diarrhea, and urinary tract infections (Karshenas *et al.,* 2024). These bacteria caused diarrhea in young cows, which later led to endometritis and inflammation of the uterus (Morris *et al.,* 2023). It is thought to be one of the most significant bacteria that cause uterine infections and the most frequent cause of cow uterine contamination in the initial weeks following calving (De Cassia Picudo *et al.,* 2019; Schouten *et al.,* 2023). Several factors, including the presence of *E. coli*, influence the frequency and severity of uterine diseases in cows (Garzón *et al.,* 2024). Recently, *E. coli* has been identified as a cause of endometritis in dairy cows carrying the *cdt, astA, ibeA, hlyA, stx, hlyE, fyuA*, and *fimH* genes, which are responsible for pathogenicity (Keyang *et al.,* 2023). It is responsible for the most common diseases in animals and was isolated from the vagina of cows (Das *et al.,* 2025). It causes serious infections in the genital tract, leading to decreased fertility, in addition to inflammation of the uterus and its lining after birth, which causes infertility due to the extended period between birth and pregnancy (Conzalez *et al.,* 2020; Agrawal *et al.,* 2021). Pathogenic *E. coli* produces Stx1 and Stx2. Shiga toxins, also known as type A-B toxins, cause hemolytic uremic syndrome and hemorrhagic colitis by preventing the production of proteins. The genes *stx1* and *stx2* encode them, due to their bloodborne transmission, these bacteria have been connected to uterine infections and endometritis in numerous mammals (Cagnoli *et al*., 2024). Compared to strains obtained from the uterus of clinically healthy animals, *E. coli* strains that cause a terminal inflammatory response were more invasive and adherent to endometrial tissue in vitro (Raheel *et al.,* 2020). Several studies in the field of livestock breeding have reported that the uterus of pregnant cows contains zoonotic *Proteus mirabilis* (Liu *et al.,* 2023). *Proteus mirabilis* possesses the *ureC* gene, which is crucial in the production of the urease enzyme, which helps raise the pH, thereby reducing the viability of other organisms. They also

possess the *zapA* gene, which works to break down the IgA (Gaston *et al*., 2021). Most studies have confirmed that the presence of the *ureC* gene increases the pathogenicity of *P. mirabilis*, and a synergistic effect is observed with high expression of the *ureC* and *zapA* genes (Phan *et al.,* 2008). Other bacteria found in uterine diseases include (*Acinetobacter* spp, *Trueperella* spp, *Fusobacteria* spp., and *Peptostrept*ococci spp. (Rosales and Ametaj, 2021). There are several methods used to diagnose infections of the uterus, endometrium, and vagina, including vaginal speculum examination (Ault *et al.,* 2019), rectal examination of the uterus, uterine biopsy, and the most important of which is the cultivation of vaginal secretions and fluids on media specific to most bacteria, which international companies provide. In recent years, genetic diagnostic methods, such as the polymerase chain reaction test, have become the best diagnostic methods (Rasheed *et al.,* 2024). In order to identify the most prominent solutions in the field of animal breeding, the *stx1*, *stx2*, *zapA*, and *UreC* genes were chosen and molecularly diagnosed due to their significance and impact on the genital system.

MATERIALS AND METHODS

Ethical approval

Under the guidance of a specialist committee, the work was approved by the University of Mosul's College of Veterinary Medicine Ethics Committee, which issued number UM.2024.039. All relevant procedures and instructions were adhered to. Remove

Study region

The study was conducted in several locations throughout the Nineveh Governorate, including the veterinary hospital in Bab Sinjar and the districts of Nimrud and Shamsiyat, from September 2024 to February 2025. These locations were picked because they are easily accessible and have a comparatively high number of dairy cows.

Isolation and identification of gram-negative bacteria

The vulva was cleaned with an antiseptic. A sterile speculum was used to guide the swab into the vagina. Swabs were collected from infected cows that were experiencing excessive discharge between 5 and 30 days postpartum (Mekibib *et al.,* 2024). The swabs were placed in Tryptone soya broth medium supplied from [HiMedia, India] until they were delivered to the Laboratory. Swabs were cultivated on blood and MacConkey agar supplied from [HiMedia, India] and incubated at 37°C for 24 h (Rasheed *et al.,* 2024). After staining, the VITIK 2 compact system was used for confirmatory diagnosis, which involves dividing the bacteria into Gram-positive and Gram-negative categories. After the diagnosis was completed, only the Gram-negative bacteria with the highest percentage were taken for study purposes. Gram-negative bacteria were subcultured in heart infusion agar to obtain single colonies, and these were saved on a Slanta (Talaro and Chess, 2018).

Antibiotic susceptibility testing

Antibiotic sensitivity testing was used, using the disc diffusion method, measuring the area of inhibition, comparing it with the standard, and selecting the most resistant isolates of 23 *E. coli and 4 Proteus mirabilis* (Naji *et al.,* 2023).

DNA Extraction

DNA was extracted according to Geneaid Biotech Ltd, Taiwan, Catalogue No: GBB100, as instructed by the manufacturer, and the obtained DNA was kept at -20°C (Rasheed *et al.,* 2024).

Amplification of DNA

Primers and their information are illuminated in Table (1). A confirmatory test

for the diagnosis of *E. coli* was performed using primers specific to the *UidA* gene forward 5" CCA AAA GCC AGA CAG AGT "3 and reverse 5"GCA CAG CAC ATC AAA GAG"3 (Al-Aalim *et al.,* 2022). At the same time, the *Stx1*and *Stx2* genes were detected (Momtaz *et al.,* 2012). A confirmatory test for the diagnosis of *Proteus mirabilis* was carried out with primers designed specifically for the *16S rRNA* gene forward 5"AGAGTTTGATCC-TGGCTCA"3 and reverse 5"TACGGTT-ACCTTGTTACGACTT"3 (Abbas *et al.,* 2015). At the same time, the *ZapA* and *UreC* genes were detected (Alchalaby *et al.,* 2025).

Table 1: Sequences of the virulence factors primer and PCR product (*stx1,stx2, UidA)* for *E.coli* and (16S RNA, *zapA & ureC)*for *Proteus mirabilis*

No	Primer	Sequence	Size bp	Reference
1	*Stx1*	5" CGA TCG TCA CTC ACT GGT TTC ATC A"3	366	Momtaz *et al.,*2012
		5"TGC CAT TCT GGC AAC TCG CGA TGC A"3		
2	*Stx2*	5"CGA TCG TCA CTC ACT GGT TTC ATC A"3	282	
		5 "GGA TAT TCT CCC CAC TCT GAC ACC"3		
3	*Uid A*	5"CCA AAA GCC AGA CAG AGT"3	623	Al-Aalim *et al.,*2022
		5"GCA CAG CAC ATC AAA GAG"3		
4	16S RNA	5"AGAGTTTGATCCTGGCTCAG"3	1500	Abbas *et al.,*2015
		5"TACGGTTACCTTGTTACGACTT"3		
5	*zapA*	5"ACCGCAGGAAAACATATAGCCC"3	540	Alchalaby *et al.,*2025
		5"GCGACTATCTTCCGCATAATCA"3		
6	*ureC*	5"GTTATTCGTGATGGTATGGG"3	317	
		5" ATAAAGGTGGTTACGCCAGA"3		

RESULTS

The results of bacterial isolation from a total of 70 samples from cows that exhibited clinical signs revealed the isolation of 48 single isolates, 10 mixed isolates, and 12 cases that did not yield any bacterial growth. The highest percentage of isolation of *Escherichia coli* bacteria was 44%, followed by *Proteus mirabilis* with 7.4%. Also, *Klebsiella* spp., *Serratia ficaria*, and *Enterobacter* spp. had the same percentage of 1.5%. Then, *Staphylococcus* spp. was represented by *5 bacteria: Staphylococcus lentus* (3%), *Staphylococcus chromogenes* (1.5%), and *Staphylococcus xylosus* (1.5%). As for the rest of the isolates, their percentages differed, as shown in Table (2); only the Gram-negative bacteria with the highest percentage were selected for study purposes.

Among 26 cases of metritis, 30 isolates were identified, with the largest share being E. coli, accounting for 50%. In the case of endometritis, among 17 cases, 10 isolates were diagnosed with E. coli, at 66.6%. Regarding vaginitis, there were four cases, with one isolate diagnosed for each of E. coli, Proteus mirabilis, and Enterococcus faecium, at a rate of 20% for each (Table 3).

In the study of the risk factors for cows contracting genital tract infections, 6 cases of retained placenta were recorded and 6 isolates were diagnosed, the highest percentage was for *E. coli* at 33.3%, and 6 cases of dystocia, at 5 isolates for each of *E. coli*, *Enterobacter* spp, *Serratia ficaria*, *Staph—xylosus* and *Enterococcus faecalis*, at a concentration of 20%. Ten cases of abortion were recorded, and 6 isolates for each of *Gardnerella vaginalis* and *Kocuria rhizophila*, at 16.6%. One case of milk fever was recorded from which *E. coli* was isolated, at a rate of 100% (Table 4).

Table 2: Frequency of gram-positive, gram-negative, and mixed bacterial infections

	Bacteria	No.	%
Gram negative	*Escherichia coli*	30	44
	Proteus mirabilis	5	7.4
	Klebsiella spp	1	1.5
	Serratia ficaria	1	1.5
	Enterobacter spp	1	1.5
Gram positive	*Staphy. Lentus*	2	3
	Staphy. chromogenes	1	1.5
	Staphy. Xylosus	1	1.5
	Enterococcus faecium	3	4.4
	Enterococcus faecalis	1	1.5
	Gardnerella vaginalis	1	1.5
	Kocuria rhizophila	1	1.5
Mix	*Bacillus spp Staphy.* .spp	2	3
	Bacillus spp +*Kocuria varians*	2	3
	Bacillus spp+*Strept* spp	2	3
	Candida spp+*Staphy.* spp	2	3
	Proteus mirabilis+*Staph.* spp	2	3.
	Enterococcus faecium+*E.coli*	8	11.7
	Kocuria spp+*E.coli*	2	3
Total		68	100

Table 3: Number of isolates from vaginitis, metritis, and endometritis together with their kinds

Isolates	Metritis (n= 26)	Endometritis (n=17)	Vaginitis (n=4)
Escherichia coli	15(50)	10(66.6)	1(20)
Proteus mirabilis	2(7)	2((13.3)	1(20)
Klebsiella spp	1(3.3)	0(0)	0(0)
Staphylococcus lentus	1(3.3)	1(6.6)	0(0)
Staphylococcus chromogenes	1(3.3)	0(0)	0(0)
Enterococcus faecium	2(7)	0(0)	1(20)
Bacillus spp *Staphylococcus* spp	2(7)	0(0)	0(0)
Proteus mirabilis+*Staphylococcus* spp	2(7)	0(0)	0(0)
Enterococcus faecium+*E.coli*	4(13.3)	2(13.3)	0(0)
Candida spp+*Staphylococcus* spp	0	0	2(40)
Total isolates	30	15	5

*Between brackets is a percentage.

Twenty-three *E. coli* samples showed complete resistance to imipenem, tetracycline, and cephalosporin antibiotics at a rate of 100%, followed by 83% resistance to neomycin. In contrast, the samples were susceptible to ofloxacin. The resistance of the isolates to the remaining types of antibiotics used varied (Table 5). As for *P. mirabilis*, they showed complete resistance to azithromycin, nitrofurantoin, and tetracycline, and were completely sensitive to the other types of antibiotics used (Table 5).

Table 4: Association of risk factors with the number of bacterial isolates

Isolates	Retained placenta (n= 6)	Dystocia (n= 6)	Abortion (n=10)	Milk fever (n=1)
Escherichia coli	2(33.3)	1(20)	0	1(100)
Serratia ficaria	0	1(20)	0	0
Enterobacter spp	0	1(20)	0	0
Staphylococcus xylosus	0	1(20)	0(0)	0
Gardnerella vaginalis	0	0(0)	1(16.6)	0
Enterococcus faecalis	0	1(20)	0	0
Kocuria rhizophila	0	0	1(16.6)	0
Bacillus spp+*Kocuria varians*	0	0	2(33.3)	0
Bacillus spp+*Streptococcus* spp	2(33.3)	0	0	0
Enterococcus faecium+*E.coli*	2(33.3)	0	0	0
Kocuria spp+*E.coli*	0	0	2(33.3)	0
Total isolates	6	5	6	1

* Between brackets is a percentage

Table 5: *Proteus mirabilis* and *E. coli* that were isolated from genital tract infections showed antibacterial resistance

Antimicrobials		Con/(µg)	Disc diffusion (mm)		No (%) of resistant *E. coli*	No (%) of resistant *Pro. mirabilis*
			R	S		
β-lactams	Imipenem (IPM)	10	≤ 19	≥ 23	23(100)	0
Macrolides	Azithromycin (AZM)	30	≤ 22	≥ 28	8(34)	4(100)
Tetracyclines	Tetracycline (TE)	30	≤ 14	≥ 19	23(100)	4(100)
Aminoglycoside	Neomycin (N)	30	≤ 12	≥ 17	19(83)	
Sulfonamides	Trimethoprim/ Sulpha-methoxazole (SXT)	25	≤ 13	≥ 17	1(4.3)	0
Miscellaneous	Chloramphenicol (C)	30	≤ 12	≥ 18	12(52)	0
Quinolone	Ciprofloxacin (CIP)	5	≤ 15	≥ 21	2(8.6)	0
	Ofloxacin (OFX)	5	≤12	≥ 16	0(0)	0
Nitrofuran	Nitrofurantoin (F)	300	≤ 14	≥ 17	4(17)	0
Cephalosporin	Cefixime (CFM)	5	≤15	≤19	23(100)	4(100)

Only 23 isolates, which exhibited the highest level of antibiotic resistance, were chosen for the research. All 23 *E. coli* isolates used in the study were positive for the genus-specific gene Uid, according to the results of antibiotic sensitivity tests (Figure 1). The *stx1* and *stx2* genes were detected in all isolates (Figures 2 and 3). The *zapA* and *ureC* genes are present in all *Pro—mirabilis* (Figures 5,6).

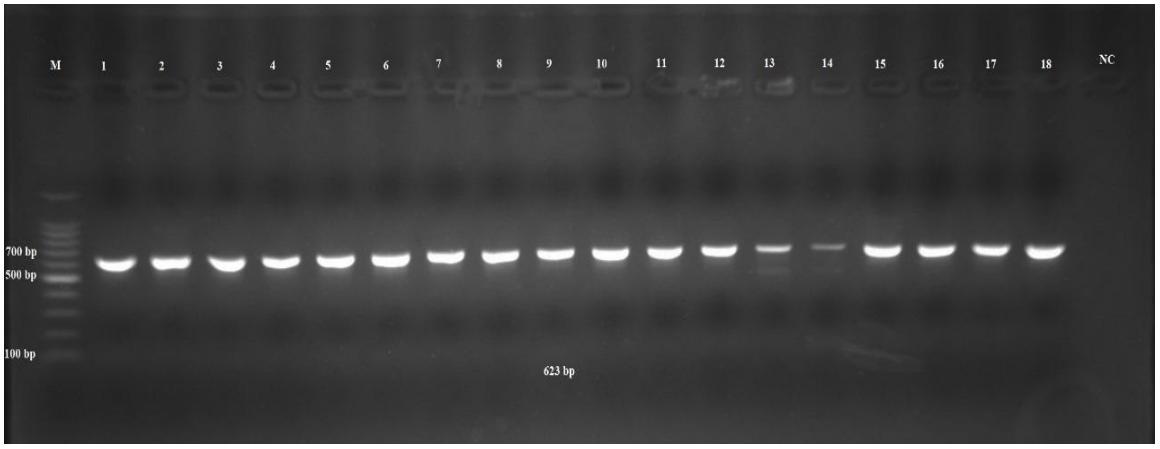

Figure 1: Detection of *the UidA* gene of E.coli M: Ladder, lanes (1-18 positive) *UidA* gene (623 bp), lanes NC are negative.

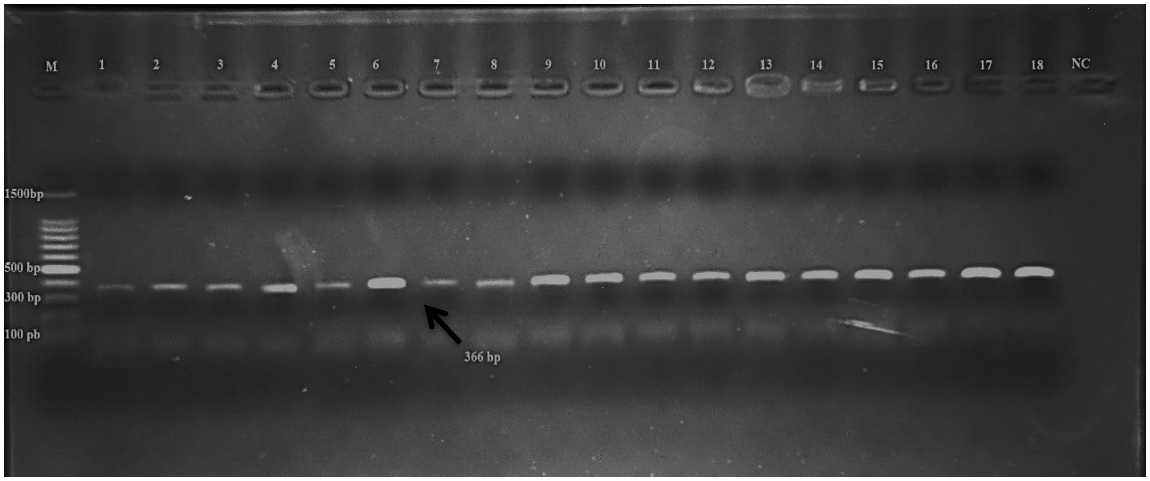

Figure 2: Detection of the *stx* 1gene of *E.coli*. M: Ladder, lanes (1-18 positive), *stx 1* gene (366 bp), lanes NC are negative.

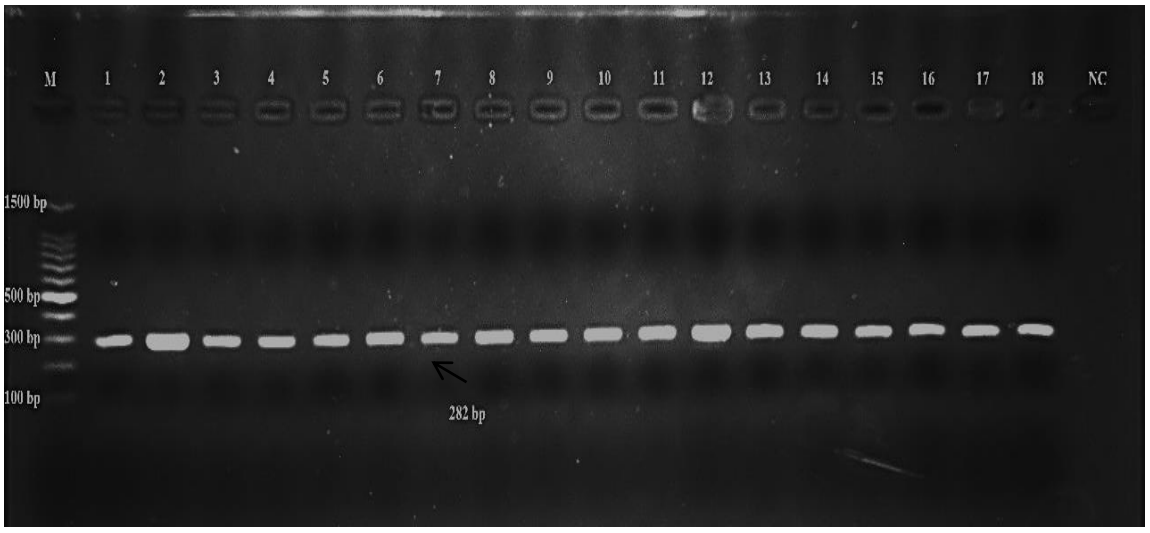

Figure 3: Detection of the *stx 2* gene of *E.coli*. M: Ladder, lanes (1-18 positive), *stx 2* gene (282 bp), lanes NC are negative.

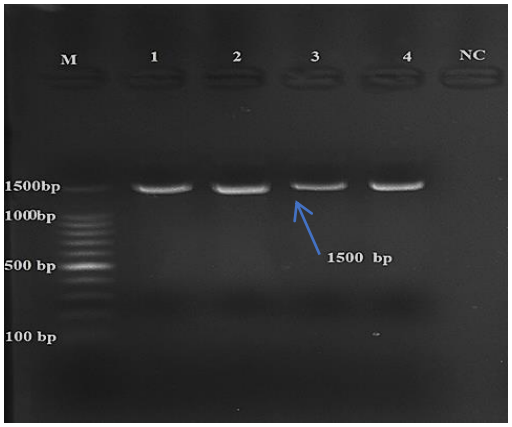

Figure 4: Molecular confirmation of *Proteus mirabilis* isolates according to the sequence of *16S rRNA* (M: Ladder, (1-4 positive) for *Proteus mirabilis* at 1500 bp).

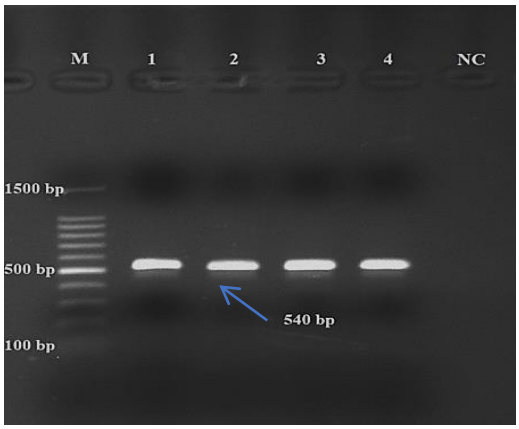

Figure 5: Detection of the *Zap A* gene of *Proteus mirabilis*. M: Ladder, lanes (1-4 positive), *ZapA* gene (540 bp), lanes NC are negative

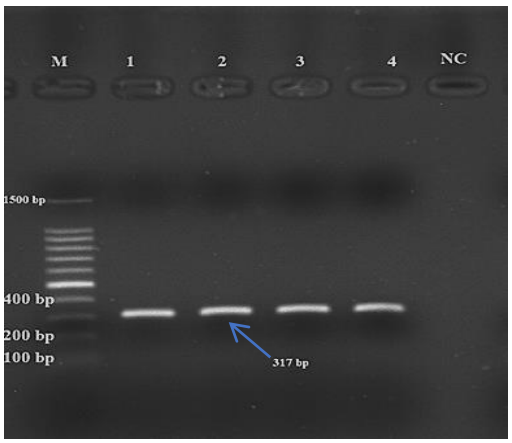

Figure 6: Detection of *the ureC* gene of *Proteus mirabilis*. M: Ladder, lanes (1-4 positive), *ureC* gene (317 bp), lanes NC are negative

DISCUSSION

Endometritis and metritis can have a significant impact on the economy of countries through their effect on reduced milk production and reproductive capacity, leading to infertility and the resulting increase in treatment costs that force farmers who raise cows to slaughter them in line with the situation (Bromfield *et al.,* 2015). Infectious bacteria in the uterus lead to inflammation and tissue lesions in the endometrium, which postpone uterine contraction. Additionally, it interferes with cows' ovulation or the development and operation of ovarian follicles following calving. Therefore, uterine disease is associated with longer intervals between calving and first birth or pregnancy, and poorer pregnancy rates (Sharma *et al.,* 2017). Because bacteria alter metabolism, their presence has a significant role in the development of endometritis and metritis (Udhayavel *et al.,* 2013). Although there are many causes of endometritis and metritis, many studies have focused on *E. coli* (Agrawal *et al.,* 2021).

E. coli accounted for 44% of the bacteria recovered from metritis and endometritis cases in our investigation, while *Proteus mirabilis* came in second at 7.4%. During calving or as a result of contamination, these bacteria enter the uterus first (Williams *et al.,* 2007). Numerous researchers in this field have confirmed this result, as *E. coli* was the most frequently isolated bacterium, at a rate of 45% (Mekibib *et al.,* 2024). Because the anus is situated adjacent to the reproductive system, numerous studies have proposed that feces may be present in the area around the reproductive system. Additionally, contaminated water sources, unhygienic environments, poor cow hygiene, and the cleanliness of animal bedding and flooring all contribute to the infection of this species (Andriniani *et al.,* 2021). As noted during parturition, the cervix, an anatomical and immunological barrier, opens, allowing

bacteria to ascend from the vagina or pass through it from the environment or feces. New research on cows indicates that uterine pathogens may spread hematogenously from the gut to the uterus. The migration of white blood cells to the uterus as calving approaches and to the uterus after calving, carrying bacteria through phagocytosis, is responsible for delivering bacteria to the uterine cavity after calving (Jones *et al.,* 2022). This was determined in our study through genetic diagnosis, as most *E. coli* isolates were positive for the presence of the stx gene, which is responsible for the production of Shiga toxin. This is, in fact, because these bacteria are transmitted from the intestines through the circulatory system to the uterus and are responsible for causing disease in cows. Additionally, the generative vascular changes that occur immediately after birth may allow pathogens floating freely in the bloodstream to enter the uterus (Joen *et al.,* 2017).

Klebsiella pneumoniae was isolated at a rate of 2.4% from cases of metritis. This rate was slightly different from what Udhayavel had obtained, which was the isolation of the aforementioned researcher from cases of endometritis at a rate of 30% after *E. coli* bacteria (Udhayavel *et al.,* 2013). While some researchers have reported the presence of *Bacillus spp* in the early postpartum period, which has been closely linked to the development of puerperal metritis in dairy cows, the presence of Bacillus bacteria in small quantities may be the result of contamination that occurs during sample collection (Bicalho *et al*., 2012). *Candida spp* appeared in our results at a low rate, due to their presence in the environment, skin, and even feces and vagina. Research has also reported the presence of yeast and uterine colonization in cows suffering from specific conditions, such as infertile cows, cows with excessive reproduction, cows with chronic endometritis, prolonged intrauterine antibiotic treatment, and those with reduced immunity (Ribeiro *et al.,* 2024).

Many bacterial species, such as *Shigella dysenteriae* serotype 1 and Stx-producing *E. coli* (STEC), produce Shiga toxins (Stxs), which are protein exotoxins that are conserved genetically, physically, and functionally (Bova *et al.,* 2023). This toxin works by damaging eukaryotic ribosomes, leading to the cessation of protein synthesis in target cells (Bova *et al.,* 2023). Vero, Swan 71, and Hela cells were found to be fatally affected by the cytotoxic effects of stx2-positive upper cervical fluid from *E. coli* isolated from the cervix. Our findings offer new insights into the presence of STEC during pregnancy (Scalise *et al.,* 2022). *E. coli* has a genome that has the aptitude to transmit or acquire important virulence factors. It is challenging to determine the impact of STEC infection on reproductive health during pregnancy because there are not many epidemiological studies on the topic (Yang *et al.,* 2020).

The primary source of human infection and the main reservoir of STEC is ruminants. Ingestion of animal-derived foods, including raw meat, milk, and dairy products, as well as intimate contact with infected animals, are the primary ways humans become infected (Treacy *et al.,* 2019).

In our study, all *P. mirabilis* isolates were positive for the *ureC* gene, which encodes the urease enzyme. This enzyme exhibits vigorous activity, capable of decomposing urea into ammonia and carbon dioxide, thereby converting the environment to an alkaline state that suits the bacterium's conditions and growth (Lian *et al.,* 2025). It also uses it as a metabolic activity to obtain some minerals and materials. The activation of urease is essentially the process of binding nickel ions to urease proteins (Stickle *et al.,* 2010). Many studies have shown that the presence of this gene in most strains indicates the importance of the function it carries as a virulence factor (Schaffer *et al.,* 2015). The *zapA* gene was also present in all isolates. The *zapA* gene

regulates the expression of IgA protease during the differentiation of swarmer cells into swarmer cells. (El-Tarabili *et al.,* 2022). Most studies have confirmed that the presence of the *ureC* gene increases the pathogenicity of *P. mirabilis*, and a synergistic effect is observed with high expression of the *ureC* and *zapA* genes (Phan *et al.,* 2008).

The majority of investigations in this sector have demonstrated that the presence of differentiated swimming cells carrying zapA is what enables bacteria to infiltrate epithelial cells (Li & Mobley, 2002). *The ZapA* gene has been considered a key virulence factor for *P. mirabilis*. Its role extends not only to the degradation of IgA, which is considered the most important defense system of the host mucosal surfaces, but also to the destruction of biologically active molecules, such as defensins, which participate in defense against fungal infections, as well as host cell structural elements like matrix proteins. It is also responsible for degrading bradykinin, which is released during inflammatory processes as part of the defense mechanism (Anéa *et al.,* 2011).

The studied isolates showed absolute resistance to the antibiotic tetracycline. This outcome may be attributed to the improper use of antibiotics without first determining the susceptibility of the isolates to them, as well as to some animal breeders who use antibiotics to promote growth and achieve high yields (Al-Marri, 2021). The existence of genes that code for the exclusion of antibiotics like tetracycline from the cell, which lowers the concentration and protection of *E. coli* ribosomes, is one of the causes of antibiotic resistance. The results collected demonstrate the limited alternatives available for treating these bacterial species. Similar problems with multidrug resistance in the same bacteria from dairy cows' uteri have also been reported in several studies (Basbas *et al.,* 2022). Regarding resistance in the study's beta-lactam group, it results from *E. coli* producing beta-lactamase, which attempts to counteract the drug's activity and eliminate its effect (Thapa *et al.,* 2020).

CONCLUSION

E. coli and *Proteus mirabilis* are important causes of genital tract infections in cows, as they carry virulence genes (*stx1,stx2, ureC,* and *ZapA*) capable of causing damage to the endometrium and metritis, which has an impact on production and the occurrence of abortions.

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التوصيف الجزيئي ونمط مقاومة المضادات الحيوية لمعظم البكتيريا سالبة الجرام المعزولة من التهابات الجهاز التناسلي في الأبقار في محافظة نينوى، العراق

فرح نوح ، بلسم يحيى رشيد

فرع الاحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق.

Email: balsamyr20@uomosul.edu.iq Assiut University website: www.aun.edu.eg

تعد اصابات الجهاز التناسلي البكتيرية من أهم المشاكل التي تواجه قطعان الماشية، مما يؤدي إلى خسائر اقتصادية، بالإضافة إلى العقم وانخفاض الإنتاج. أُجري بحثنا بهدف عزل ودراسة جزيئية لأهم البكتيريا سالبة الجرام المسببة لالتهابات الجهاز التناسلي في الأبقار، ودراسة حساسية هذه البكتيريا لأهم المضادات الحيوية، وتحديد أهم عوامل الضراوة. اعطت جراثيم الايشيريكيا القولونيه أكثر نسبة عزل وبواقع ٤٤ % تلتها جراثيم المتقلبات الرائعة *Proteus mirabilis* ٧,٤٪ وكانت النسبة الاقل لجراثيم الكلبسيللا والسيريشيا والانتيروبكتر ١,٥٪. ومن خلال الدراسة، كانت بكتيريا الإشريكية القولونية الأكثر عزلًا في حالات التهاب بطانة الرحم والتهاب المهبل. كما عُزلت بنسبة عالية في حالات احتباس المشيمة وعسر الولادة، والذي يُعد عاملًا مُهيئًا لالتهابات الجهاز التناسلي بنسبة ٣٣,٣٪ و٢٠٪ على التوالي. أظهرت الإشريكية القولونية مقاومةً مطلقةً للمضادات الحيوية إيميبينيم، وتتراسايكلين، وسيفيكسيم. بينما أظهرت بروتيوس ميرابيلس مقاومةً مطلقةً للأزيثروميسين، وتتراسايكلين، والنيتروفورانتوين. وُجدت جينات عوامل الضراوة *Stx1* و *Stx2* و *UidA* في جميع الإشريكية القولونية. بينما احتوت جميع عزلات المتقلبات الرائعه على جينات الضراوة *zapA* و*ureC*.

الاستنتاجات:تعتبر جراثيم الايشيريكيا القولونيه والمتقلبات الرائعة مسببات مهمة لحدوث حالات العقم والاجهاض لامتلاكها عوامل ضراوة *stx1,stx2,ureC* و *zapA* مما يتسبب في خسائر في الانتاج وقلة اللحوم لذا من المهم التعرف على هذه المسببات ودراسة اهم تأثيرات عوامل الضراوة التي تسببها في الجهاز التناسلي في الابقار.