Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Identification and Antimicrobial Susceptibility of Predominant Bacterial Isolates from Urogenital Tract Infection in Infertile men in Al-Diwaniyah City.

Rabab Chyad AL- baderi [1] Ibtisam Habeeb Al-Azawi [2]

Received: 20/01/2026; Revised: 20/02/2026; Accepted: 17/03/2026

Abstract:
Bacterial infections have been recognized as an important contributing factor to male infertility. *Escherichia coli* is strongly implicated in the development of male infertility. The study aimed to isolate and identify prevalent bacterial species responsible for urogenital tract of infertile men and study their antibiotic resistance pattern and genetic virulence profiles . A total of 240 clinical samples (seminal fluid and mid-stream urine) were collected from male infertile attending a private laboratory in Al-Diwaniyah City between December 2024 and July 2025 , the samples were cultured on blood agar and MacConkey agar and incubated aerobically for 24 hours at 37°C . Bacterial isolate were identified biochemically . Presumptive *E.coli* isolates were confirmed using the VITEK2 system for identification and antibiotic susceptibility testing . Molecular confirmation was achieved via polymerase chain reaction (PCR) targeting the *16S rRNA* Gene. Confirmed isolates were screened by PCR for the specific virulence genes.

Bacterial growth was observed in only 45(18.75 %) of these samples . Fourteen isolates were identified as *E.coli* . VITEK2 confirmed identification (100% certainty) and revealed a high susceptibility to imipenem (100%) ,Piperacillin-tazobactam and Amikacin (85%) nitrofurantoin (78%) , Trimethoprim and sulfamethoxazol (71%), Lower Susceptibility was noted for ciprofloxacin (28%) and Ceftazidime (14%). virulence gene screening identified the *fimH* gene in all isolate(100%) while the *papC* and recx gene were detected in 42.8%and 57% of isolates ,respectively.

The study revealed a significant presence of virulent and multidrug-resistant *E.coli* from urogenital tract of infertile male

Keywords: Male infertile , Uropathogenic Esherichia coli , Antibiotic susceptibility test , *fimH , papC , recX Genes*

Introduction:

Infertility is defined as the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse [1] and represents a significant global health problem. In males, infertility may arise from multiple factors, including endocrine disorders, lifestyle influences, varicocele, ejaculatory dysfunction, hypogonadism, excessive alcohol consumption, genetic abnormalities, sperm dysfunction, and urogenital tract infections, which are considered classical causes of male infertility[2]. Among these factors, infections have been widely recognized as an important etiological cause. A wide range of microorganisms, particularly bacteria and viruses, have

been identified as urogenital tract pathogens capable of inducing subfertility. Infections caused by various Gram-negative and Gram-positive bacteria are estimated to account for approximately 15% of cases of primary male infertility[3].

Microorganisms can have an indirect impact on the male reproductive system by releasing reactive oxygen species from infection-induced inflammation, which has been connected to sperm DNA fragmentation and male infertility, or directly by causing motile sperm agglutination, reducing acrosome reaction capacity, and altering cell morphology [4].

Escherichia coli is a common Gram-negative, lactose-fermenting bacillus characterized by a single circular chromosome. It is a facultative anaerobe that normally inhabits the human colon but is capable of causing a wide range of diseases in humans[5]. *E. coli* is also the most frequent etiological agent of urogenital tract infections and has been implicated in the development of male infertility[6]. Several studies have demonstrated that *E.coli* infection exerts significant detrimental effects on male fertility, particularly by impairing sperm quality. These effects include alterations in sperm motility parameters, externalization of phosphatidylserine, disruption of the acrosome reaction, and abnormal sperm morphology. Furthermore, *E. coli* has been shown to exert direct harmful effects through rapid adhesion to human spermatozoa[7, 8].

The observed morphological changes involve sperm surface structures, especially the plasma membrane of the midpiece and neck regions, as well as both the inner and outer acrosomal membranes. These structural abnormalities suggest that morphological defects may contribute to *E. coli*–induced sperm immobilization[9]. In addition, *E. coli* has been reported to induce apoptotic pathways and to cause disruption of the mitochondrial membrane.

Alterations in acrosome morphology and function appear to represent a key mechanism by which this pathogen compromises the fertilizing capacity of human spermatozoa. Early investigations have further confirmed the ability of *E. coli* to adhere to sperm cells and negatively affect sperm motility parameters[10].

Materials and methods:

Methods:

Samples collection:

A total of 240 clinical samples were collected from study participants comprising 120 mid – stream urine specimen and 120 seminal fluid sample . The participations were admitted to the private laboratories in Al- Diwanyiah City during the period from December 2024 to July 2025.

In accordance with safety handling protocols, midstream urine samples were collected from participation groups (male infertility) using sterile screw-cap containers, while seminal fluid were

collected into sterile wide –mouthed plastic containers. All samples were promptly transported to the laboratory for processing . The seminal fluid sample were subsequently incubated at 37°C for 30 minutes to facilitate normal liquefaction b prior to microbiology culture and analysis

The Exclusion Criteria of the Patients:

History of antibiotic use within the three days preceding sample collection .

Bacterial-isolates-Identification:

All collected specimens were inoculated onto primary culture media, including sheep blood agar and MacConkey agar, in accordance with standard microbiological procedures. The inoculated plates were incubated aerobically at 37 °C for 24 hours.

Preliminary identification of bacterial isolates was based on colony morphology, Gram staining characteristics, and conventional biochemical tests, including oxidase and IMViC assays.

Final confirmation of bacterial identity, along with comprehensive antimicrobial susceptibility testing, was carried out using the automated VITEK® 2 Compact system (bioMérieux, France) with appropriate Gram-negative identification (GN ID) and antimicrobial susceptibility testing (AST) cards.

DNA-Extraction and molecular confirmation of *Escherchia coli* isolate

Genomic DNA was extracted and purified in accordance with the manufacturer's instructions (Geneaid, Korea). For molecular confirmation, the extracted DNA was subjected to conventional polymerase chain reaction (PCR) targeting the species-specific *16S rRNA* gene. The reaction was carried out using specific primer pairs, with primer sequences and expected amplicon sizes listed in Table 1.

PCR amplification was performed in a thermal cycler (Bio-Rad, USA) under the following conditions: an initial denaturation step at 95 °C for 5 minutes for one cycle, followed by 30 amplification cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 1 minute. A final extension step was conducted at 72 °C for 5 minutes. These cycling conditions were optimized and established by the researcher in the present study.

Table -1 Primer sequences targeting the *16SrRNA* gene for bacterial identification

Gene	Primer Sequences (5'-3')		Product size	Ref
16SrRNA E.coli	F	AGAGTTTGATCCTGGCTCAG	1500	[11]
	R	CTACGGCTACCTTGTTACGA		

Detection of Virulence Genes by PCR .

The confirmed *E. coli* isolates were further screened for the presence of three virulence-associated genes (*fimH*, *papC*, and *recX*) using conventional polymerase chain reaction (PCR). Specific primer pairs for each target gene, including their sequences and expected amplicon sizes, are presented in Table 2. Each gene was amplified in a separate PCR reaction.

PCR amplification was carried out in a thermal cycler (Bio-Rad, USA) under the following conditions: an initial denaturation step at 95 °C for 5 minutes for one cycle, followed by 30 cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 1 minute. A final extension step was performed at 72 °C for 5 minutes. These cycling parameters were optimized based on established protocols and primer-specific requirements.

Table -2 specific primer sequences used for virulence genes detection in *E.coli* isolate .

Gene	Primer Sequences (5'-3')		Product size	Ref
fimH Gene	F	TGCAGAACGGATAAGCCGTGG	508	[12]
	R	GCAGTCACCTGCCCTCCGGT		
papC Gene	F	GACGGCTGTACTGCAGGGTGTGGCG	328	[13]
	R	ATATCCTTTCTGCAGGGATGCAATA		
recx Gene	F	GGGCAAAAATGGCCCAGAAG	267	In this study
	R	CCATATTTTCGCGTCGCCTG		

Statistical-Analysis.

The data obtained in the present study were organized and statistically analyzed using the Statistical Package for the Social Sciences (SPSS), version 27. The chi-square test was applied to assess the significance of qualitative variables. Differences were considered statistically significant when the p-value was less than 0.05[14].

Results:

Isolation & the identification bacterial isolates :

The results of the present study showed that bacterial growth was observed in 45 out of 240 clinical samples (18.75%) collected from (mid –stream urine and seminal fluid) of male infertile while 195(81.25%) samples showed no significant growth of aerobic bacteria Figure -1

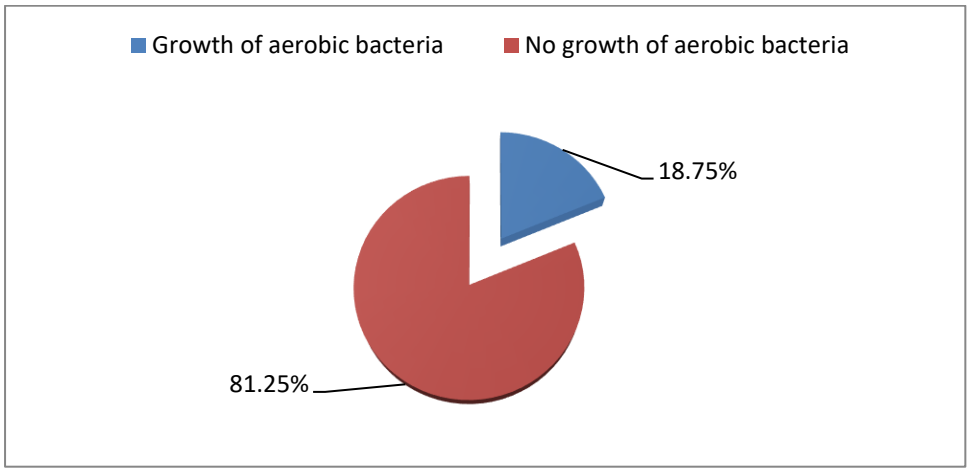

Fig - 1 Distribution of clinical sample based on presence of aerobic bacterial growth.

Out of the 45 culture –positive samples , 14 isolates (31.1% positive culture) were successfully identified as *E. coli* based on conventional biochemical tests and distinctive colonial morphylogy on macConkey and blood agar .The identity of all 14 presumptive *E.coli* isolate were further confirmed by using the automated VITEK 2 compact system (BioMérieux, France) the other 31 positive sample (68.8%) yielded growth of other aerobic bacterial species such as *Klebsellia pneumophila 8* (18%) , *pseudomonas aurginosa* 4(9%)and other growth (42%), figure -2

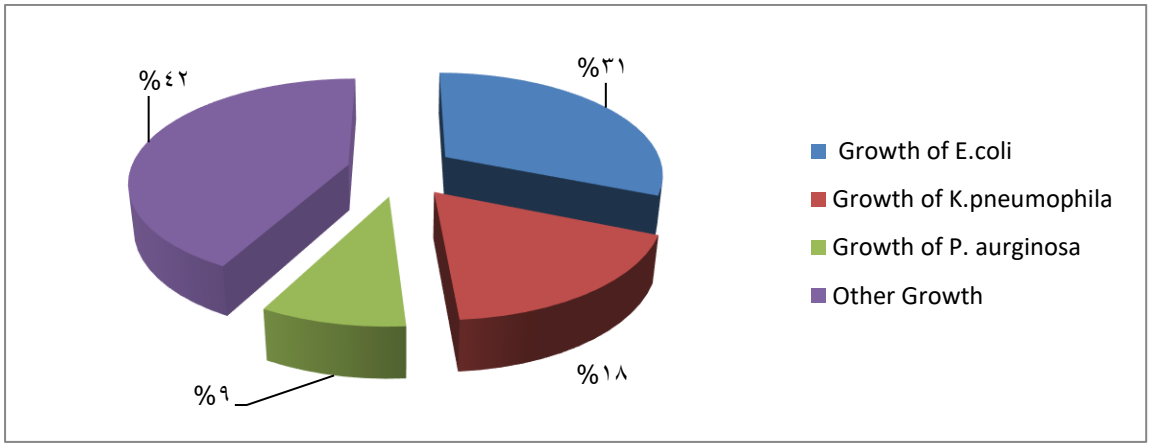

Fig -2 Distribution of isolated bacteria *E.coli* versus other aerobic bacterial species .

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed for all *E. coli* isolates using the automated VITEK® 2 system with AST-N222 cards. The susceptibility profiles are presented in Figure -3

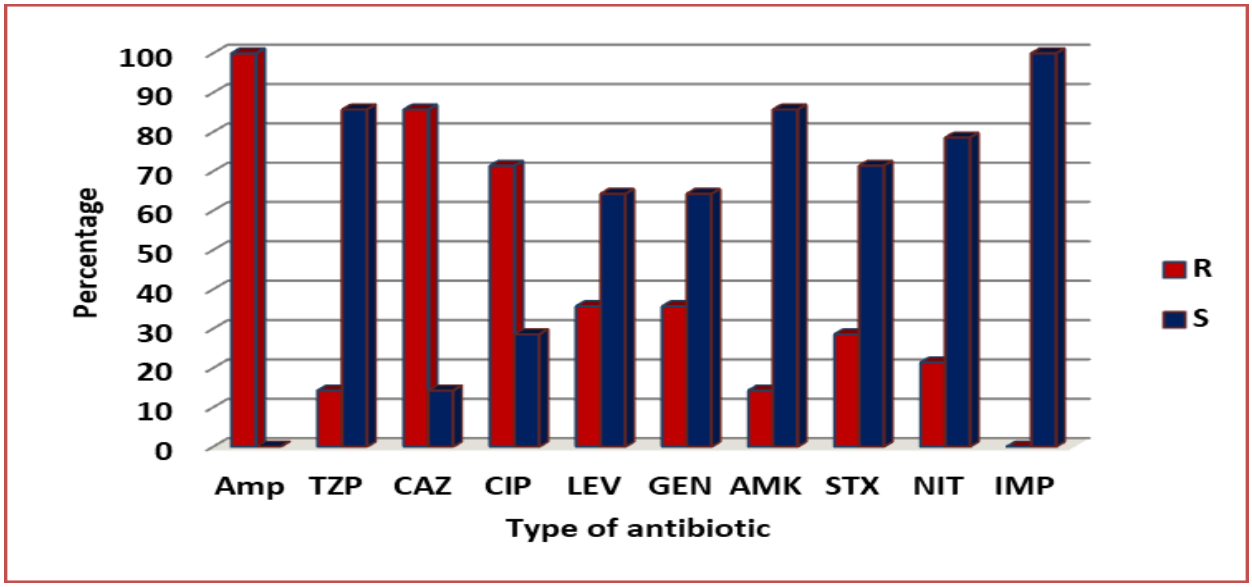

Figure -3 Antibiotic susceptibility testing of *E.coli* clinical isolates

Molecular confirmation of *Escherichia coli* by *16S rRNA Gene* Amplification.

All Fourteen bacterial isolates of *E.coli* which were identified by conventional bacteriological methods , and were subjected to molecular confirmation .Genomic DNA was extracted from each isolate . PCR assay for roughly 1500 bp *16S rRNA* gene sequencing. As shown in figure -4, all isolate tested positive for that gene (100%).

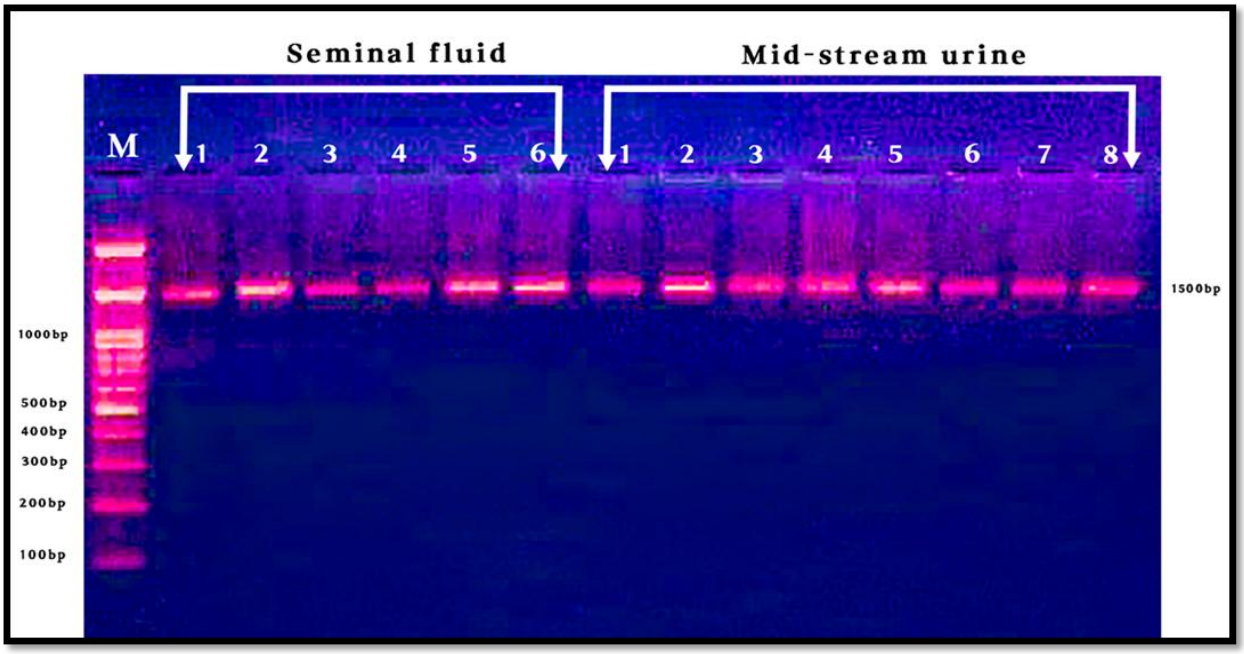

Fig-4 Positive isolates of E.coli for 16SrRNA gene on the agarose gel. Stained with Red safe. Electrophoresis of the product PCR was carried out on the agarose gel at a concentration of (2%) at 70 volts for 80 minutes. M(marker ladder (3000-100bp). Lanes (1-14) : positive E.coli clinical isolates showing (1500) bp amplicon Corresponding to *16SrRNAgene* .

Molecular detecting for virulence genes of *Escherichia coli* .

Specific PCR primers were used to detect virulence genes (*fimH*, *papc* and *recX* genes) in E.coli isolate urogenital tract of male infertility .The PCR products were visualized using gel electrophoresis show in Figures 5,6 and 7.

In the present study, the *fimH* gene was detected in all 14 (100%) *E. coli* isolates. The *papC* gene was identified in 6 isolates (42%), while the *recX* gene was present in 8 isolates (57%).

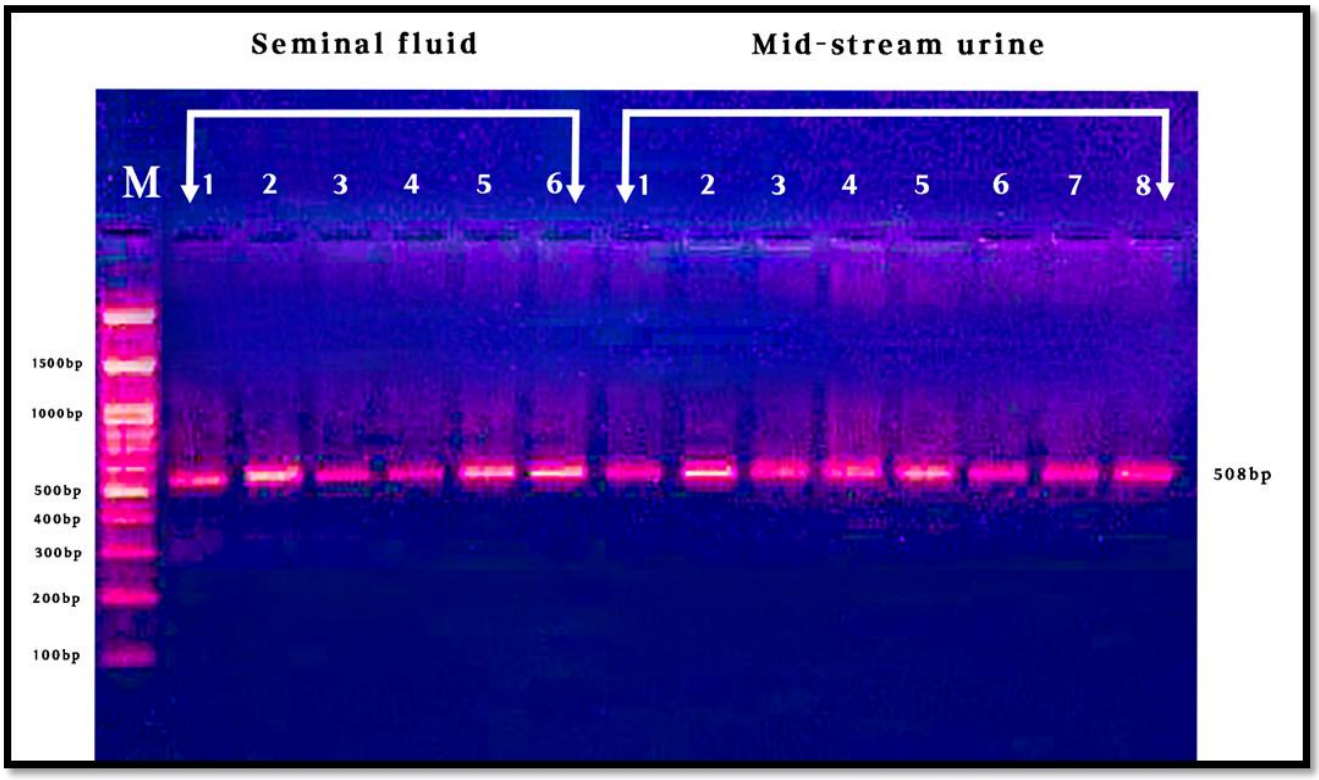

Fig-5 Positive isolates of *E.coli* for *fimH* gene on the agarose gel. Stained with Red safe. Electrophoresis of the product PCR was carried out on the agarose gel at a concentration of (2%) at 70 volts for 80 minutes. M(marker ladder (3000-100bp). Lanes (1-14) : positive E.coli clinical isolates showing (508 bp) bp amplicon Corresponding to *fimH* Gene .

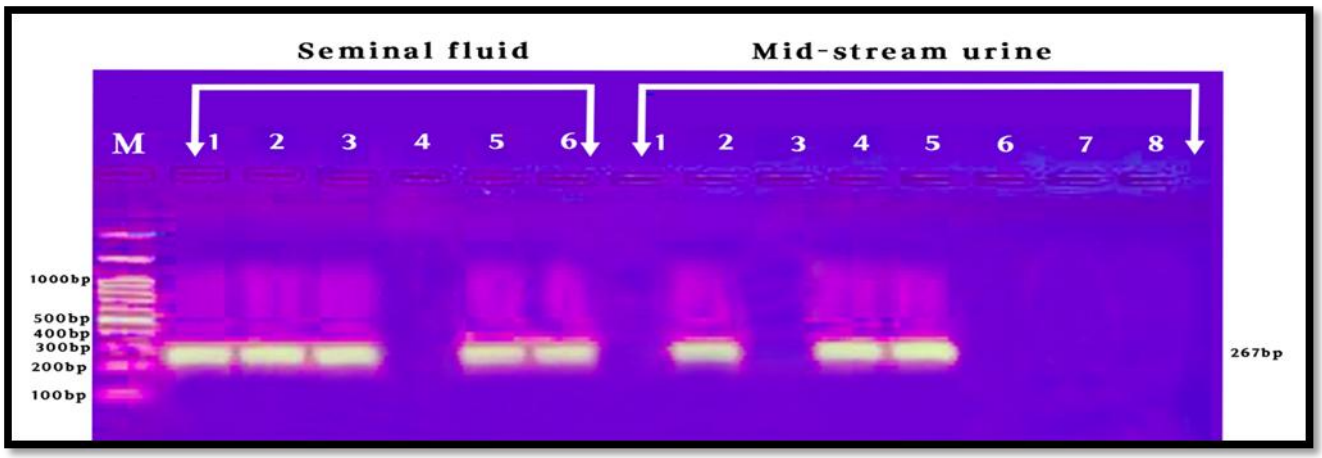

Fig -6 Positive isolates of *E.coli* for *recx* gene on the agarose gel. Stained with Red safe. Electrophoresis of the product PCR was carried out on the agarose gel at a concentration of (2%) at 70 volts for 80 minutes. M(marker ladder (3000-100bp). Lanes (1,2,3,5,6) from seminal fluid and lanes (1, 3,4) from mid-stream urine positive E.coli clinical isolates showing (267 bp) bp amplicon Corresponding to *recX* gene .

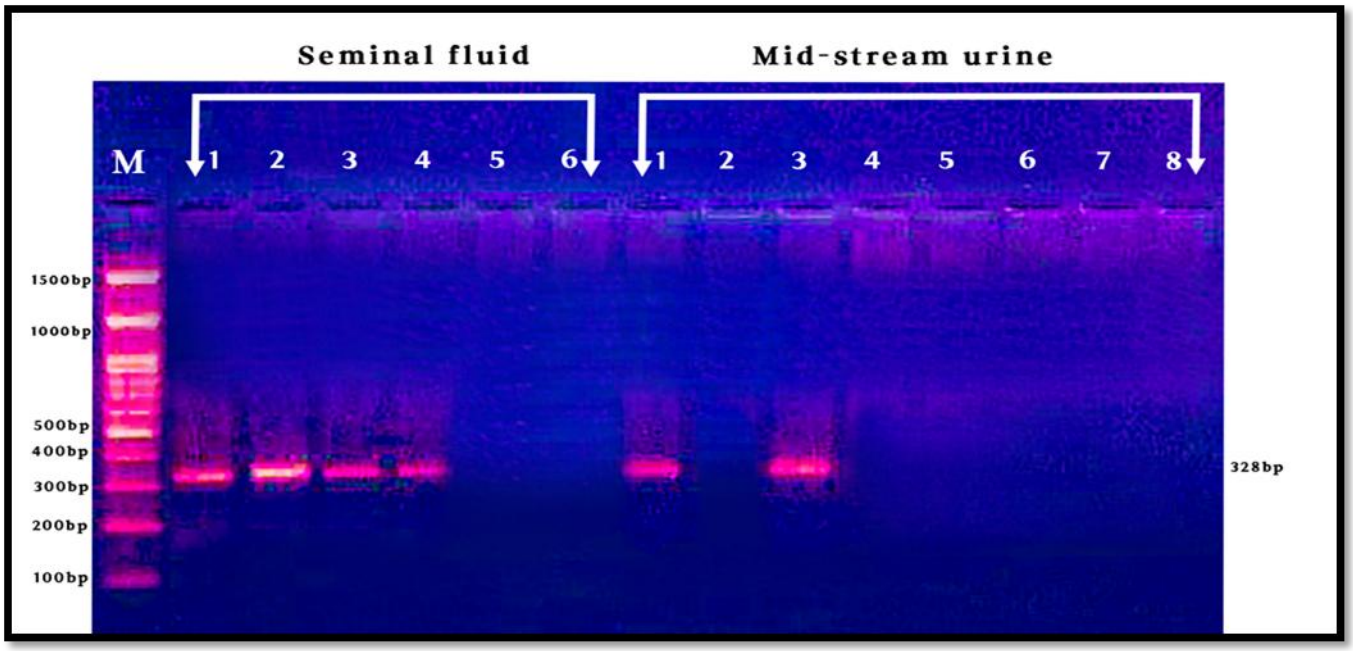

Fig -7 Positive isolates of *E.coli* for *pap C* gene on the agarose gel. Stained with Red safe. Electrophoresis of the product PCR was carried out on the agarose gel at a concentration of (2%) at 70 volts for 80 minutes. M(marker ladder (3000-100bp). Lanes (1,2,3,4) from seminal fluid and lanes (1,3) from mid-stream urine positive E.coli clinical isolates showing (328bp) bp amplicon Corresponding to *PaPC gene*

Discussion:

The male reproductive tract, with the exception of the urethra, is normally free of aerobic bacteria. The presence of pathogenic bacteria in seminal plasma, or an elevated bacterial load, is generally considered indicative of an active infection in the male reproductive system[15]. Such infections typically originate from the patient's urinary tract or may be transmitted by a sexual partner[16].

In the current study *E.coli* was identified as the causative agent in 31.11% of male urogenital tract infection among infertile men . urogenital tract infection (UTI) in(31.11%) of male infertile. The prevalence rate of *E.coli* (31.11 %) in this study is consistent with previous reports of high prevalence rate such as 40% and 41.9% [17,18] though studies have reported lower incidences [19-21]

Rapid antimicrobial susceptibility testing (AST) is critical for selecting appropriate antibiotic therapy , which is essential for reducing mortality associated with antibiotic resistant bacteria and slowing the development of further resistance **[**22**]** . In this analysis , the isolated E.*coli* strains exhibited complete susceptibility 100% to imipenem and high susceptibility rates of 85% to both piperacillin and amikacin. Variable susceptibility was observed for nitrofurantoin , trimethoprim–sulfamethoxazole , gentamicin and levofloxacin .Notably , all isolate (100%) were resistance to ampicillin. The resistance profile aligns with other research . One study reported that E.coli was

sensitive to imipenem , nitrofurantoin ,amikacin and piperacillin -tazobactam while displaying high –level resistance to ampicillin , ciprofloxacim and ceftazidime [23] . Similary , a study from Iraq found that *E.coli* isolated from semen sample of infertile men were resistant to ceftazidime and ampicillin but show moderate sensitive to norfloxacin [24]. Another study documented susceptibility rate of 76.9% to nitrofurantoin , 69.2% to levofloxacin , 61.5% to gentamicin [25]. Another study documented susceptibility rates of 76.9% to nitrofurantoin, 69.2% to levofloxacin, 61.5% to gentamicin [26] . Most *E.coli* isolates exhibited a multidrug- resistant (MDR) Phenotype whereas no extensively drug-resistance (XDR)isolates were identified .This finding consistent with other studies [27,28] which reported that *E.coli* isolated from urinary tract were frequently MDR but not XDR .

The pathogenicity of uropathogenic *E. coli* (UPEC) in urinary tract infections (UTIs) is largely determined by its virulence factors, which influence colonization, invasion, and proliferation within the umbrella cells of the urothelium [29]. Molecular methods are commonly employed to detect virulence genes, such as *fimH*, which encodes the FimH adhesin, a key component of type 1 fimbriae in *E. coli*. FimH facilitates bacterial adhesion to host cells by binding to mannose-containing glycoproteins on epithelial surfaces, thereby promoting stereo chemical interactions that enhance bacterial attachment and colonization of the urothelium . In addition, FimH contributes to biofilm formation and mediates quorum sensing among invading bacterial cells, leading to increased virulence in UPEC strains [30].

In the current study 100% *E.coli* isolated had *fimH* Gene which are consistent with study from Kirkuk city ,,Iraq who reported that (100%) of their isolate were carrying fimH adhesion Gene [31]. similarly study from Baghdad ,Iraq who reported that 100% of UPEC isolate harbored fimH adhesion Gene [32]

In the present study, genes encoding adhesive systems, which are among the most important virulence factors of *E. coli* in urinary tract infections (UTIs), were also examined. The *papC* gene, a critical virulence determinant in uropathogenic *E. coli* (UPEC), encodes an outer membrane usher protein essential for the assembly of P-fimbriae (pyelonephritis-associated pili), which mediate bacterial adhesion to host tissues. *papC* facilitates the secretion and orderly assembly of P-fimbria subunits by forming a channel in the outer membrane [33]. The findings of the current study was (42.8%) that consistent with those of previous investigations, which reported *papC* positivity in 45% and 45.8% of *E. coli* isolates, respectively [34,35].

Furthermore , this study show presence *recX gene* , avirulence factor with more specialized pathogenic role . The *recx* gene encoded to soluble spermatotoxic factors know sperm immobilization factor (SIF) which soluble protein binding to sperm , particularly at the head region ,causing immediate immobilization and sever e morphological damage also effects on sperm motility [36].

This study show the *recx gene* was identified in 8 out of 14 clinical isolates (57%). The presence and prevalence of these virulence genes (*fimH, papc* and *recx* genes) underscore the clinical and pathogenic significant of UPEC strains in AL-Diwwaniyah population may harbor genetic factor directly .

This notable prevalence underscores its potential clinical relevance in male urogenital infections, suggesting that a significant subset of UPEC strains in the Al-Diwaniyah population may carry a genetic factor directly linked to sperm damage and male infertility .

Conclusion :

This study demonstrated the presence of *E.coli* in the urogenital tract of infertile men. All 14 isolate were conclusively identified by 16SrRNA sequencing .Virulence gene profiling revealed a distinct pattern . characterized by the presence of *fimH , papc and recx* gene that coupled with a significant antibiotic resistance profile ,these finding highlight the potential pathogenic role of UPEC strains in male infertility.

Acknowledgement

I am grateful to the Medical College of Al-Qadisiyah University for providing the resources to meet all my study needs. I would also like to extend my sincere gratitude to all the participants in this study, without whom this research would not have been possible.

References

[1] Al-Hussaini RM, Al-Azawi IH. Gene Expression of fimH and recX among Klebsiella pneumoniae Associated with Genitourinary Tract Infection and Their Impact on Male Infertility. Egypt J Med Microbiol. 2025;34(4)

[2] Jafari H, Mirzaiinajmabadi K, Roudsari RL, Rakhshkhorshid M. The factors affecting male infertility: A systematic review. Int J Reprod Biomed. 2021;19(8):681-96

[3] Alzaidi JR, Kareem AA. The impact of urogenital tract infectious bacteria on male fertility. Med J Babylon. 2024;21(2):476-80.

[4] Khanjar RHA, Al-Azawi IH. Diagnosis of Neisseria gonorrhea Using Molecular Method in Infertile Iraqi Male.

[5] Basavaraju, M., and B. S. Gunashree. "Escherichia coli: an overview of main characteristics." *Escherichia coli-old and new insights* (2022): 1-21.

[6] Schuppe HC, Pilatz A, Hossain H, Diemer T, Wagenlehner F, Weidner W. Urogenital infection as a risk factor for male infertility. Dtsch Arztebl Int. 2017;114(19):339-46.

[7] Kumar V, Garg N. Effect of Escherichia coli on semen quality of infertile human male. Virol Immunol J. 2019;3(3):000214.

[8] Marchiani S, Baccani I, Tamburrino L, Mattiuz G, Nicolò S, Bonaiuto C, et al. Effects of common Gram-negative pathogens causing male genitourinary-tract infections on human sperm functions. Sci Rep. 2021;11(1):19177.

[9] Sarwar, Muhammad Kaleem. *LIPIDOMICS AND MEMBRANE AQUAPORINS IN BOVINE SPERM*. Diss. 2024.

[10] Chegini Z, Khoshbayan A, Kashi M, Shahraki RZ, Didehdar M, Shariati A. The possible pathogenic mechanisms of microorganisms in infertility: a narrative review. Arch Microbiol. 2025;207(2):27.

[12] Sultana KF, Akter A, Saha SR, Ahmed F, Alam S, Jafar T, et al. Bacterial profile, antimicrobial resistance, and molecular detection of ESBL and quinolone resistance gene of uropathogens causing urinary tract infection in the southeastern part of Bangladesh. Braz J Microbiol. 2023;54(2):803-15

[13] Liu X, Liu H, Li Y, Hao C. Association between virulence profile and fluoroquinolone resistance in Escherichia coli isolated from dogs and cats in China. J Infect Dev Ctries. 2017;11(4):306-13.

[14] Daniel WW, Cross CL. Biostatistics: a foundation for analysis in the health sciences. 11th ed. Hoboken: Wiley; 2018

[15] Cyoia PS, Rodrigues GR, Nishio EK, Medeiros LP, Koga VL, Pereira APD, et al. Presence of virulence genes and pathogenicity islands in extraintestinal pathogenic Escherichia coli isolates from Brazil. J Infect Dev Ctries. 2015;9(10):1068-75

[16] Guiton R, Drevet JR. Viruses, bacteria and parasites: infection of the male genital tract and fertility. Basic Clin Androl. 2023;33(1):19.

[17]Colella M, Topi S, Palmirotta R, D'Agostino D, Charitos IA, Lovero R, et al. An overview of the microbiota of the human urinary tract in health and disease: current issues and perspectives. Life (Basel). 2023;13(7):1486

[18] Sasikumar, S., et al. "Study of microbial infection in Asthenozoospermia patients." (2014): 37-48.

[19]Bhatt CP, Mishra S, Bhatt A, Lakhey M. Bacterial pathogens in semen culture and their antibiotic susceptibility pattern in vitro. Int J Biomed Res. 2015;6(11):909-14

[20] Ali, Md Sahadat, Fatima Tuz Zohora Mony, and Jonathan D. Eisenback. "Glycerol Stock Solution Preparation for Long-Term Bacterial Storage." (2025).

[21] Shash RYM, Mohamed GAA, Shebl SE, Shokr M, Soliman SA. The Impact of Bacteriospermia on Semen Parameters Among Infertile Egyptian Men: A Case–Control Study. Am J Mens Health. 2023;17(3):15579883231176621.

[21] Ekhaise FO, Richard FR. Common bacterial isolates associated with semen of infertile patients. Iraqi J Med Sci. 2011;9:301-7.

[22]Anton-Vazquez V, Suarez C, Planche T .Impact of rapid susceptibility testing on antimicrobial therapy and clinical outcomes in Gram-negative bloodstream infections. J Antimicrob Chemother. 2022;77(3):771-81.

[23] Vemireddy S. Antibiotic Susceptibility of Escherichia coli in UTI. Master's thesis. Brooklyn: Long Island University; 2022.

[24] Faisal AJ, Salman HA. Determination of semen quality and antibacterial susceptibility pattern of bacteria isolated from semen of Iraqi subjects. Microbiol Biotechnol Lett. 2021;49(4):587-93.

[25] Hassan DINA, El-Moaafy MA, Saleh HH, Abdelrahman AA, Abdallah SE. Characterization of Quinolone Resistance Genes in Uropathogenic Escherichia coli Isolates, in Egypt. Rec Pharm Biomed Sci. 2022;6(2):163-72.

[26] Nasrallah YS, Anani M, Omar HH, Hashem AA. Microbiological profiles of semen culture in male infertility. Hum Androl. 2018;8(2):34-42

[27] Al-Azawi IH. Detection and Characterization of hly, cdtI and stxI genes, in MDR uropathogenic and intestinal E. coli isolates, Hilla city, Iraq. AL-Qadisiyah Med J. 2023;19(1):24-30

[28] Al-Azawi IH, Al-Bidiri MS. Distribution of integron III and phylogenic clade among MDR uropathogenic E. coli from patient in Al-Diwaniyah City, Iraq. Wiad Lek. 2022;75(5):1254-60.

[29] Whelan S, Lucey B, Finn K. Uropathogenic Escherichia coli (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment. Microorganisms. 2023;11(9):2169.

[30] Foroogh N, Rezvan M, Ahmad K, Mahmood S. Structural and functional characterization of the FimH adhesin of uropathogenic Escherichia coli and its novel applications. Microb Pathog. 2021;161:105288.

[31] Aljebory IS, Mohammad KA. Molecular detection of some virulence genes of escherichia coli isolated from uti patients in kirkuk city, iraq. J Glob Pharma Technol. 2019;11(3):349-55.

[32] Hamid SAA, Khoshabeh RM. Antibiotic resistance, biofilm formation, and identification of FimH and FimA adhesion genes in uropathogenic Escherichia coli (UPEC) isolated from patients in Baghdad province. Iraqi J Sci. 2024;[54-5546

[33] Isidro-Coxca MI, Ortiz-Jiménez S, Puente JL. Type 1 fimbria and P pili: regulatory mechanisms of the prototypical members of the chaperone-usher fimbrial family. Arch Microbiol. 2024;206(9):373.

[34] Montes-Robledo A, Buelvas-Montes Y, Baldiris-Avila R. Description of extraintestinal pathogenic Escherichia coli based on phylogenetic grouping, virulence factors, and antimicrobial susceptibility. Iran J Microbiol. 2023;15(4):503-13.

[35] Asif S, Khan M, Arshad MW, Shabbir MI. PCR optimization for beginners: a step by step guide. Res Mol Med. 2021;9(2):81-102.

[36] Answal, Monika, and Vijay Prabha. "Escherichia coli recombinant sperm immobilizing factor RecX as a potential vaginal contraceptive." *Reproductive Biology and Endocrinology* 16.1 (2018): 88.