ORIGINAL ARTICLE

# Gene Expression of *fimH* and *recX* among *Klebsiella pneumoniae* Associated with Genitourinary Tract Infection and Their Impact on Male Infertility

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***Key words:***
***K. pneumoniae, male infertility, fimH, sperm immobilizing factor, genitourinary tract infection, gene expression.***

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## ABSTRACT

***Background****: Klebsiella pneumoniae is a significant pathogen responsible for urinary tract infections (UTIs). Bacterial infections of the male genitourinary tract account for 15% of male infertility.* ***Objective****: This research examined certain strains of K. pneumoniae that express the recX and fimH genes, assessing their impact on sperm and its relationship to male infertility.* ***Methodology****: Total 200 samples were collected (100 semen and 100 urine) from the infertile men with genitourinary tract infection, and 200 samples from fertile men suffering from UTI from Al-Najaf, Al-Diwaniya and Karbala governorates. All samples' cultures were conducted for all patients and the control groups. PCR assay method was employed to detect fimH and recX genes. Real-Time PCR used to evaluate the genes expression in K. pneumoniae.* ***Results****: Only 17 (8.5%) of the suspected isolates were identified as K. pneumoniae from patients and 17 (8.5%) from control. The isolates were divided as follows: 13 isolates from urine and 4 isolates from semen among patients, and only 17 isolates from urine in control group. The prevalence of genes among K. pneumoniae isolates was fimH (64.7%), while recX was (94%) in patients. Among control group it was (88%), (58.8%) for fimH and recX, respectively. Patients group exhibited a significant rise in the expression levels of all studied genes (recX =5.516254 and fimH=3.919551) as compared with genes expression of controls samples (recX=1.089123 and fimH=1.434213).* ***Conclusions****: Urinary tract infection may impact on sperm parameters and this led to male infertility.*

## INTRODUCTION

Infertility is the inability of couples to have children after one year of marriage[1]. Poor semen quality, which can result from compromised testicular function or sperm transiting via the male reproductive canal, is the main cause of male infertility. The inflammation with its two phases Acute and chronic, are thought to account for around 15-35% of male infertility cases, possibly due to their adverse impact on spermatozoa; nevertheless, the correlation between inflammation, infections, and diminished semen quality remains inadequately defined[2]. The *Enterobacteriaceae spp*. are prevalent infections of the urogenital tract and may adversely affect male fertility[3].

One of the most major causes of male infertility, accounting for 8% to 35% of cases, is a male urogenital tract infection (UTI). The function of spermatozoa may be affected by microorganisms in a number of ways, including (a) direct adhesion with sperm cells, (b) accumulation and agglutination of motile sperm, (c) reduction of the acrosome reaction ability. (d) changes in cell morphology. (e) Cause a localized inflammatory response that raises reactive oxygen species (ROS) levels. (f) Sperm autoantibody induction. (d) Cytotoxic molecule production. In addition to the long-term antibiotic therapy of an infection, it may result in sperm defects[4].

Urinary tract infections are considered multi-microbial infections, which occur via various strains of bacteria and fungi. The members of *Enterobacteriaceae* especially *Klebsiella pneumoniae* and *Escherichia coli* are considered the most prevalent bacteria in urinary infections[5]. The main *K. pneumoniae* pathogenicity is established from different virulence agents that permit it to attack innate immunity and preserve infections in a human host. The major virulence agents that have a significant role in pathogenesis include capsular polysaccharides, lipopolysaccharides, pili, and siderophores[6].

Its ability to form one of the virulent factors such as biofilms enable the bacteria to evade immune responses, and acquire more chance for resistance through gene transfer horizontally and made it a significant challenge in clinical pathogeesity[7]. Treatment of infections is more difficult by biofilms with significant challenges, which increase both innate and acquired antibiotic resistance, especially in virulent strains[8].

Due to the increase of multidrug-resistant strains, the CDC has identified *Klebsiella pneumoniae* as a pathogen of urgent concern. *K. pneumoniae* has a

special mechanism in genitourinary tract infections (UTIs) by tipping type 1 pili with the two-domain *fimH* adhesin[9].

The adhesive characteristics of *K. pneumoniae* are primarily facilitated by typeI,III fimbriae, which are consist from the globular proteins which allow *K. pneumoniae* to adhere to vital host cells, marking the first phase of the infectious steps[10].

*fimH* genes were crucial for the invasion, colonization with persistence of bacterial isolated from in urinary tract infections. The expression originates from a chromosomal *fim* gene cluster including 8 genes, but 3 types of fimbriae are classified under the chaperone-usher category and are encoded by 5 genes, along with other genetic processes in *K. pneumoniae* (*mrk* A, B, C, D, and F)[11].

*Klebsiella pneumoniae* with inhibitor effect on sperm motility have been attributed to release of sperm immobilizing factor (SIF)[12]. One of the components thought to be responsible for the detrimental effects of *E. coli* on sperm nature motility is the 56 kello Dalton Sperm-Immobilizing-Factor (SIF)[13]. Public health officials will need to come up with a developed plan to fight growing antibiotic resistance and create new urinary tract infection treatment guidelines with specialized protocols[14].

This study aimed to investigate the prevalence of the Fimbrial Adhesin type-1 gene (*fimH*) and Sperm immobilizing factor-encoding gene (*recX*) among *Klebsiella pneumoniae.*

## METHODOLOGY

**Study design:** Case-control study.

**Sample selection**

Four hundred clinical samples were collected from patients and control, comprising 100 urine and 100 seminal fluid samples from patients and 100 urine and 100 of seminal fluid samples from the control group. The patients were admitted to the Fertility Center in Al-Sadder Medical City and received outpatient care from private laboratories in Al-Najaf, Al-Hamza, and Karbala governorates during the period from December 2023 to July 2024.

In accordance with safety handling protocols, midstream urine samples were collected from patients and control groups and transferred into sterile screw-cap containers, while seminal fluid was gathered in a sterile plastic vial. The sample was subsequently transferred to the laboratory and incubated at 37°C for 30 minutes to facilitate normal liquefaction of the seminal fluid. All samples were streaked following established protocols utilizing to identification of *K. pneumoniae* on selective and differential media (MacConkey, blood agar) and incubated at 37°C for 24 hours under aerobic conditions[15]. The isolates were confirmed through conventional biochemical tests, with validation provided by the Viteck-II compact system (Biomerieux, France).

**Polymerase Chain Reaction:**

*Klebsiella pneumoniae* isolates were undergone to DNA extraction following the instructions provided by the manufacturer of the Genomic DNA Extraction Kit (FavorPrep, Austria). The Nanodrop instrument (THERMO, USA) was utilized to measure DND concentration and its purity and of DNA for each isolate.

Polymerase-chain-reaction (PCR) was designed for screening the fimbrial adhesin type 1 gene (*fimH*) and SIF-encoding gene (*recX*). In the current study, all primers were dependent on the Promega company, USA. Primers details are tabulated in Table 1.

**Gene Expression**

The quantitative Real-Time PCR technique was used to analyze the gene expression of the fimbrial adhesin type 1 gene (*fimH*) and SIF-encoding gene (*recX*) in *K. pneumoniae* strains. Briefly, 96-well plates were filled with 100 µl of each bacterial solution, and they were then aerobically incubated for 18 hours at 37 °C. Then, adhering cells were scraped off using LB broth after each well was cleaned with nuclease-free water. Following instructions, an RNA extraction kit (*TransZol*Up plus RNA kit) was used to extract the total RNA of the strains that had been collected. The purified RNA was transformed into cDNA using aspecific kit (*TransZol*Up plus RNA kit).

Using Go Taq® qPCR Master Mix Kit RT-PCR equipment (Korea), every cDNA employed as a template in a final volume (20 µl) that contained 2 µl of cDNA, 10 p/mol of every primers as mention in(Table1), and 10µl of CXR dye PCR Master-Mix (Go Taq® qPCR Master Mix Kit). The relative expression of the *fimH* and *recX* genes was determined using the ΔΔCт technique, and the 16S-rRNA was employed as a house-keeping gene as a standardize for mRNA expression levels[17, 18].

**Statistical analysis:** Chi square and P-value ($P \leq 0.05$).

**Table 1: Sequencing Primers**

| No. | Gene | | Primer sequencing | Amplicon size(bp) | Annealing Temp./Time | Ref. |
|---|---|---|---|---|---|---|
| 1 | *fimH* | F | GCCAACGTCTACGTTAACCTG | 180 | 60°C /1min. | [16] |
| | | R | ATATTTCACGGTGCCTGAAAA | | | |
| 2 | *recX* | F | TACATATGGGCGCGTCCGGCAGC | 266 | 56°C/30 sec. | This study |
| | | R | TTGGATCCTTACTCCTCGGCTTCGT | | | |

## RESULTS

**Isolation and identification of *K. pneumoniae* from patients and control**

The results in the present study revealed that 120 (60%) specimens had shown positive bacterial growth from which only 17 (8.5%) were identified to be *K. pneumoniae* and 103 (51.5%) other bacteria, while 80 (40%) specimens showed no growth from infertile men. From the control group 99 (49.5%) specimens showed positive bacterial growth from which only 17 (8.5%) were identified to be *K. pneumoniae* and 82 (41%) were other bacteria, while 101 (50.5%) specimens revealed no growth (Table 2 and Table 3).

**Seminal fluid analysis**

Two hundred seminal fluid samples (100) from infertile men and (100) samples from control were analyzed according to WHO[19], laboratory guidelines for the testing and processing of human sperm (Table4).

**Table 2: Distribution of *K. pneumoniae* isolates among infertile group**

| Sample source | Total no. | Bacterial growth | | | P-value (P≤0.05) |
|---|---|---|---|---|---|
| | | No growth | Growth | *K. pneumoniae* | |
| **Seminal fluid** | 100 (50%) | 63 (63%) | 33 (33%) | 4 (4%) | < 0.0001* |
| **Urine** | 100 (50%) | 17 (17%) | 70 (70%) | 13 (13%) | < 0.0001* |
| **Total** | 200 (100%) | 80 (40%) | 103 (51.5%) | 17 (8.5%) | < 0.0001* |
| **P-value (P≤0.05)** | | < 0.0001* | < 0.0001* | 0.0290* | |
| ***Significant difference by chi-square** | | | | | |

**Table 3: Distribution of *K. pneumoniae* isolates among control group**

| Sample source | Total no. | Bacterial growth | | | P-value (P≤0.05) |
|---|---|---|---|---|---|
| | | No growth | Growth | *K. pneumoniae* | |
| **Seminal fluid** | 100 (50%) | 94 (94%) | 6 (6%) | 0 (0%) | < 0.0001* |
| **Urine** | 100 (50%) | 7 (7%) | 76 (76%) | 17 (17%) | < 0.0001* |
| **Total** | 200 (100%) | 101 (50.5%) | 82 (41%) | 17 (8.5%) | < 0.0001* |
| **Probability (P≤0.05)** | | < 0.0001* | < 0.0001* | < 0.0001* | |
| ***Significant by chi-square** | | | | | |

**Table 4: Characteristics of seminal fluid from infertile and control groups**

| Parameters | | Infertile group (n=100) | Control group (n=100) | P value |
|---|---|---|---|---|
| **Volume (ml)** | Normal (≥ 1.5) | 44 (44%) | 83 (83%) | < 0.0001* |
| | Abnormal (< 1.5) | 56 (56%) | 17 (17%) | < 0.0001* |
| | P value | 0.2301$^{NS}$ | < 0.0001* | |
| **Spermatic concentration** | Normal (≥ $10^6$/ml) | 22 (22%) | 90 (90%) | < 0.0001* |
| | Abnormal (< $10^6$/ml) | 78 (78%) | 10 (10%) | < 0.0001* |
| | P value | < 0.0001* | < 0.0001* | |
| **Total sperm count (million)** | Normal (≥ 39) | 19 (19%) | 78 (78%) | < 0.0001* |
| | Abnormal < 39) | 81 (81%) | 22 (22%) | < 0.0001* |
| | P value | < 0.0001* | < 0.0001* | |
| **Motility (%)** | Normal (≥ 32%) | 23 (23%) | 85 (85%) | < 0.0001* |
| | Abnormal (< 32%) | 77 (77%) | 15 (15%) | < 0.0001* |
| | P value | < 0.0001* | < 0.0001* | |
| **Morphology (%)** | Normal (≥ 4%) | 33 (33%) | 79 (79%) | < 0.0001* |
| | Abnormal (< 4%) | 67 (67%) | 21 (21%) | < 0.0001* |
| | P value | < 0.0001* | < 0.0001* | |
| **Pus cell** | Normal (less than 1) | 7 (7%) | 43 (43%) | < 0.0001* |
| | Abnormal | 93 (93%) | 57 (57%) | 0.0033* |
| | P value | < 0.0001* | 0.1615$^{NS}$ | |
| *Significant by chi-square. .NS:no-significant | | | | |

**Molecular study of *fimH*-1 and *recX* genes**

Conventional PCR technique was carried out to detect *fimH*-1 and *recX* genes. *fimH*-1 gene were studied in all 34 *K. pneumoniae* strain. The results found 16/17 (94%) were genetic positively to *fimH* gene in infertile men (12 of which was positive urine samples and 4 positive semen samples). The size of the PCR product was approximately 180 bp, as pointed out in Figure 1.

The results of the control group showed that 15/17 (88%) were positive to *fimH* gene. PCR product was roughly (180bp) in size, the results were shown in Figure (2).

Sperm Immobilizing Factor-encoding gene (*recX*) gene were detected in all 34 *K. pneumoniae* isolates. The results showed that 9/17 (53%) were positive to *recX* gene of *K. pneumoniae* isolated from infertile men (5 of which was positive urine samples and 4 positive semen samples). PCR product was roughly (266bp) in size, the results are shown in Figure (3).

The results of control group showed that 10/17 (58.8%) were positive to *recX* gene. The product size of PCR approximately (266bp) as pointed in Figure (4).

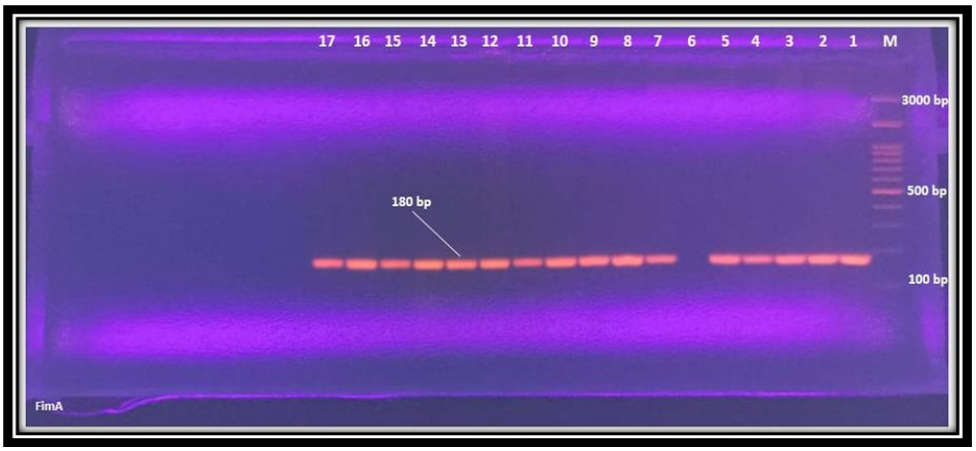


**Fig. 1:** Agarose gel electrophoresis (1.5%) of RCR amplified of *fimH* gene (180bp) of *Klebsiella pneumonia* from patients for (55) min at (70) volt M: ladder (DNA marker). Number (1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,17) positive *Klebsiella pneumonia* isolates, while only number (6) negative.

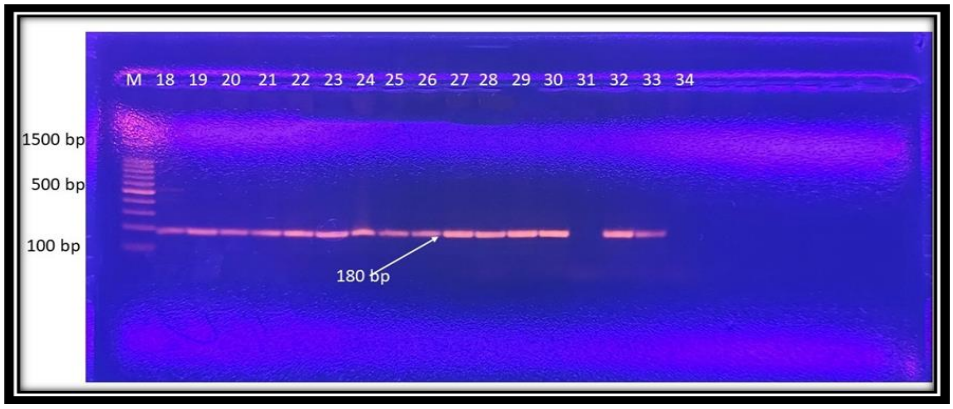


**Fig. 2:** Agarose gel electrophoresis (1.5%) of RCR amplified of *fimH* gene (180bp) of *Klebsiella pneumonia* from control for (55) min at (70) volt M: ladder (DNA marker). Number (18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33) positive *Klebsiella pneumonia* isolates, while only number (31, 34) negative.

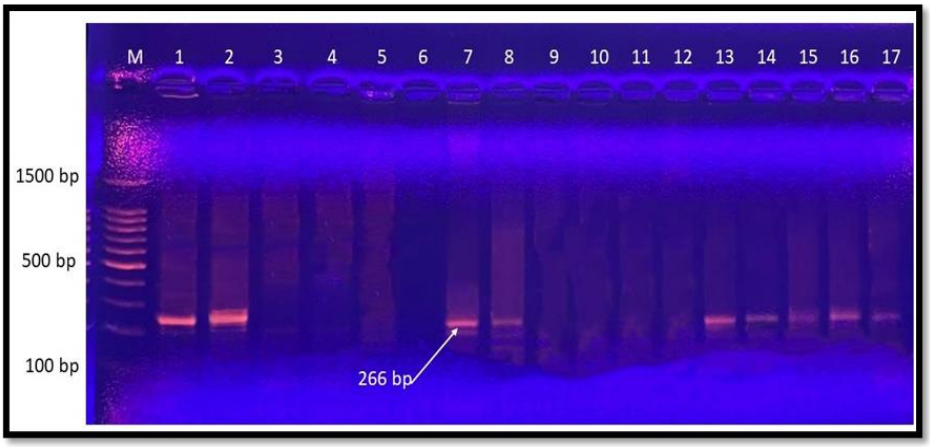


**Figure 3:** Agarose gel electrophoresis (1.5%) of RCR amplified of *recX* gene (266bp) of *Klebsiella pneumonia* from patients for (55) min at (70) volt M: ladder (DNA marker). Number (1,2,7,8,13,14,15,16,17) positive *Klebsiella pneumonia* isolates, while number (3,4,5,6,9,10,11,12) negative *Klebsiella pneumonia* isolates.

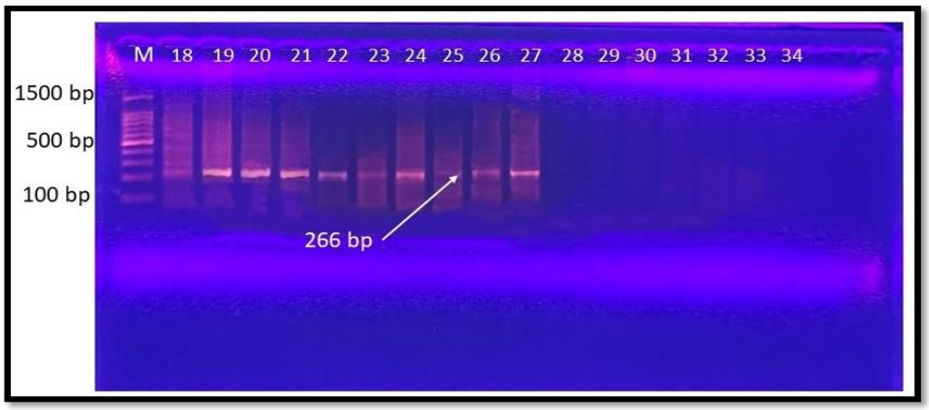


**Fig. 4:** Agarose gel electrophoresis (1.5%) of RCR amplified of *recX* gene (266bp) of *Klebsiella pneumonia* from control for (55) min at (70) volt M: ladder (DNA marker). Number (18, 19, 20, 21, 22, 23, 24, 25, 26, 27) positive *Klebsiella pneumonia* isolates, while number (28, 29, 30, 31, 32, 33, 34) negative isolates.

**Gene expression of *fimH* and *recX* genes**

The expression of *fimH* and *recX* genes in *Klebsiella pneumoniae* strain was identified by (RT-PCR) technique. Nine isolates dependent on the experimental group were yielded from infertile and control growth cultures. The present study revealed that the patients group showed significant differential gene expression of the target genes (*recX*, and *fimH*), which representing a significant difference with a p-value of ≤ 0.05. Patients group exhibited the highest significant in the expression levels of all studied genes (*recX* =5.516254 and *fimH*=3.919551) as compared with genes expression of controls samples (*recX*=1.089123 and *fimH*=1.434213) as explained in Tables (5) and (6), as well as shown by Figures (5) and (6).

**Table 5: *fimH* Fold Gene Expression between Control and Patients versus the reference gene (16 SRNA)**

| Groups | N | *fimH* Expression levels (2^-(ΔΔCt)) | | |
|---|---|---|---|---|
| | | Mean | SD | SE |
| **Controls** | 9 | 1.434213 | 0.443854 | 0.147951 |
| **Patients** | 9 | 3.919551 | 1.538613 | 0.543982 |
| **P value** | 0.001103* | | | |
| * represent a significant difference under p-value ≤ 0.05. SD, SE: Standard Deviation and Error | | | | |

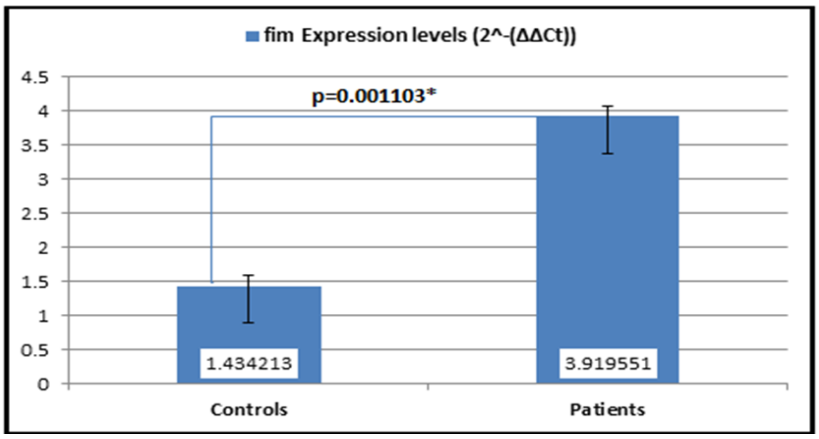


**Fig. 5:** *fimH* Fold Gene Expression between Control and Patients versus the reference gene (16 SRNA)

**Table 6: *recX* Fold Gene Expression between Control and Patients versus the reference gene (16 SRNA)**

| Groups | N | *recX* Expression levels (2^-(ΔΔCt)) | | |
|---|---|---|---|---|
| | | Mean | SD | SE |
| **Controls** | 9 | 1.089123 | 0.40553 | 0.13517 |
| **Patients** | 9 | 5.516254 | 0.947355 | 0.334941 |
| **P value** | 0.00000* | | | |
| * represent a significant difference under p-value ≤ 0.05. SD, SE: Standard Deviation and Error | | | | |

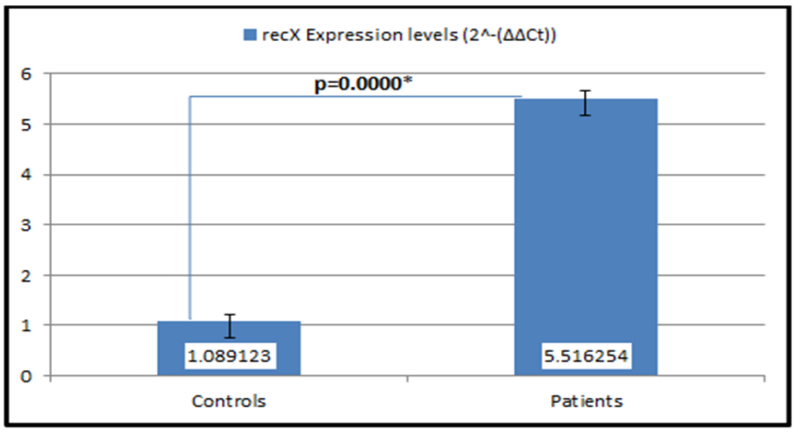


**Fig. 6:** *recX* Fold Gene Expression between Control and Patients versus the reference gene (16 SRNA)

## DISCUSSION

Among the most common bacterial diseases are urinary tract infections (UTIs). *Klebsiella pneumoniae* is becoming a major multidrug-resistant pathogen, especially in hospital environments and healthcare settings[20].

Many studies reported that the *Enterobacteriaceae* family was the most prevalent cause of UTI in humans[21, 22]. Many variables help *Enterobacteriaceae* adhere to uroepithelium. Such bacteria cause adhesion pili and fimbriae to form on the uroepithelial mucosa[23].

Our result, demonstrated only 17 (8.5%) of collected isolates were identified as *Klebsiella pneumoniae* from infertile men, closely related result was conducted by Faisal and Salman[24] in Baghdad, who found the rate of *Klebsiella spp.* among infertile men was (15%).

Additionally, a research done in Irbil, Iraq, reported that *Klebsiella pneumoniae* infection rate was (28%)[25]. Other study in Kirkuk reported the rate of *K. pneumoniae* was (26%)[26]. In Al-Diwaniya a report showed the prevalence rate of *K. pneumoniae* isolates was (35.29%) and were obtained from patients with UTI[27].

Sperm concentration is a significant semen characteristic that contributes to male infertility[28]. Our study indicated that concentration of sperm was decreased in infected semen relative to control group. Other research refer to the bacterio-spermia as a probable causative organism[29].

Sperm morphology and motility are significant determinants of semen parameters[28]. Sperm abnormalities in its morphology have been noted in individuals with semen infection or UTIs. These infections lead to many errors represented by either elongation or diminished acrosomal inducibility in addition to tapering of the sperm head and neck with anomalies of the sperm tail[30]. Poor sperm morphology is often linked to sperm nuclear abnormalities resulting from inflammatory or viral conditions of the urogenital tract.

Infertile patients have high numbers of pus cells. Although the distribution, origin, and function of pus cells in semen remain unclear immunologically, pyospermia is considered one of the most significant causes of male infertility. Pyospermia has been shown to have detrimental impacts on semen parameters and even on in vitro fertilization (IVF), according to research[31]. The local inflammatory response brought on by microorganisms triggers and activates leukocytes and inflammatory signals, including cytokines and reactive oxygen species, which can harm sperm and greatly increase male infertility[31].

The results of study in Iraq by Khadim and Bermani[32], Regarding the pus cell percentage, during bacterial infection leads to increase significantly in their percentage ($P \geq 0.05$), concluding that the bacterial infection of the genital tract in infertile men leads to poor-semen health parameters. A frequently noted occurrence is bacterial adhesion to the acrosome, resulting in acrosomal disintegration that halts essential fertilization processes. Zhang *et al*.[33] elucidated that bacterial adhesion to the sperm surface can raise the cellular load, thereby hindering sperm motility. Bacteria immobilized by adhesion may communicate with other bacteria and attracted it, leading to the formation of complexes that agglutinate and create structures that may obstruct the movement of spermatozoa[34].

Bacteria that may attach to the sperm surface include polymeric sticky threads known as "pili or fimbriae," facilitating first contact and subsequent colonization[35]. Pili are characterized as virulence components that enable interbacterial aggregation, biofilm formation, or allow particular identification of host-cell receptors[36].

A study done by Alquraishi[37] in Wasit governorate, showed that (42.8%) were positive with *fimH* genes among *K. pneumoniae* isolated from UTI.

A significant reduction in sperm motility is seen over time due to drastic changes in morphological sperm production of soluble spermatotoxic agents[38], like sperm immobilizing factor (SIF), which hinder sperm movement[39]. A link between male infertility has also been documented to other Enterobacteriaceae genus, like *Klebsiella pneumoniae*, which express the *rec-X* SIF-coding gene or exhibit highly similar genomic sequences[40].

In one study, the research suggested that the sperm damage ways happen by bacteria passing through the expression of the flagellin and pilin proteins to mannose receptors on human sperm surface (spermatozoa) as a type of virulence factor for bacterial stability[41]. As well as, The increasing of *fimH* gene expression plays crucial role in the linking of bacteria to surfaces, leading to strong biofilm formation[42].

## CONCLUSION

Genitourinary tract infection may impact on sperm parameters and this led to male infertility.

**Recommendations**

Study limitations: Difficulty in sample collections so the number was few, short time of study, cost, difficult diagnosis. Further investigations may be undertaken to ascertain the genetic sequence of *K. pneumoniae* for phylogenetic analysis.

**Assignment**

All the participants provided informed consents for inclusion in the study and were assured that all the information provided would be used solely for the purposes of this study and treated confidentially.

**Ethical Approval Declaration**

The research was proper accordance to the ethical standards established in the Declaration of Helsinki. Prior to sampling, consent was obtained from the patient or their partner. The research methodology, subject information, and permission form received approval from the College of Medicine, Al-Qadisiyah University.

**Acknowledgment**

We would like to thank College of Medicine, Department of Medical Microbiology, Clinical sites, and the patients for their participation.

**Competing interests**

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

This manuscript has not been previously published and is not under consideration in another journal.

**Funding:** Authors did not receive any grants from funding agencies.

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