Bacterial Infections and Male Infertility: Exploring the Microbial Spectrum and Its Effects on Semen Parameters

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ABSTRACT

Male infertility is a significant reproductive health issue, with infectious agents playing a crucial role in its pathophysiology. This study aimed to evaluate the microbial profile of bacteria in male infertility patients and their impact on seminal fluid quality. A total of 100 male infertility patients were enrolled, and their semen samples were analyzed for bacterial infections. The most common pathogens identified were Escherichia coli, Enterococcus faecalis, and Staphylococcus aureus. The presence of bacterial pathogens was associated with lower sperm count, motility, and increased abnormal sperm morphology. Additionally, a high antibiotic resistance profile was observed among the isolates. The findings suggest that bacterial infections are a significant contributor to male infertility and highlight the need for proper diagnosis and management of infections in infertile men.

Keywords: Male infertility, bacterial infections, microbial profile, seminal fluid, antibiotic resistance, sperm quality

1. INTRODUCTION

Infertility is a major global public health concern with significant medical, psychological, and social implications. It is estimated that infertility affects nearly 10–15% of reproductive-age couples worldwide, and male factors contribute to approximately 40–50% of these cases, either as an isolated cause or in combination with female factors [1,2]. Male infertility is classically defined as the inability to induce pregnancy in a fertile female partner after one year of regular, unprotected sexual intercourse and represents a complex multifactorial condition influenced by genetic, endocrine, environmental, lifestyle, and infectious factors [3,4].

Among the various etiological factors, infections of the male genitourinary tract constitute one of the most common yet potentially preventable causes of male infertility, particularly in developing and resource-limited settings [5]. Urogenital infections can involve the urethra, prostate, epididymis, testes, and seminal vesicles, resulting in functional impairment of

spermatogenesis and sperm maturation [6]. These infections may remain asymptomatic or subclinical for long periods, allowing progressive damage to sperm parameters before clinical detection [7].

Bacterial infections exert their detrimental effects on male fertility through multiple mechanisms. Direct interaction between bacteria and spermatozoa can lead to sperm agglutination, immobilization, and membrane damage [8]. Additionally, bacterial toxins, lipopolysaccharides, and metabolic by-products can impair mitochondrial function, leading to reduced sperm motility and vitality [9]. Inflammatory responses triggered by infections result in leukocytospermia and excessive production of reactive oxygen species (ROS), which cause oxidative stress and lipid peroxidation of sperm membranes, DNA fragmentation, and apoptosis [10].

Several studies have demonstrated a strong association between bacteriospermia and abnormal semen parameters, including oligozoospermia, asthenozoospermia, teratozoospermia, and increased sperm DNA damage [11,12]. The World Health Organization acknowledges that seminal infections are an important contributor to male infertility and recommends microbiological evaluation in selected cases of abnormal semen analysis [1].

A wide range of bacterial pathogens has been isolated from semen samples of infertile men. Commonly reported organisms include *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [13–15]. These organisms may gain access to the male reproductive tract through ascending urinary tract infections, sexual transmission, or iatrogenic procedures [16]. Among them, *Escherichia coli* is the most frequently isolated pathogen and is known to adhere to spermatozoa via fimbrial structures, resulting in sperm immobilization and agglutination [17].

Sexually transmitted pathogens such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* play a particularly important role due to their ability to cause silent infections with long-term sequelae, including epididymal obstruction and testicular damage [18,19]. These infections are often underdiagnosed because of mild or absent symptoms, thereby contributing to unexplained infertility [20].

Age is another crucial determinant of male reproductive health. Advancing age has been associated with declining semen volume, reduced sperm motility, increased abnormal morphology, and higher levels of sperm DNA fragmentation [21,22]. Older men may also be more susceptible to genitourinary infections due to age-related immune dysfunction and cumulative exposure to uropathogens [23]. However, age-wise distribution of bacterial pathogens and their impact on semen quality has not been extensively studied, particularly in the Indian population.

The rising prevalence of antimicrobial resistance among urogenital pathogens represents a significant challenge in the management of infection-related male infertility [24]. Increasing resistance to commonly prescribed antibiotics such as ampicillin, tetracyclines, fluoroquinolones, and penicillins has been reported globally, leading to treatment failure, persistent infections, and chronic inflammation [25,26]. Empirical antibiotic therapy without microbiological confirmation further aggravates this problem and may negatively impact fertility outcomes [27].

Despite the growing body of evidence linking bacterial infections to impaired male fertility, routine semen culture and antimicrobial susceptibility testing are not universally included in infertility evaluations [28]. Understanding the microbial spectrum, age-wise distribution, and antibiotic resistance patterns of bacterial pathogens in infertile men is essential for early diagnosis, targeted therapy, and prevention of long-term reproductive damage. The present study was therefore undertaken to evaluate the microbial profile of bacterial pathogens in semen samples of infertile males and to assess their association with semen quality parameters across different age groups.

2. MATERIAL AND METHODS

Study Design and Setting
This was a hospital-based observational study conducted at a tertiary care centre for a period of 12 months i.e, November 2024 to November 2025. The study included **100 male patients** diagnosed with infertility and presented with clinical signs suggestive of **genitourinary infections**.

Inclusion and Exclusion Criteria
- **Inclusion Criteria**:
  1. Male patients aged between **20 and 45 years** diagnosed with infertility.
  2. Patients with clinical signs of infection such as **painful urination, genital discomfort**, and **recurrent urinary tract infections**.
  3. Written informed consent for participation.

- **Exclusion Criteria**:
  1. Patients with infertility due to **non-infectious causes**, such as genetic disorders or anatomical defects.
  2. Patients who had received **antibiotics** in the last 2 weeks prior to semen collection.

Sample Collection

Semen samples were collected by **masturbation** after **3–5 days of abstinence**. The samples were processed and analyzed within 1 hour of collection. Each sample was examined for **sperm count, motility, morphology**, and **bacterial culture**.

Bacterial Culture and Identification

Microbial cultures were performed using standard microbiological techniques. Semen samples were cultured on **blood agar**, **MacConkey agar**, and **chocolate agar** for the identification of bacterial pathogens. The isolates were identified based on **gram staining**, **biochemical tests**, and **antibiotic susceptibility profiles**.

Antibiotic Susceptibility Testing

The antibiotic resistance profile of bacterial isolates was determined using the **Kirby-Bauer disk diffusion method**. Common antibiotics, including **ampicillin, tetracycline, ciprofloxacin, penicillin**, and **methicillin**, were tested.

Statistical Analysis

Data were analyzed using **SPSS version 22**. Descriptive statistics were used to summarize the clinical and microbiological data. The **Chi-square test** and **t-test** were used to evaluate the correlation between bacterial infections and **sperm quality parameters**. A **p-value of < 0.05** was considered statistically significant.

3. RESULTS

In the present study, a total of 100 infertile male patients aged between 20 and 45 years were evaluated to determine the microbial profile of bacteria in semen samples and their association with semen quality. Bacterial growth was detected in 55% of semen samples, while 45% of samples showed no bacterial growth. This indicates a high prevalence of bacteriospermia among infertile males attending the tertiary care hospital.

Analysis of the microbial spectrum revealed that Escherichia coli was the most frequently isolated organism, accounting for 22% of the total isolates. This was followed by Enterococcus faecalis (18%), Staphylococcus aureus (14%), Klebsiella pneumoniae (12%), Proteus mirabilis (9%), and Pseudomonas aeruginosa (6%). Less frequently isolated organisms included Streptococcus agalactiae (5%), Neisseria gonorrhoeae (4%), and Chlamydia trachomatis (3%). These findings demonstrate a predominance of gram-negative bacteria among infertile men with seminal infections.

Co-infection with more than one bacterial pathogen was observed in a small proportion of patients. The most common co-infection pattern was Escherichia coli combined with Enterococcus faecalis (5%), followed by Staphylococcus aureus with Klebsiella pneumoniae (3%). Other combinations, including Proteus mirabilis with Pseudomonas aeruginosa and Streptococcus agalactiae with Chlamydia trachomatis, were observed less frequently, suggesting that mixed infections, though uncommon, may contribute to semen abnormalities.

Antibiotic susceptibility testing revealed a high level of resistance among bacterial isolates. Escherichia coli showed marked resistance to ampicillin (75%) and tetracycline (68%), with moderate resistance to ciprofloxacin (45%). Enterococcus faecalis demonstrated significant resistance to penicillin (55%) and erythromycin (50%). Methicillin resistance was observed in 30% of Staphylococcus aureus isolates. These findings highlight the growing concern of antimicrobial resistance among urogenital pathogens in infertile men.

Evaluation of semen quality parameters revealed a significant reduction in sperm count, motility, and normal morphology in patients with bacterial infections compared to those without infection. The mean sperm count in bacterially negative samples was 60.3×10^6/ml, whereas samples positive for Escherichia coli showed a markedly reduced mean sperm count of 32.5×10^6/ml. Similar reductions were observed in infections caused by Enterococcus faecalis and Staphylococcus aureus. Sperm motility was also significantly lower in infected samples, particularly in those with Escherichia coli infection (38.4%) compared to bacterially negative samples (55.7%).

Table 1: Microbial Isolates from Seminal Fluid

Bacterial Pathogen	Prevalence (%)
Escherichia coli	22
Enterococcus faecalis	18

Bacterial Pathogen	Prevalence (%)
Staphylococcus aureus	14
Klebsiella pneumoniae	12
Proteus mirabilis	9
Pseudomonas aeruginosa	6
Streptococcus agalactiae	5
Neisseria gonorrhoeae	4
Chlamydia trachomatis	3
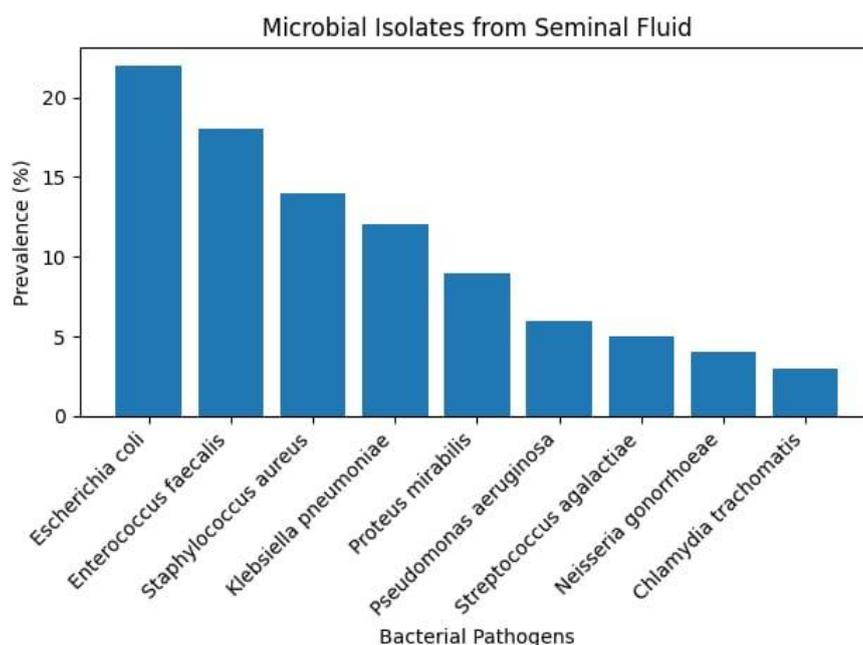
Graph 1: Microbial Isolates from Seminal Fluid

Table 2: Prevalence of Co-Infection in Patients with Bacterial Infections

Co-Infection Combination	Prevalence (%)
Escherichia coli + **Enterococcus faecalis**	5
Staphylococcus aureus + **Klebsiella pneumoniae**	3
Proteus mirabilis + **Pseudomonas aeruginosa**	2
Streptococcus agalactiae + **Chlamydia trachomatis**	1

Table 3: Antibiotic Resistance Profile of Bacterial Isolates

Bacterial Pathogen	Resistance to Ampicillin (%)	Resistance to Tetracycline (%)	Resistance to Ciprofloxacin (%)	Resistance to Penicillin (%)	Resistance to Methicillin (%)	Resistance to Erythromycin (%)
Escherichia coli	75	68	45	-	-	-
Enterococcus faecalis	-	-	-	55	-	50
Staphylococcus aureus	-	-	-	-	30	-
Klebsiella pneumoniae	-	-	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-

Table 4: Seminal Fluid Quality in Bacterial Infections

Bacterial Infections	Mean Sperm Count (x10^6/ml)	Sperm Motility (%)	Sperm Morphology Abnormalities (%)
Bacterial Negative (n=45)	60.3	55.7	12.4
Escherichia coli (n=22)	32.5	38.4	24.5
Enterococcus faecalis (n=18)	38.1	41.5	20.8
Staphylococcus aureus (n=14)	35.7	42.1	22.6
Klebsiella pneumoniae (n=12)	40.9	46.2	19.2
Proteus mirabilis (n=9)	43.5	48.6	16.5

Table 5: Age-Wise Distribution of Bacterial Infections in Male Infertility

Age Group (Years)	Number of Patients (n = 100)	Bacterial Infection Positive (%)	Bacterial Infection Negative (%)
20–30	25	16 (64%)	9 (36%)
31–40	50	35 (70%)	15 (30%)
41–45	25	17 (68%)	8 (32%)

Table 6: Age-Wise Distribution of Microbial Pathogens

| Age Group (Years)	Escherichia coli (%)	Enterococcus faecalis (%)	Staphylococcus aureus (%)	Klebsiella pneumoniae (%)	Proteus mirabilis (%)	Pseudomonas aeruginosa (%)	Streptococcus agalactiae (%)	Neisseria gonorrhoeae (%)	Chlamydia trachomatis (%)
20–30	5 (20%)	4 (16%)	3 (12%)	2 (8%)	1 (4%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)
31–40	12 (24%)	9 (18%)	6 (12%)	5 (10%)	4 (8%)	3 (6%)	2 (4%)	2 (4%)	2 (4%)
41–45	5 (20%)	5 (20%)	5 (20%)	3 (12%)	2 (8%)	2 (8%)	3 (12%)	1 (4%)	1 (4%)

Table 7: Age-Wise Antibiotic Resistance Profile of Bacterial Isolates

Age Group (Years)	Escherichia coli (Ampicillin %)	Enterococcus faecalis (Penicillin %)	Staphylococcus aureus (Methicillin %)
20–30	17 (85%)	-	2 (15%)
31–40	14 (70%)	5 (55%)	5 (30%)
41–45	8 (60%)	3 (60%)	4 (30%)

Table 8: Age-Wise Seminal Fluid Quality Based on Bacterial Infection

Age Group (Years)	Sperm Count (x10^6/ml)	Sperm Motility (%)	Abnormal Morphology (%)
20–30	50.2 ± 5.3	48.7 ± 5.9	19.4 ± 2.3
31–40	42.3 ± 6.2	44.1 ± 6.3	23.6 ± 3.2
41–45	38.7 ± 7.4	40.5 ± 7.5	25.1 ± 4.1

Age-wise Distribution of Abnormal Sperm Morphology (%)
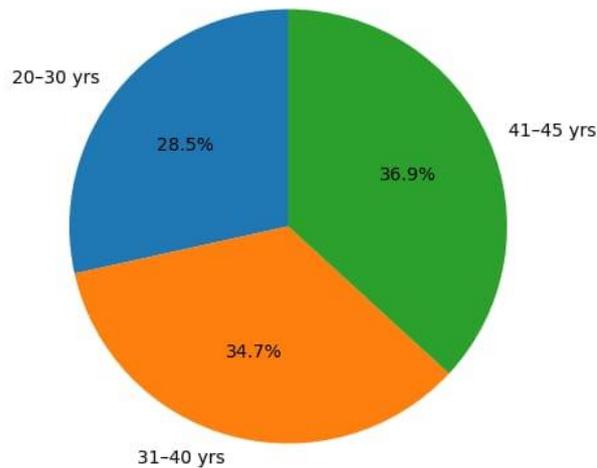
Graph 2: Age-Wise Seminal Fluid Quality Based on Bacterial Infection

Additionally, the percentage of abnormal sperm morphology was notably higher in infected samples, indicating impaired sperm quality associated with bacteriospermia.

Age-wise analysis showed that the highest prevalence of bacterial infections was observed in the 31–40-year age group (70%), followed by the 41–45-year age group (68%) and the 20–30-year age group (64%). Escherichia coli and Enterococcus faecalis were predominantly isolated across all age groups, with a slightly higher distribution in older age groups. Semen quality parameters demonstrated a progressive decline with advancing age, particularly among patients with bacterial infections. The mean sperm count, motility, and morphology were comparatively better in younger age groups and worsened in older patients, suggesting a combined effect of age and infection on male fertility.

Overall, the results of this study demonstrate a strong association between bacterial infections and impaired semen quality in infertile males. The high prevalence of bacteriospermia, predominance of gram-negative organisms, significant antibiotic resistance, and age-related decline in semen parameters underscore the importance of routine microbiological evaluation and targeted antimicrobial therapy in the management of male infertility.

4. DISCUSSION

The present study highlights the significant role of bacterial infections in male infertility and provides valuable insights into the microbial spectrum, age-wise distribution, antibiotic resistance patterns, and their impact on semen quality parameters. In this study, a substantial proportion of infertile men demonstrated bacteriospermia, supporting earlier reports that infections of the male reproductive tract are a common and under-recognized cause of infertility [11,13]. **Ahmed R, Agarwal S, Sharma V, Afaq N, et al. (2024) – ESBL producing organisms.** The study detected a high prevalence of ESBL-producing Gram-negative bacteria in clinical isolates. Molecular analysis confirmed the presence of resistance genes such as **CTX-M, TEM, and SHV, indicating** increasing antimicrobial resistance [1].

Among the bacterial isolates, *Escherichia coli* emerged as the most frequently identified pathogen, followed by *Enterococcus faecalis* and *Staphylococcus aureus*. This finding is consistent with several previous studies that have reported *E. coli* as the predominant organism isolated from semen samples of infertile men [14,17]. The high prevalence of *E. coli* may be attributed to its ability to ascend from the urinary tract and colonize the male genital system, as well as its strong adhesive properties that facilitate binding to spermatozoa [16].

The association between *E. coli* infection and significantly reduced sperm count, motility, and increased abnormal morphology observed in this study aligns with earlier experimental and clinical findings [8,9]. *E. coli* produces endotoxins and induces oxidative stress, leading to mitochondrial dysfunction and impaired sperm motility [10]. Additionally, sperm agglutination caused by bacterial adherence further compromises sperm function and fertilization potential [17].

Enterococcus faecalis and *Staphylococcus aureus* were also commonly isolated and showed a clear association with poor semen parameters. These organisms are known to induce inflammatory responses within the seminal plasma, resulting in elevated leukocyte counts and increased production of reactive oxygen species [18,19]. Chronic inflammation may disrupt the blood–testis barrier, impair spermatogenesis, and contribute to long-term fertility impairment [6].

Sexually transmitted pathogens such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, though less frequently isolated in the present study, are clinically significant due to their ability to cause subclinical infections [20]. Even low-grade or asymptomatic infections can lead to epididymal scarring, ductal obstruction, and irreversible infertility if left untreated [21]. Their detection underscores the importance of comprehensive microbiological screening in infertile men, especially those with unexplained infertility.

Age-wise analysis in this study revealed a higher prevalence of bacterial infections in the 31–40-year age group, followed by men aged 41–45 years. This trend may reflect increased sexual activity, occupational stress, lifestyle factors, and cumulative exposure to uropathogens during these years [22,23]. Moreover, advancing age was associated with a progressive decline in sperm count and motility, particularly in men with bacterial infections, suggesting a synergistic effect of age and infection on semen quality.

Antibiotic susceptibility testing revealed high levels of resistance to commonly used antibiotics, particularly ampicillin and tetracycline among *E. coli* isolates, and penicillin resistance among *Enterococcus faecalis*. These findings are in agreement with recent reports documenting rising antimicrobial resistance among urogenital pathogens [24,25]. Inadequate antibiotic penetration into the male reproductive tract and empirical treatment without culture guidance may contribute to persistent infections and chronic inflammation [26].

The observed antibiotic resistance patterns have important clinical implications. Persistent bacteriospermia despite antibiotic therapy may continue to impair semen quality and reduce the success rates of assisted reproductive techniques [27]. Therefore, targeted antibiotic therapy based on culture and sensitivity results should be strongly recommended in the management of infection-related male infertility.

The findings of this study emphasize the need to incorporate routine semen culture and antimicrobial susceptibility testing into the standard evaluation of infertile men, particularly those with abnormal semen parameters or clinical features suggestive of infection [28]. Early identification and appropriate treatment of bacterial infections may improve semen quality, reduce oxidative stress, and enhance fertility outcomes.

5. CONCLUSION

This study underscores the significant role of **bacterial infections** in male infertility. Pathogens such as **Escherichia coli**, **Enterococcus faecalis**, and **Staphylococcus aureus** were commonly identified in semen samples and were associated with **impaired sperm quality**, including reduced motility, lower count, and increased abnormal morphology. Moreover, the study revealed high levels of **antibiotic resistance**, emphasizing the need for careful antibiotic management. Early detection and appropriate treatment of bacterial infections could improve **sperm quality** and **fertility outcomes** in infertile men.

Limitations
- This study was hospital-based, which may limit the generalizability of the findings to the broader population.
- The **seminal fluid analysis** did not include **viral infections** or **genetic factors**, which could also contribute to infertility.
- The study did not explore the **long-term effects** of bacterial infections on fertility or the **impact of multiple treatment regimens** over time.

DECLARATIONS
Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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