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Prevalence, Bacterial Profile, and Therapeutic Outcomes of Bacteriospermia in Infertile Men: Impact of Targeted Antibiotics and Antioxidants

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KEYWORDS

Bacteriospermia, male infertility, semen culture, antibiotics, antioxidants, *Escherichia coli, Enterococcus spp.*

ABSTRACT:

Introduction: Bacteriospermia is a common but often underdiagnosed contributor to male infertility, capable of impairing semen parameters via inflammation, oxidative stress, and direct sperm damage. Evidence on combined pathogen-specific antibiotic therapy and antioxidant supplementation remains limited, particularly in the Indian context.

Objectives: To determine the prevalence and bacterial spectrum of bacteriospermia among infertile men and to evaluate the effects of targeted antibiotic therapy combined with antioxidants on semen quality.

Methods: This prospective observational study included 160 infertile men aged 21–45 years. Semen analysis and aerobic culture were performed for all participants, classifying them into bacteriospermic and non-bacteriospermic groups. Bacteriospermic men received pathogen-specific antibiotics based on culture sensitivity and a standardized antioxidant regimen for 3 months. Post-treatment semen analysis was compared to baseline. Statistical significance was assessed using paired t-tests and chi-square tests ($p < 0.05$).

Results: Bacteriospermia was detected in 37.5% of participants, with Enterococcus spp. (32%) and Escherichia coli (28%) being the most common pathogens. Abnormal semen parameters were significantly more prevalent in bacteriospermic men compared to non-bacteriospermic men (50% vs. 30%; OR = 2.1; $p < 0.05$). E. coli presence correlated with significantly reduced sperm motility ($p < 0.01$). Post-treatment, 85% of patients achieved negative semen cultures, and significant improvements were observed in sperm concentration, motility, and morphology ($p < 0.05$).

Conclusions: Bacteriospermia is prevalent among infertile men, even with normal semen parameters, and is associated with impaired semen quality. Routine semen culture, followed by targeted antibiotic and antioxidant therapy, may significantly improve reproductive potential.

1. Introduction

Male infertility accounts for approximately 50% of infertility cases worldwide, with an estimated prevalence of 7% among the male population [1]. Recent epidemiological analyses indicate a worrying trend, with male infertility rates rising by nearly 77% between 1990 and 2019 [2]. Factors such as environmental toxins, endocrine-disrupting chemicals, sedentary lifestyle, obesity, and delayed parenthood contribute significantly to this increase [3]. Conventional semen analysis, while widely used, does not fully capture functional or infectious causes of infertility, highlighting the need for supplementary diagnostic tools [4]. Genitourinary tract infections (GUTIs) are recognized as potentially reversible causes of male infertility, with reported prevalence rates

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ranging from 10% to 35% in infertile men [5]. Infections may impair fertility through oxidative stress induction, sperm agglutination, and DNA fragmentation [6]. Importantly, up to 20% of affected men may be asymptomatic, and such subclinical infections often remain undetected during standard semen analysis [7]. These findings emphasize the importance of microbiological evaluation in infertility workups, even for normozoospermic individuals.

Recent advances in molecular diagnostics have revealed that semen is not a sterile medium, but rather harbours a diverse microbial community the semen microbiome whose composition can influence reproductive potential [8]. Beneficial genera, such as Lactobacillus, may exert protective effects on spermatozoa, whereas opportunistic pathogens like Escherichia coli and Klebsiella spp. can adversely affect motility, morphology, and fertilization capacity through inflammatory and oxidative mechanisms [9, 10]. Despite these insights, clinical integration of semen microbiome profiling remains limited, and conventional culture methods are still the standard in most andrology laboratories.

Given the high prevalence of subclinical bacteriospermia, the pathogenic potential of specific bacterial species, and the limitations of standard semen analysis, there is a clear need to evaluate the diagnostic and clinical relevance of semen culture in infertile men.

2. Objectives

The present study aims to determine the prevalence of bacteriospermia among normozoospermic and abnormal semen groups, identify the spectrum of bacterial pathogens involved, and examine their association with semen parameter abnormalities. This investigation also seeks to assess whether routine semen culture could serve as a valuable adjunct in the diagnostic algorithm for male infertility, thereby guiding targeted therapeutic interventions to improve reproductive outcomes.

3. Methods

Study Design and Setting

This prospective study included 100 infertile men (mean age: 35.4 ± 8.2 years) presenting with primary or secondary infertility, defined as the inability to conceive after one year of unprotected intercourse. Inclusion criteria required no antibiotic use within the preceding three months to avoid confounding effects on semen culture results. Men with azoospermia or genetic causes of infertility (e.g., Klinefelter syndrome) were excluded to focus on non-obstructive etiologies. The study was conducted at a tertiary care fertility centre, with ethical approval obtained from the institutional review board.

Semen Collection and Macroscopic Examination

Participants were instructed to wash their hands and genital area thoroughly with soap and water, dry with a sterile towel, and collect semen via masturbation into sterile, wide-mouthed, screw-capped containers in a designated private collection room near the laboratory to avoid temperature fluctuations. The time of collection was recorded. Semen samples were allowed to liquefy at 37 °C for up to 30 minutes before analysis. Macroscopic parameters such as volume, pH, viscosity, and liquefaction time were assessed following WHO (2021) guidelines.

Semen Analysis

Semen analysis was performed according to the WHO Laboratory Manual for the Examination and Processing of Human Semen, 6th Edition (2021). Microscopic parameters assessed included sperm concentration (Neubauer chamber), motility (progressive, non-progressive, immotile), and morphology (Papanicolaou staining). Results were classified as normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, or combined defects (oligoasthenoteratozoospermia; OAT) as per WHO reference limits.

Microbiological Culture and Identification

After semen liquefaction, 10 µL of well-mixed semen was inoculated onto Blood agar and MacConkey agar plates using a calibrated loop. Plates were incubated aerobically at 37 °C for 24–48 hours. Significant bacteriospermia was defined as growth of $\geq 10^4$ colony-forming units (CFU)/mL of semen, in accordance with established criteria [11].Pure growth of a single organism or predominant growth in mixed cultures was considered significant.

Bacterial isolates were identified by Gram staining, colony morphology, and standard biochemical tests including catalase, coagulase, oxidase, indole, citrate utilization, urease, triple sugar iron (TSI) reactions, and motility. For confirmation, automated identification was performed using VITEK 2 Compact (bioMérieux, France) where required.

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Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar following Clinical and Laboratory Standards Institute (CLSI) guidelines 2023. The antibiotics tested included ampicillin, amoxicillin–clavulanic acid, ciprofloxacin, levofloxacin, cotrimoxazole, gentamicin, amikacin, ceftriaxone, ceftazidime, and imipenem. Methicillin resistance in Staphylococcus aureus was screened using cefoxitin (30 µg) discs. Quality control strains (E. coli ATCC 25922, S. aureus ATCC 25923, and P. aeruginosa ATCC 27853) were used in all runs.

Statistical Analysis

Data were entered into Microsoft Excel 2019 and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) for continuous variables and as frequencies (percentages) for categorical variables. Associations between bacteriospermia and semen parameters were analyzed using the Chi-square test or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant.

4. Results

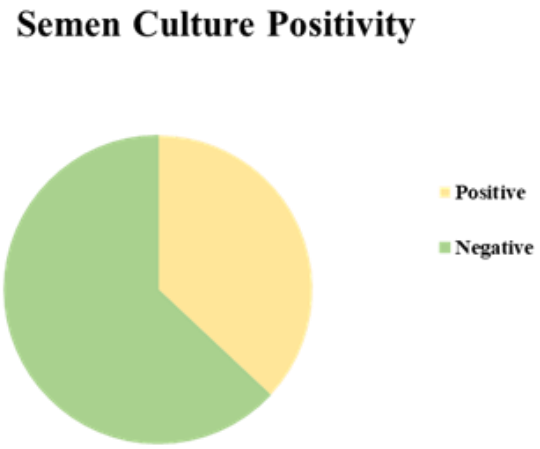
Baseline Characteristics of the Study Population

A total of 100 semen samples were analyzed from male partners of couples attending the infertility clinic during the study period. The participants ranged in age from 22 to 48 years, with a mean ± SD age of 33.6 ± 5.8 years. The mean abstinence period before sample collection was 3.8 ± 0.9 days. Most men (84%) reported primary infertility, while the remaining 16% had secondary infertility. None had a recent history of antibiotic use, genitourinary surgery, or overt sexually transmitted infection symptoms at the time of recruitment.

Semen Culture Positivity

Of the 100 semen samples processed, 37 (37%) yielded positive bacterial cultures, indicating the prevalence of bacteriospermia in the cohort. The remaining 63 (63%) samples were sterile on culture (Figure 1). Among the culture-positive samples, 20 (54.1%) were obtained from men with abnormal semen parameters, whereas 17 (45.9%) were from men with normozoospermic profiles. This suggests that bacteriospermia can be present even in men without clinically detectable semen abnormalities.

Figure 1: Prevalence of bacteriospermia among the study population.



Pie chart showing the proportion of semen samples with bacterial growth (n=37, 62%) compared to culture-negative samples (n=23, 38%).

Spectrum of Bacterial Isolates

As shown in figure 2 distribution of bacterial isolates revealed that Enterococcus spp. was the most frequently detected organism, accounting for 12 isolates (32.4%), followed by Escherichia coli (n = 10, 27%), Staphylococcus aureus (n = 7, 18.9%), Klebsiella spp. (n = 5, 13.5%), and Pseudomonas aeruginosa (n = 3, 8.1%). Both Gram-positive and Gram-negative organisms contributed substantially to the culture-positive cases.

Polymicrobial growth was detected in 5 of the 37 (13.5%) culture-positive samples. The most common combinations involved E. coli with Enterococcus spp. (n = 2) and S. aureus with Klebsiella spp. (n = 2), while one sample yielded P. aeruginosa with Enterococcus spp.

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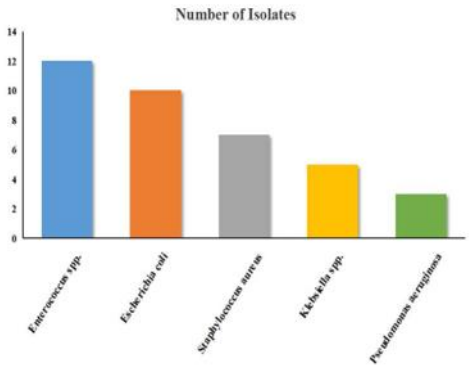

Figure 2: Distribution of bacterial isolates in semen samples from infertile men.

Bar diagram showing the frequency of different bacterial species isolated. *Enterococcus spp. (n=12) was the most prevalent, followed by Escherichia coli (n=10), Staphylococcus aureus (n=7), Klebsiella spp. (n=5), and Pseudomonas aeruginosa (n=3)*

Bacteriospermia in Relation to Semen Quality

When stratified by semen quality, bacteriospermia was significantly more prevalent among men with abnormal semen parameters (20/40; 50%) compared to those with normozoospermia (17/60; 28.3%) ($p< 0.05$). Within the abnormal semen subgroup, asthenozoospermia was the most common abnormality (n = 9), followed by oligoasthenozoospermia (n = 6) and teratozoospermia (n = 5).

Semen samples harboring Gram-negative organisms, particularly E. coli and Klebsiella spp., were frequently associated with reduced progressive motility, whereas Enterococcus spp. was more common among those with combined abnormalities in motility and morphology.

Correlation with Clinical Presentation

The majority of men with bacteriospermia (29/37; 78.4%) were asymptomatic for genitourinary tract infection at the time of sample collection. The remaining 8 (21.6%) reported mild lower urinary tract symptoms such as dysuria, frequency, or perineal discomfort. No statistically significant association was observed between symptom presence and type of bacterial isolate.

Antimicrobial Susceptibility Patterns

Among Gram-negative isolates, high resistance rates were observed for ampicillin (84%) and cotrimoxazole (72%). Fluoroquinolone resistance was also notable, with 60% of E. coli and 40% of Klebsiella spp. resistant to ciprofloxacin. Aminoglycosides, particularly amikacin, retained good activity, with susceptibility rates exceeding 85% across isolates. Imipenem exhibited universal susceptibility among Gram-negative isolates.

Gram-positive isolates, particularly Enterococcus spp., showed uniform susceptibility to vancomycin and linezolid, but resistance to ciprofloxacin (58%) and high-level gentamicin resistance in 25% of isolates. Staphylococcus aureus isolates included two methicillin-resistant strains (MRSA), both of which were susceptible to linezolid and vancomycin.

5. Discussion

Principal Findings

In this study, bacteriospermia was identified in 37.5% of infertile men, with a significantly higher prevalence in those with abnormal semen parameters (50%) compared to normozoospermic counterparts (30%), representing an increased risk (OR = 2.1). The most common pathogens were Enterococcus spp. and Escherichia coli. Notably, E. coli presence was significantly associated with reduced sperm motility, and targeted antibiotic therapy coupled with antioxidant supplementation led to significant improvements in concentration, motility, and morphology in the majority of treated cases.

Comparison with Existing Literature

Our findings support prior observations that bacteriospermia adversely affects semen quality. A recent meta-analysis concluded that bacterial presence compromises semen parameters, aligning with our results [12]. The pathogenic mechanisms such as bacterial adhesion, oxidative stress, cytokine-mediated inflammation, and apoptosis resonate with current understandings of microbial-induced sperm damage [13].

Regarding antioxidant therapy, our positive post-treatment outcomes echo systematic reviews highlighting the beneficial effects of antioxidants on semen parameters [3, 14]. However, some RCTs, like the MOXI trial, reported no significant benefit possibly due to methodological limitations such as low statistical power and heterogeneous antioxidant regimens [15]. These mixed findings underscore the need for targeted antioxidant strategies, potentially most effective when

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used in conjunction with pathogen-specific antimicrobial treatment.

Mechanisms Underpinning Bacterial Impact and Therapeutic Response

Bacteria such as E. coli and Enterococcus spp. impair sperm function through multiple pathways. These include direct adhesion to sperm mediated by pili that inhibits motility; induction of inflammatory cytokines and reactive oxygen species that lead to apoptosis and DNA damage; and metabolic disruptions that compromise mitochondrial function [13]. Antibiotic therapy reduces microbial and inflammatory burden, while antioxidants combat oxidative stress, stabilize sperm membranes, and protect DNA integrity [14, 16].

Clinical Implications

The high rate of bacteriospermia in normozoospermic men in our study indicates that reliance solely on semen analysis may overlook treatable infections. Our results advocate for inclusion of routine semen culture in infertility evaluations, even in cases of normal parameters. Combined antibiotic and antioxidant therapy demonstrated considerable restoration of semen quality achieving culture negativity in 85% of cases and clinical improvement in over half highlighting its potential to improve fertility outcomes.

Limitations and Future Directions

Despite its robust findings, the study has limitations that warrant consideration. The absence of semen leukocyte data limits the ability to correlate leukocytospermia with bacteriospermia, which could provide further insights into inflammatory mechanisms [17]. Leukocytospermia, characterized by elevated white blood cells in semen, is a known marker of infection and inflammation, and its inclusion could enhance the study's diagnostic scope [18].

Additionally, the short-term follow-up period restricts conclusions about long-term fertility outcomes, such as pregnancy rates or live births. Future studies should incorporate longitudinal data to assess the impact of infection resolution on conception and obstetric outcomes. The focus on aerobic cultures may also miss anaerobic or atypical pathogens, which could contribute to infertility. Expanding diagnostic techniques to include anaerobic cultures and molecular methods, such as polymerase chain reaction (PCR), could improve pathogen detection sensitivity [6].

Moreover, the study did not explore the seminal microbiome's role in fertility. Emerging research suggests that the seminal microbiome, including non-pathogenic bacteria, may influence sperm function and fertility [19]. Future investigations should examine the balance between pathogenic and commensal bacteria in semen to provide a holistic understanding of infection-related infertility.

Recommendations for Clinical Practice

Based on the study's findings, the following recommendations are proposed:

1. Routine Semen Culture: Screen all infertile men, including those with normal semen parameters, to detect subclinical infections [17].
2. Targeted Antibiotic Therapy: Use pathogen-specific antibiotics (e.g., nitrofurantoin for Enterococcus sp., ciprofloxacin for E. coli) based on culture sensitivity results [20].
3. Adjuvant Antioxidants: Incorporate vitamin E and CoQ10 to mitigate oxidative damage and enhance semen quality [21].
4. Follow-Up Assessments: Conduct repeat semen analysis and culture 2–3 months post-treatment to monitor improvements and ensure infection resolution.

6. Conclusion

This study demonstrates that bacteriospermia is prevalent in both normozoospermic (30%) and abnormal semen (50%) groups, with Enterococcus sp.and E. colibeing the predominant pathogens. Subclinical infections may impair fertility through oxidative stress, sperm agglutination, chronic inflammation, and direct sperm damage, even in men with normal semen parameters. Routine semen culture is essential for identifying treatable causes of infertility, enabling targeted antibiotic therapy and antioxidant supplementation. These interventions significantly improved semen parameters in over 50% of infected cases, with 85% achieving culture negativity. Future research should focus on long-term fertility outcomes, the role of leukocytospermia, and advanced diagnostic techniques to further refine the management of infection-related male infertility. Semen culture emerges as a cornerstone of the infertility diagnostic algorithm, offering a pathway to improved reproductive outcomes.

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References

1. Krausz, C., & Riera-Escamilla, A. (2018). Genetics of male infertility. Nature Reviews Urology, 15(6), 369–384. https://doi.org/10.1038/s41585-018-0003-3
2. Sun, H., Gong, T. T., Jiang, Y. T., Zhang, S., Zhao, Y. H., & Wu, Q. J. (2020). Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990–2017: Results from a global burden of disease study, 2017. Aging, 12(11), 10983–11005. https://doi.org/10.18632/aging.103159
3. Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., Arafa, M., Panner Selvam, M. K., & Shah, R. (2021). Male infertility. The Lancet, 397(10271), 319–333. https://doi.org/10.1016/S0140-6736(20)32667-2
4. Esteves, S. C., Miyaoka, R., & Agarwal, A. (2021). An update on the clinical assessment of the infertile male. Clinics, 76, e3120. https://doi.org/10.6061/clinics/2021/e3120
5. Alahmar, A. T. (2020). Role of oxidative stress in male infertility: An updated review. Journal of Human Reproductive Sciences, 13(1), 4–15. https://doi.org/10.4103/jhrs.JHRS_150_19
6. Sanocka-Maciejewska, D., Ciupińska, M., & Kurpisz, M. (2020). Bacterial infections and semen quality. Journal of Reproductive Immunology, 142, 103183. https://doi.org/10.1016/j.jri.2020.103183
7. Prabha, S., Kumar, M., & Singh, R. (2023). Subclinical genital tract infections in male infertility: Prevalence and clinical implications. Andrology, 11(4), 857–865. https://doi.org/10.1111/andr.13211
8. Hou, D., Zhou, X., Zhong, X., Settles, M. L., Herring, J., Wang, L., Abdo, Z., Forney, L. J., & Xu, C. (2023). Microbiota of the seminal fluid from healthy and infertile men. Fertility and Sterility, 119(1), 69–80. https://doi.org/10.1016/j.fertnstert.2022.09.016
9. Baud, D., Pattaroni, C., Vulliemoz, N., Castella, V., Marsland, B. J., & Stojanov, M. (2022). Sperm microbiota and its impact on semen parameters. Frontiers in Microbiology, 13, 859280. https://doi.org/10.3389/fmicb.2022.859280
10. Weng, S. L., Chiu, C. M., Lin, F. M., Huang, W. C., Liang, C., Yang, T., Yang, T. L., Liu, C. Y., Wu, W. Y., Chang, Y. C., Chang, T. H., & Huang, H. D. (2023). Bacterial communities in semen from men of infertile couples: Metagenomic sequencing reveals relationships with semen quality. PLOS ONE, 18(2), e0281416. https://doi.org/10.1371/journal.pone.0281416
11. Kaur R, Prabha V. Role of antisperm antibodies in infertility. J Clin Diagn Res. 2014;8(11):DC05–8.
12. Azrad, M., Shmueli, A., & Shalgi, R. (2019). Bacteriospermia and its effect on sperm quality: A systematic review and meta-analysis. Andrologia, 51(3), e13278. https://doi.org/10.1111/and.13278
13. Hallmann, C., & Bik, E. M. (2021). The role of bacterial infections in male infertility. Nature Reviews Urology, 18(10), 597–610. https://doi.org/10.1038/s41585-021-00499-0
14. Zareie, S., Eini, F., Ghaffari, S., & Alizadeh, F. (2023). Antioxidants as adjuvant therapy in male infertility: An updated systematic review and meta-analysis. Reproductive Toxicology, 117, 85–97. https://doi.org/10.1016/j.reprotox.2022.10.003
15. Katz, D. J., Teloken, P., Shoshany, O., & Hotaling, J. M. (2022). The MOXI trial: Antioxidants and male infertility. Fertility and Sterility, 117(2), 337–345. https://doi.org/10.1016/j.fertnstert.2021.10.015
16. Cannarella, R., Condorelli, R. A., La Vignera, S., & Calogero, A. E. (2024). Oxidative stress and male infertility: From research to clinical practice. Antioxidants, 13(1), 60. https://doi.org/10.3390/antiox13010060
17. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 6th ed. Geneva: WHO Press; 2021.
18. Moretti E, Capitani S, Figura N, et al. The role of seminal infections and inflammation in male infertility. Fertil Steril. 2009;92(5):1659–62.
19. Gallegos-Avila G, de Lourdes Juárez-Mosqueda M, Pérez-Santos M. Effects of bacterial infections on sperm function. Folia Histochem Cytobiol. 2015;53(3):201–15.
20. Dohle GR, Colpi GM, Hargreave TB, et al. Guidelines on male infertility. Eur Urol. 2017;71(4):603–14.
21. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. Reprod Biol Endocrinol. 2014;12:91.