Kufa Journal for Nursing Sciences
Open Access Full Text Article

Publisher
University of Kufa, Faculty of Nursing

Received: 05 May, 2025
Accepted: 23 June, 2025
Published: 28 June, 2025
Vol. 15(1), 2025: 110 – 121

https://doi.org/10.36321/kjns.vi20251.19499
ORIGINAL RESEARCH

Semen Evaluation and Sperm DNA Fragmentation in Males Infected with Burkholderia cepacia Complex

Kawther M. H. Al-Karaawi [1], Zina M. A. Alshami [2], Haider M. A. Samaka[3]

[1] Medical Microbiology department, College of Medicine, Jabir ibn Hayyan Medical University, Najaf, Iraq.
[2] Department of Family and Community Medicine, College of Medicine, Jabir ibn Hayyan Medical University, Iraq.
[3] Department of medical science, College of Medicine, Jabir ibn Hayyan Medical University, Najaf, Iraq.

CORRESPONDING AUTHOR: Kawther M. H. Al-Karaawi, Medical Microbiology department, College of Medicine, Jabir ibn Hayyan Medical University, Najaf, Iraq. Email: kawther.m.halboos@imu.edu.iq

ABSTRACT

Background: Burkholderia cepacia complex (BCC) is a multi-drug resist pathogen, its presence in semen, may be a factor in infertility by triggering the generation of reactive oxygen species (ROS), causing sperm DNA fragmentation.

Objectives: The current study aimed to investigate semen quality in the presence of Burkholderia cepacia compared to semen quality associated with other bacterial strains.

Methodology: In this case-control study, semen analysis was performed on 105 men to evaluate their infertility status due to the presence of infection. According to WHO guideline, 5th edition, 2010, semen was evaluated for all parameters. Sperm DNA fragmentation was also assessed to evaluate sperm DNA integrity. Using the VITEK 2 system, the isolated Burkholderia strain was identified, and its antibiotic resistance was tested.

Results: Among the 105 semen samples collected, bacterial isolates were found in 57 cases, with 8 isolates confirmed to be Burkholderia cepacia. The group infected with Burkholderia cepacia showed a significant reduction in progressive motility ($p<0.05$). And 75% of Burkholderia cases were isolated from immunocompromised individuals. 50% of the isolates exhibit weak biofilm formation. Antibiotic susceptibility testing using VITEK AST demonstrated 100% sensitivity to amikacin, gentamicin, meropenem, and ciprofloxacin, while sulfamethoxazole demonstrated the highest level of resistance.

Conclusion: As a result of the association between Bcc infection and compromised sperm function, microbiological screening should be considered in cases of unexplained male infertility.

Keywords: Semen analysis, Sperm DNA fragmentation, Oxidative stress, Bacterial infection, Male infertility.

INTRODUCTION

Burkholderia is a multidrug-resistant pathogen that poses a serious and urgent threat to public health, affecting nearly all areas of modern medicine [(1)]. Burkholderia consists of approximately 20 closely related bacterial species, termed Burkholderia cepacia complex (Bcc), that remain inadequately studied in terms of their medical significance and impact on human health [(2, 3)], particularly in immunocompromised patients and in those afflicted with chronic illnesses [(1, 4)].

Burkholderia is a rod, Gram-negative, motile, and obligately aerobic bacterium; the lipopolysaccharides (LPS) in the outer membrane play important roles in the pathogenicity and resistance [(4, 5)]. Additionally, Burkholder can utilize a wide range of nutrients for growth and metabolism.

How to Cite: Al-Karaawi, K. M. H. ., Alshami, Z. M. A., & Samaka, H. M. A. (2025). Semen evaluation and sperm DNA fragmentation in males infected with burkholderia cepacia complex. *Kufa Journal for Nursing Sciences, 15*(1), 110-121. https://doi.org/10.36321/kjns.vi20251.19499

Many members of the BCC share this characteristic, enabling them to adapt and thrive in diverse environments [6]. Burkholderia possesses several virulence factors, including biofilm formation, iron acquisition, enzymatic activity, and the ability to evade the host defenses by modulation of the immune response to avoid clearance [2, 5]. Bcc arose in the early 1980s as an opportunistic, obligate pathogen in humans; however, recently, it has been increasingly isolated as a human pathogen due to its ability to cause serious infections. Bcc species very rarely infect healthy individuals but can cause severe disease in immunocompromised individuals, including pregnant women, children, the elderly, and patients with cancer or other chronic illnesses [1]. Indeed, the BCC members are widespread and isolated from diverse clinical and environmental sources, including human samples, hospital environments, medical devices, water, soil, and various plants [7]. However, Bcc has been isolated from semen; this is relatively uncommon and usually associated with a genital tract infection or previous medical procedure such as using catheters or surgery [8]. The Bcc species exhibit intrinsic resistance to β-lactams, aminoglycosides, cationic antimicrobial peptides, and polymyxins. Additionally, they employ multiple resistance mechanisms against various other antibiotic classes, including quinolones, tetracyclines, chloramphenicol, and trimethoprim [1]; the high levels of resistance of this bacterium to various antimicrobial agents harshly restrict treatment options for affected patients [2].

The presence of bacteria in body fluids such as seminal fluid is well known to induce oxidative stress (OS) and inflammation; both are significant in causing sperm DNA fragmentation by increasing reactive oxygen species (ROS) and inflammatory cytokines, which in turn destroy DNA [9]. In addition to the effects of virulence factors such as proteases, lipopolysaccharides, pili, and mucin-binding adhesins, the heat-labile hemolysin also plays a significant role, exhibiting both phospholipase C and sphingomyelinase activities [10].

Burkholderia cepacia produce enzymes or toxins that directly compromise sperm cell membrane and DNA integrity or elicit an immune response by activating leukocytes to release reactive oxygen species (ROS), further destroying sperm DNA [11]. The presence of Burkholderia cepacia in seminal fluid requires treatment with appropriate antibiotics; the sensitivity of the bacteria to antibiotics must be determined because of their known resistance to some antibiotics, which may be considered another cause for sperm DNA fragmentation [12].

Several studies stated the effect of bacterial infection on sperm quality [12,13,14, and 15].

AIMS OF THE STUDY

The current study aimed to investigate semen quality in the presence of Burkholderia cepacia compared to semen quality associated with other bacterial strains.

METHODOLOGY

Design of study and participants

This case control study was conducted following the World Health Organization guidelines(WHO, 2010). Semen samples were collected with the approval of Medicine College / Jabir Ibn Hayyan University for Medical and Pharmaceutical sciences Bioethics Committee (No. 56, July 7, 2024). A total of 105 males participated in the study, all of whom visited either governmental fertility centers or private fertility clinics between July and November 2024. The participants were divided into two groups: the study group included 57 males and the control group included 27 healthy males, samples from 21 patients were excluded from the study due to the presence of gram positive bacteria. Before sample collection, each participant completed a questionnaire form that included age, weight, parenthood status, chronic disease history, and family fertility history.

Study group: consisted of males aged 20 to 45 with mean age (30.28) years who pursued semen evaluation for either infection assessment or fertility testing.

Control group: consisted of individuals with normal semen who had no infections or underlying diseases.

Study limitations

This study has several limitations, such as the relatively limited sample size, which may limit the extrapolation of the results to broader population groups. A second significant limitation is the difficulty in identifying cases of DNA fragmentation resulting from unknown causes, such as vitamin D3 deficiency, exposure to harmful environmental factors and radiation, and a history of alcohol consumption, which may often not be disclosed by the participants.

Sample collection

The study period started in July 2024 to January 2025. Seminal samples were obtained from patients undergoing routine semen analysis seeking fertility in the private andrology laboratory, the Fertility Center in Al-Seder Medical City, and Al-Hakim General Hospital in Najaf City. Participants were guided to collect semen samples by ejaculation after 3 to 5 days of sexual abstinence. Patients were instructed to urinate before ejaculation to avoid possible contamination from the urine or external genitalia. During sample collection, the use of lubricants was prevented, as they may affect the sample characteristics [16].

Semen preparation

Semen samples were divided aseptically into 3 parts; the first part was incubated at 37°C for 30 minutes to allow liquefaction, and a 10 µl aliquot was used for semen analysis, including pH, sperm concentration, sperm motility, and morphology; the analysis was performed according to the World Health Organization guideline [17]. The second part (50 µl) was used for testing sperm DNA fragmentation, and the third part of the samples was sent aseptically to the microbiology laboratory for bacterial culturing [18].

DNA fragmentation assessment

The test is based on the sperm chromatin dispersion assay (SCDA). Regulated DNA denaturation, followed by nuclear protein extraction, leads to partial deproteinization in which the DNA loops extend, forming chromatin-dispersion halos. However, either there is no dispersion halo or the halo does not produce any spermatozoa nucleoids whose sperm dioxide is fragmented.

The kit was used according to the instruction attached to it from the company (WWW.IVFCO.IR):

The sperm sample was diluted with phosphate buffer saline (PBS) until it reached a concentration of 20 million per ml. If the count of sperm is low, the semen centrifuged for 5-7 minutes at 1200 rpm and use the sediments. The agarose tube was put in a water bath at a temperature of 95-100˚C for 5 minutes until the gel inside was completely dissolved. Then, the tube was kept at 37˚C for 2 minutes. Immediately after, 50 µl from the sperm sample was transferred to the agarose tube and mixed gently with a micropipette. following directly, 25 µl was taken and put on the slides and covered by cover slid with no bubbles and put in the refrigerator for 5 minutes at a temperature of 4˚C. Then the cover slide was removed horizontally and gently. the slide was placed in solution A (denaturation solution) for 7 minutes at room temperature. then in solution B (lysis solution) for 15 minutes at room temperature. Then placed the slides horizontally in distilled water for 5 minutes. the slide was applied at the concentrations of 70%, 90%, and 100%, respectively, for 2 minutes for each concentration of ethanol. Allow it to dry on filter paper. C solution was added (staining process) and incubated for 75 seconds. then, removed completely. D solution was applied and incubated for 3 minutes. Then, the stain was removed by tilting. At the end, solution E was added and incubated for 2 minutes. Excess stain was removed with distilled water and allowed to dry at room temperature. The counting is under a light microscope at 40×, and in our study 300 sperms were counted for each sample. The DNA-

fragmented sperm appears without a halo, and the sperm with the halo appears without the DNA fragment Figure 1.

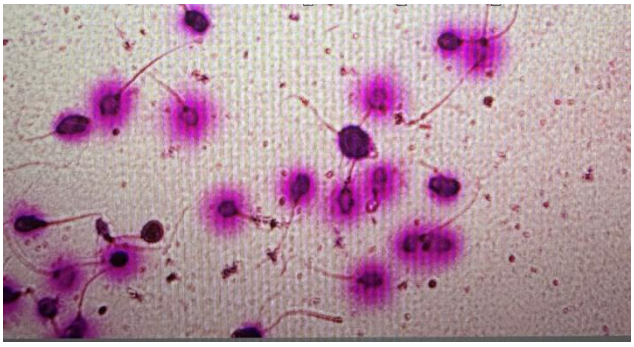

Figure (1): DNA Fragmentation Pattern of sperm: a. fragmented sperms (no halo), b. medium damage (small halo), c. normal sperm (big halo).

Semen culture

To conduct bacterial culture, the semen sample was transferred aseptically to the microbiology laboratory less than 3 hours after sample collection. A loopful of the sample was cultured on MacConkey agar, and the culture media was incubated for 18-24 hours at 37°C [19]. The identification of the isolates was performed using different biochemical tests, including catalase, oxidase, triple sugar iron agar (TSI), H2S production test, Simmons Citrate, motility test, and urease test. Culture media used included (MacConkey agar, brilliant green agar, and xylose lysine deoxycholate agar [20]. Further species identification was done using the VITEK 2 system according to the manufacturer's instructions.

Detection of biofilm by microtiter plate

The microtiter plate method was used to detect bacterial biofilm formation as recommended by Stepanovi´c et al. and Kuinkel et al., using Muller Hinton broth (supplemented with 1% glucose) to adjust the bacterial suspension to 0.5 McFarland (108 cfu/ml). This bacterial suspension was 20-fold diluted to reach 5*106 cfu/ml. Add 20 µl of bacterial suspensions to 180 µl of MHB supplemented with 1% glucose and inoculate into a 96-well sterile microplate to reach 5*105cfu/ml as the final concentration. The microtiter plate was incubated at 37°C for 24 hours, the formed biofilm on the wall of the microplate was fixed by methanol for 20 min, and stained by using 150 µl of safranin for 15 minutes, and the wells were washed twice with phosphate buffer saline (PBS) pH (7.2); the plate was dried at 600C for one hour. The excess dye was resolubilized using 150 µl of 95% ethanol (figure 2). The concentration of the biofilm formation was measured using a spectrophotometer microtiter plate reader at 570 nm. The well containing sterile MHB medium was used as a blank for calculating (OD) [21].

The optical density cutoff value (ODc) was determined using the formula [ODc = Average OD of blank + (3 × Standard Deviation (SD) of blank)]. And for each bacterial isolate, the biofilm formation was calculated as follows: [OD isolate = Average OD of the isolate – Odc] [21], If the result obtained from this calculation was negative, it was adjusted to zero, signifying the absence of biofilm production. Conversely, a positive value confirmed biofilm formation.

According to the formula above, the results can be categorized based on optic density measurements after calculating the cutoff value (ODc). [21]

1. [OD ≤ Odc] "indicate no biofilm production."
2. [ODc < OD ≤ 2 × ODc] "indicate weak biofilm production."
3. [2 × ODc < OD ≤ 4 × Odc] "indicate moderate biofilm production"
4. [OD > 4 × Odc] "indicate strong biofilm production."

Statistics

Statistical analysis was performed using [insert software, e.g., SPSS version X.X or R version X.X]. Categorical variables, such as DNA fragmentation categories, were expressed as frequencies and percentages. The differences in distribution among the groups were evaluated using the chi-square test.

When an overall significant difference was found among more than two groups, post hoc pairwise chi-square tests were conducted to identify which specific group differences were significant. To

adjust for multiple comparisons, a Bonferroni correction was applied, setting the adjusted significance level at α = 0.0167 (i.e., 0.05 divided by 3 pairwise comparisons). P value less than 0.005 was considered significant.

RESULTS

Fifty-seven gram-negative bacterial strains were isolated from a total of 105 samples collected from males with suspected secondary infertility; 8 (14.03%) isolates were confirmed as members of the Burkholderia cepacia complex, and 49 (85.96%) cultures had gram-negative bacteria other than BCC.

The number of isolated BCC was higher at 6/8 (75%) among immunocompromised individuals, as summarized in Table 1, with significant differences between the groups ($p < 0.05$).

The data showed that eight patients had ages ranging from 22 to 37, two patients had no underlying condition, and six had different underlying conditions. Weak biofilm formation was found in 4/8 (50%) of isolates. Table 2.

Two samples only showed a high number of leukocytes (more than 1×106/ml) that was considered a case of leukocytospermia according to the definition of the WHO, as given in figure 2.

Analysis of semen sample parameters of the study group indicated only progressive motility with a significant difference ($p < 0.05$) compared to the control group, while other parameters, such as total sperm concentration, volume, and viscosity, were non-significant ($p > 0.05$).Table 3.

An evaluation of sperm DNA fragmentation among the participants revealed statistically significant differences in sperm quality ($p<0.05$). However, the results suggest that Bcc infection has a significantly different impact on semen quality or sperm DNA integrity when compared to other Gram-negative bacterial infections. (Table 4).

results of DNA fragmentation were statistically significant, according to the Post hoc pairwise chi-square tests with Bonferroni correction (α = 0.0167) results were significant for the BCC group and the control group ($p = 0.0023$), also the difference with other Gram-negative group and the control group ($p < 0.000001$). however, a non-significant difference was observed between the BCC group and the other Gram-negative group ($p = 0.702$) (Table 5).

antimicrobial susceptibility test was performed using VITEK , antibiotics including cefepime, cefazoline, ceftazidime, ceftriaxone, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole, and piperacillin / tazobactam were evaluated. As shown in Figure 3, the study isolates were sensitive to amikacin, gentamicin, meropenem, and ciprofloxacin (100% sensitivity), while trimethoprim/sulfamethoxazole exhibited the highest resistance levels among the tested antibiotics. According to figure 3.

DISCUSSION:

Bacterial infection are frequently recorded and pathogens were isolated from semen of infertile men, consistent epidemiological links have been established between bacterial infections and male infertility with alterations in semen. a recent study reported that bacterial infections directly contribute to 15% of male infertility [22]. BCC has been isolated from different clinical samples, such as skin infections, bacteremia, soft tissue infections, and respiratory tract infections, as reported in several studies [23, 24]. Based on current knowledge, this is the first study in Iraq to isolate Burkholderia cepacia from semen and show how it damages sperm DNA integrity and semen parameters, which in turn affects male fertility. Burkholderia cepacia complex is an invasive bacteria that can reach the body's sterile site, especially among immunocompromised patients [25], and BCC outbreaks are being recorded globally in hospitals and other healthcare facilities [26]. The emergence of multidrug-resistant Burkholderia cepacia is associated with high mortality rates, particularly among immunocompromised patients. Traveling to regions experiencing outbreaks of drug-

resistant Burkholderia cepacia poses significant health risks for such individuals [27].

This study demonstrates that sperm motility may be impeded by bacterial infections in semen, which has the potential to result in infertility. Other semen parameters, such volume, viscosity, and sperm concentration, on the other hand, were largely unaffected. These findings are consistent with earlier research showing that infection largely affects sperm motility instead of other factors. In both natural and assisted fertilization, this impairment may notably reduce reproductive success since progressive motility is essential for sperm to reach and pierce the egg [11, 28]. But as stated in other research, bacterial infections have a negative impact on each sperm parameter and could serve as a major factor in decreased fertility and reproductive potential [29]. One of the most notable findings in this study is the high rate of DNA damage in males infected with BCC compared to uninfected people and those with other gram-negative bacteria ($p < 0.05$). This effect on sperm function consequently leads to the failure of egg fertilization and failure to achieve pregnancy [30]. This is likely to Burkholderia cepacia have a direct effect on sperm DNA integrity either through inducing oxidative stress (OS), by stimulation of the inflammatory pathway [31] or by releasing bacterial virulence factors and toxins [10]. Biofilm production and toxin secretion [32] are the principal causes of tissue damage and therapy failure during infection [33]. Recent studies highlighted the effect of increased ROS on sperm DNA damage, which is a significant factor in male fertility [34]. One prominent study conducted by Syed Waseem Andrabi [35] explored that one of the most common causes of male infertility is reproductive tract infection. Staphylococcus aureus, Pseudomonas aeruginosa, Ureaplasma urealyticum, Chlamydia trachomatis, and Mycoplasma are the most common types of organisms that cause urogenital tract infection that may cause excessive ROS generation, which leads in increased oxidative stress that is well-recorded in reproductive biology as a key participant to DNA damage, mainly in sperm cells, even though sperm have definite antioxidant defenses [36]. Furthermore, most Bcc-positive culture results were for individuals with immune-suppressing conditions, reinforcing the idea that the weakened immune system makes them more vulnerable to persistent infections [37], and data showed that 50% of immunocompromised patients did not exhibit abnormal increases in WBC count in the semen because of the failure of the immune system to stimulate immune reaction. Since most patients show symptoms due to an immune response to infection, those with immunosuppression may show an ineffective response; as a result, the body struggles to resist the infection in the early stages, increasing the risk of chronic infection. Accordingly, routine investigation of bacterial infection is essential for immunocompromised individuals to avoid chronic infection.

The most significant aspect of Burkholderia infections is their difficulty treating them due to their significant antibiotic resistance [38]. In this study, antibiotic susceptibility testing confirmed resistance to multiple antibiotics, limiting treatment options and increasing therapy failure.

One of the key mechanisms for antimicrobial resistance in Burkholderia species is their ability to form a biofilm, which not only enhances their resistance to antibiotic agents but also helps them evade the immune system's action and facilitates the exchange of genetic materials with other bacterial strains; as a result, biofilm formation overcomplicates the eradication process and improves bacterial survival in hostile environments [39]. At the same time, some of the isolates showed weak biofilm production, while others demonstrated no detectable biofilm production. Remarkably, none of the tested samples showed strong or moderate biofilm production, which disagrees with most studies [40, 41, and 42]. However, it is possible that the effect of seminal fluids and immunity will compromise BCC's ability to form a

strong biofilm. This is due to its possession of a highly efficient immune system [26, 43].

CONCLUSIONS:

This study confirms the importance of bacterial isolation and identification in cases of unexplained infertility and their effect on the level of sperm DNA fragmentation. The presence of Bcc in semen warrants clinical caution, as it is highly associated with persistent infections and secondary infertility in males. A comprehensive survey is required to help healthcare institutions detect BCC outbreaks early and determine potential sources. Future studies should focus on novel therapeutic strategies containing antioxidant treatment or biofilm disruptors to reduce BCC's effect on sperm quality.

Declarations

Ethical approval and consent to participate

This case-control study was conducted following the World Health Organization guidelines (WHO, 2010). Semen samples were collected with the approval of the Medicine College/Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences Bioethics Committee (No. 56, July 7, 2024).

Authors contribution

All authors contributed equally.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

REFERENCES:

1. Lauman, P., & Dennis, J. J. (2021). Advances in phage therapy: targeting the Burkholderia cepacia complex. *Viruses*, 13(7), 1331.
2. Sousa, S. A., Feliciano, J. R., Pita, T., Guerreiro, S. I., & Leitão, J. H. (2017). Burkholderia cepacia complex regulation of virulence gene expression: a review. *Genes*, 8(1), 43.
3. Holden, M. T., Seth-Smith, H. M., Crossman, L. C., Sebaihia, M., Bentley, S. D., Cerdeno-Tarraga, A. M., ... & Parkhill, J. (2009). The genome of Burkholderia cenocepacia J2315, an epidemic pathogen of cystic fibrosis patients. *Journal of bacteriology*, 191(1), 261-277.
4. Leite, F. C., Machado, A. B. M. P., Lutz, L., Vieira, M. I., & Barth, A. L. (2011). Molecular identification of Burkholderia cepacia complex and species distribution among cystic fibrosis patients seen at the reference center in Southern Brazil. *Clinical and Biomedical Research*, 31(2).
5. Nelson, J. W., Butler, S. L., Krieg, D., & Govan, J. R. (1994). Virulence factors of Burkholderia cepacia. *FEMS immunology and medical microbiology*, 8(2), 89-97.
6. Sanz-García, F., Gil-Gil, T., Laborda, P., Ochoa-Sánchez, L. E., Martínez, J. L., & Hernando-Amado, S. (2021). Coming from the wild: multidrug resistant opportunistic pathogens presenting a primary, not human-linked, environmental habitat. *International Journal of Molecular Sciences*, 22(15), 8080.
7. Tavares, M., Kozak, M., Balola, A., & Sá-Correia, I. (2020). Burkholderia cepacia complex bacteria: a feared contamination risk in water-based pharmaceutical products. *Clinical microbiology reviews*, 33(3), 10-1128.
8. Stettler, G. R., Preslaski, C., Lawless, R., Cohen, M., & Platnick, B. (2023). Burkholderia cepacia infection in an immunocompetent patient following pancreaticoduodenectomy. *The American Surgeon™*, 89(4), 1099-1101.
9. Cocuzza, M., Sikka, S. C., Athayde, K. S., & Agarwal, A. (2007). Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis. *International braz j urol*, 33, 603-621.
10. Hutchison, M. L., Poxton, I. R., & Govan, J. R. (1998). Burkholderia cepacia produces a hemolysin that is capable of inducing apoptosis and degranulation of mammalian phagocytes. *Infection and immunity*, 66(5), 2033-2039.

11. Marchiani, S., Baccani, I., Tamburrino, L., Mattiuz, G., Nicolò, S., Bonaiuto, C., ... & Baldi, E. (2021). Effects of common Gram-negative pathogens causing male genitourinary-tract infections on human sperm functions. *Scientific reports*, 11(1), 19177.

12. Eini, F., Kutenaei, M. A., Zareei, F., Dastjerdi, Z. S., Shirzeyli, M. H., & Salehi, E. (2021). Effect of bacterial infection on sperm quality and DNA fragmentation in subfertile men with Leukocytospermia. *BMC Molecular and Cell Biology*, 22, 1-10.

13. Wang, S., Zhang, K., Yao, Y., Li, J., & Deng, S. (2021). Bacterial infections affect male fertility: A focus on the oxidative stress-autophagy axis. *Frontiers in cell and developmental biology*, 9, 727812.

14. Hassan, E. A., Sadeek, Y. K., Abdelghany, T. M., & Gadel-Rab, A. G. (2024). Influence of bacterial infection on human sperm. *Journal of Bioscience and Applied Research*, 10(6), 137-150.

15. Rusz, A., Pilatz, A., Wagenlehner, F., Linn, T., Diemer, T. H., Schuppe, H. C., ... & Weidner, W. (2012). Influence of urogenital infections and inflammation on semen quality and male fertility. *World journal of urology*, 30, 23-30.

16. Moretti, E., Capitani, S., Figura, N., Pammolli, A., Federico, M. G., Giannerini, V., & Collodel, G. (2009). The presence of bacteria species in semen and sperm quality. *Journal of assisted reproduction and genetics*, 26, 47-56.

17. World Health Organization. (2010). World health statistics 2010. *World Health Organization*.

18. Karthikeyan, M., Kubera, N. S., & Singh, R. (2021). Association of Semen Bacteriological Profile with Infertility:–A Cross-Sectional Study in a Tertiary Care Center. *Journal of Human Reproductive Sciences*, 14(3), 260-266.

19. Hakim, Y., Abbas, A. A., Abakar, A. D., Siddig, H. M., AlHassan, Y. M., & Siddig, A. T. (2017). Gram Negative Bacteria and Their Effect on Some Semen Parameters Of Infertile Men Attending Fertility Center at Wad Medani (November 2015-2016).

20. Matthaiou, D. K., Chasou, E., Atmatzidis, S., & Tsolkas, P. (2011). A case of bacteremia due to Burkholderia cepacia in a patient without cystic fibrosis. *Respiratory Medicine CME*, 4(3), 144-145.

21. Kırmusaoğlu, S. (2019). Biofilm and Screening Antibiofilm Activity of Agents. *Antimicrobials, antibiotic resistance, antibiofilm strategies and activity methods*, 99.

22. Lakhe, G., Nair, N., Pareek, C., & Ugemuge, S. (2024). Bacteriospermia-Related Male Infertility: A Case Report on Diagnostic and Therapeutic Approaches. *Cureus*, 16(6).

23. Folescu, T. W., da Costa, C. H., Cohen, R. W. F., Neto, O. C. D. C., Albano, R. M., & Marques, E. A. (2015). Burkholderia cepacia complex: clinical course in cystic fibrosis patients. *BMC pulmonary medicine*, 15, 1-6.

24. Kwayess, R., Al Hariri, H. E., Hindy, J. R., Youssef, N., Haddad, S. F., & Kanj, S. S. (2022). Burkholderia cepacia infections at sites other than the respiratory tract: A large case series from a tertiary referral hospital in Lebanon. *Journal of Epidemiology and Global Health*, 12(3), 274-280.

25. Luk, K. S., Tsang, Y. M., Ho, A. Y. M., To, W. K., Wong, B. K. H., Wong, M. M. L., & Wong, Y. C. (2022). Invasive Burkholderia cepacia complex infections among persons who inject drugs, Hong Kong, China, 2016–2019. *Emerging Infectious Diseases*, 28(2), 323.

26. Wigby, S., Suarez, S. S., Lazzaro, B. P., Pizzari, T., & Wolfner, M. F. (2019). Sperm success and immunity. *Current topics in developmental biology*, 135, 287-313.

27. Sullivan, K. E., Bassiri, H., Bousfiha, A. A., Costa-Carvalho, B. T., Freeman, A. F., Hagin, D., ... & Tang, M. L. (2017). Emerging infections and pertinent infections related to travel for patients with primary immunodeficiencies. *Journal of clinical immunology*, 37, 650-692.

28. Lazem, A. A., Al-Kaseer, E., Al-Diwan, J. K., & Al-Hadithi, T. S. (2010). Effect of infection on semen parameters in a sample of Iraqi infertile males.

Journal of the Faculty of Medicine Baghdad, 52(3), 274-276.

29. Zeyad, A., Amor, H., & Hammadeh, M. E. (2017). The impact of bacterial infections on human spermatozoa. *International Journal of Women's Health and Reproduction Sciences*, 5(4), 243-252.

30. Coughlan, C., Clarke, H., Cutting, R., Saxton, J., Waite, S., Ledger, W., ... & Pacey, A. A. (2015). Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. *Asian journal of andrology*, 17(4), 681-685.

31. Drevinek, P., Baldwin, A., Lindenburg, L., Joshi, L. T., Marchbank, A., Vosahlikova, S., ... & Mahenthiralingam, E. (2010). Oxidative stress of Burkholderia cenocepacia induces insertion sequence-mediated genomic rearrangements that interfere with macrorestriction-based genotyping. *Journal of Clinical Microbiology*, 48(1), 34-40.

32. Leitão, J. H., Sousa, S. A., Ferreira, A. S., Ramos, C. G., Silva, I. N., & Moreira, L. M. (2010). Pathogenicity, virulence factors, and strategies to fight against Burkholderia cepacia complex pathogens and related species. *Applied Microbiology and Biotechnology*, 87, 31-40.

33. Hendry, J., Elborn, J. S., Nixon, L., Shale, D. J., & Webb, A. K. (1999). Cystic fibrosis: inflammatory response to infection with Burkholderia cepacia and Pseudomonas aeruginosa. *European Respiratory Journal*, 14(2), 435-438.

34. Hussain, T., Kandeel, M., Metwally, E., Murtaza, G., Kalhoro, D. H., Yin, Y., ... & Kalhoro, M. S. (2023). Unraveling the harmful effect of oxidative stress on male fertility: A mechanistic insight. *Frontiers in endocrinology*, 14, 1070692.

35. Andrabi, S. W., Ara, A., Saharan, A., Jaffar, M., Gugnani, N., & Esteves, S. C. (2024). Sperm DNA Fragmentation: causes, evaluation and management in male infertility. *JBRA assisted reproduction*, 28(2), 306.

36. Walke, G., Gaurkar, S. S., Prasad, R., Lohakare, T., & Wanjari, M. (2023). The impact of oxidative stress on male reproductive function: exploring the role of antioxidant supplementation. *Cureus*, 15(7).

37. Govan, J. R. W., Hughes, J. E., & Vandamme, P. (1996). Burkholderia cepacia: medical, taxonomic and ecological issues. *Journal of medical microbiology*, 45(6), 395-407.

38. Rhodes, K. A., & Schweizer, H. P. (2016). Antibiotic resistance in Burkholderia species. *Drug Resistance Updates*, 28, 82-90.

39. Gunardi, W. D., Timotius, K. H., Natasha, A., & Evriarti, P. R. (2021). Biofilm targeting strategy in the eradication of Burkholderia infections: a mini-review. *The Open Microbiology Journal*, 15(1).

40. Narayanaswamy, V. P., Duncan, A. P., LiPuma, J. J., Wiesmann, W. P., Baker, S. M., & Townsend, S. M. (2019). In vitro activity of a novel glycopolymer against biofilms of Burkholderia cepacia complex cystic fibrosis clinical isolates. *Antimicrobial agents and chemotherapy*, 63(6), 10-1128.

41. Ghafil, J. A., İbrahim, B. M. S., & Zgair, A. K. (2022). Coating indwelling urinary catheters with moxifloxacin prevents biofilm formation by Burkholderia cepacia. *Polymers in Medicine*, 52(1), 5-9.

42. Karthikeyan, R., Singh, B. R., Yadav, A., Agri, H., Jayakumar, V., OR, V., & Sinha, D. K. (2023). Antimicrobial Susceptibility Testing and Biofilm Production of Burkholderia cepacia Complex Organisms from Ultrasound Gels in India. *Act Scie Micro*, 6(2), 27-36.

43. Edstrom, A. M., Malm, J., Frohm, B., Martellini, J. A., Giwercman, A., Morgelin, M., ... & Sørensen, O. E. (2008). The major bactericidal activity of human seminal plasma is zinc-dependent and derived from fragmentation of the semenogelins. *The Journal of Immunology*, 181(5), 3413-3421.

TABLES & Figures:

Table (1): Distribution of Participants According to Immune Status

Immune status	With BCC	Gram-negative other than BCC	Control group	Total
Immunocompromised individuals	6(75%)*	4(8.1%)	3(11.11%)	13(15.47%)
immunocompetent individuals	2(25%)	45(91.83%)	24(88.88%)	71(84.52%)
Total	8(9.52%)	49(58.33%)	27(32.14%)	84(100%)

*** ($p<0.05$).**

Table (2): General Description of Patient's Data Associated with BCC

Patient number	Age	Underlying condition	Medication	Leukocyte count	DNA fragmentation level	Biofilm production
1	27	Lung cancer	Chemotherapy	1.2×10^6/ml	Abnormal >30%	Negative
2	31	None	None	53×10^2/ml	Abnormal >30%	Weak
3	22	* DM-type 1	Insulin injection	1.32×10^6/ml	Moderate (15-30) %	Negative
4	27	Colon cancer	Chemotherapy	44×10^2/ml	Moderate (15-30) %	Negative
5	27	SLE	Corticosteroid	12×10^2/ml	Moderate (15-30) %	Weak
6	37	Non	Non	2.6×10^6/ml	Moderate (15-30) %	Weak
7	26	Rheumatoid arthritis	Corticosteroid	1.6×10^6/ml	Normal <15 %	Weak
8	36	* DM- type 1	Insulin injection	3×10^2/ml	Moderate (15-30) %	Negative

*** DM- type 1; Diabetic Meletus type 1, SLE; Systemic lupus erythematous.**

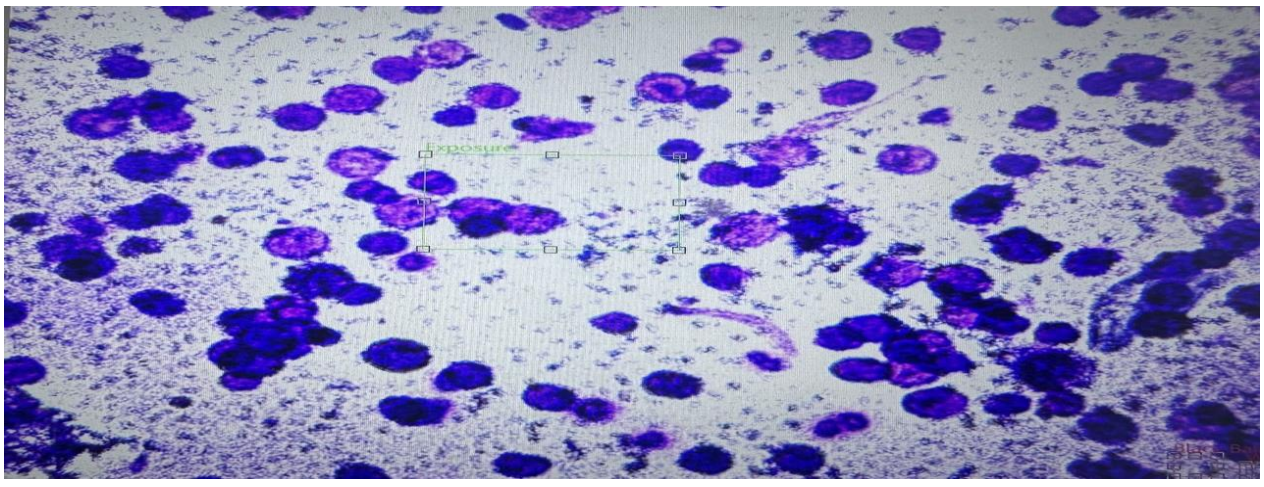

Figure (2): Semen sample with leukocytospermia

Table (3): The Effect of Burkholderia Cepacia Complex Infection on Semen Parameters Compared to the Control Group

Semen parameters		Type of infection			P value
		Burkholderia cepacia infection group	Control group	Other Gram-negative bacteria	
Viscosity	Normal	7 (87.5%)	22(81.48%)	37(75.51%)	0.83370255
	Moderate	0 (0%)	2(7.4%)	6(12.24%)	
	High	1 (12.5%)	3(11.11%)	6(12.24%)	
Volume (ml)	≥1.5(normal)	8 (100%)	25(92.59%)	46(93.87%)	0.7367549
	<1.5(abnormal)	0 (0%)	2(7.4%)	3(6.12%)	
Total sperm count (10^6)	≥15(normal)	4 (50%)	22 (81.48%)	36(73.46%)	0.20484534
	<15(abnormal)	4 (50%)	5(18.51%)	13(26.53%)	
Progressive Motility	≥32%(normal)	2 (25%)	24(88.88%)	14(28.57%)	0.00000124*
	<32%(abnormal)	6 (75%)	3(11.11%)	3571.42%)	

*(p<0.05).

Table (4): The Effect of Burkholderia Cepacia Complex Infection on the Rate of DNA Fragmentation

Type of infection	DNA fragmentation percentage			Total
	Normal<15 %	Moderate (15-30) %	Abnormal >30%	
Burkholderia cepacia infection group	1(12.5%)	5(62.5%)	2(25%)	8
Other Gram-negative bacterial infection group	7(14.28%)	23(46.93%)	19(38.77)	49
Control group	19(70.37%)	8(29.62%)	0(0%)	27
Total		84 (100%)		
p value		*0.00000373		

* (p<0.05).

Table (5): post hoc pairwise comparisons using chi-square tests, with Bonferroni correction applied

Comparison	p-value	Significant (α = 0.0167)
BCC vs. Other Gram-negative	0.702	No
BCC vs. Control	0.0023	Yes
Other Gram-negative vs. Control	9.4×10^{-7}	Yes

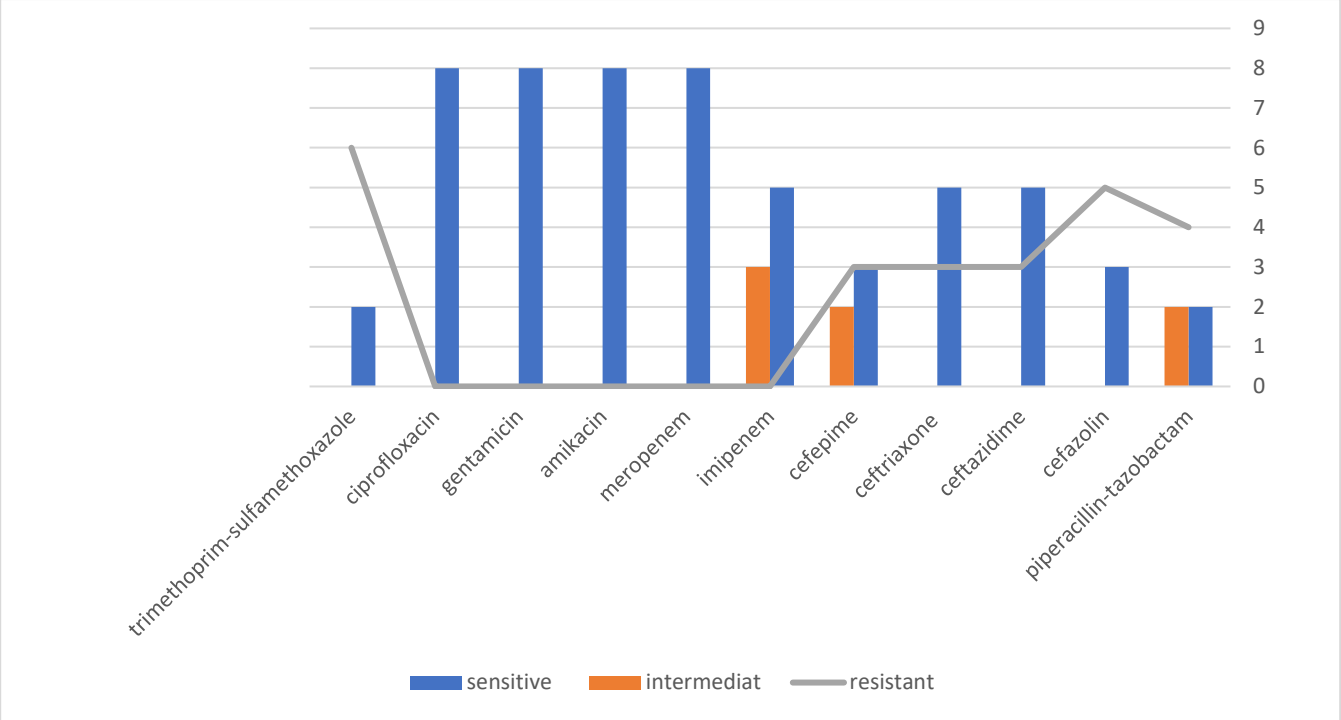

Figure (3): Antibiotic susceptibility test profile of Burkholderia cepacia complex