



MICROBIAL DYNAMICS IN PERITONEAL FLUID OF INFERTILE WOMEN: PREVALENCE, SENSITIVITY, AND RESISTANCE PATTERNS

Obstetrics & Gynaecology

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ABSTRACT

Backgrounds: Microbial infections of the female reproductive tract are known to play significant role in causing infertility. In the present investigation the microbiological profile of the peritoneal fluid from infertile women was determined and the antimicrobial susceptibility patterns of the isolated microorganisms were investigated. **Methodology:** This is an observational analytical study comprising of 80 infertile women who underwent diagnostic laparoscopy at SMS Medical College and Zanana Hospital Jaipur. Peritoneal fluid samples were obtained and the cytological, microbiological and antimicrobial susceptibility analysis was performed. **Results:** There was a generally high prevalence of people aged between 25 and 35 (73.75%) and primary infertility was more prevalent (57.5%). In 41.25% of cases, the peritoneal cytology revealed inflammatory changes. The most common pathogens were *Mycobacterium tuberculosis* (26.25%), *Neisseria gonorrhoeae* (8.75%) and *Chlamydia trachomatis* (6.25%). The most sensitive organism for Rifampicin was *M. tuberculosis*, whereas *N. gonorrhoeae* was found to be the most sensitive to Kanamycin. Azithromycin, Doxycycline and Ofloxacin were found to be most effective against *C. trachomatis*. **Conclusion:** The results suggest that microbial infections, especially *Mycobacterium tuberculosis* could be an important factor in the etiology of infertility. Routine microbiological analysis and antimicrobial susceptibility testing can be helpful in the early diagnosis and appropriate management of infection-related infertility.

KEYWORDS

Infertility, Peritoneal Fluid, *Mycobacterium Tuberculosis*, *Neisseria Gonorrhoeae*, *Chlamydia Trachomatis*, Antimicrobial Resistance.

INTRODUCTION

According to the World Health Organization (2023), the definition of infertility, as the state of not being able to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse, is considered a disease of the male or female reproductive system. It impacts people of all ages, social and economic status, and geographical locations, posing a major public health problem around the world. Lifestyle modifications, environmental exposures, metabolic disorders, reproductive tract infections, and chronic diseases are all attributed to the increasing prevalence of infertility, which is associated with delayed childbearing (Stanhiser et al., 2023). In addition to clinical relevance, the psychological, social and economic impacts of infertility are significant, and it is important that this condition is fully researched, diagnosed, managed and also equitably accessed for reproductive healthcare services (Cox et al., 2024). Infections of the reproductive tract are significant but often underappreciated causes of infertility, with many infections going undiagnosed or untreated, leading to long-term damage of the reproductive organs (Unemo et al., 2024). One of the most frequent infectious causes of infertility is pelvic inflammatory disease (PID) and the more often a woman has an infection in the pelvis, the higher her chance of developing PID (Workowski et al., 2021). There are a number of pathogens associated with the risk of tubal factor infertility, such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycobacterium tuberculosis*. These microorganisms can cause chronic inflammation, epithelial damage, fibrosis and adhesion, and immune-mediated damage to tissues, which results in tubal occlusion, and a decrease in the fertility potential of the tubes. Persistent infections can also change the microenvironment of the reproductive tract, which can negatively impact the process of fertilization and implantation (Nagpal et al., 2024; White et al., 2025). The PF is a dynamic biological fluid that surrounds the uterus, the ovaries and fallopian tubes, and mirrors the physiological and pathological events that take place in the pelvic cavity (Reis et al., 2022). Because of its proximity to the reproductive organs, PF has become an important specimen to study infertility and other gynecological diseases. Recent research has shown that changes in the composition of PF can reveal crucial mechanisms of disease processes that could not be easily identified using traditional clinical evaluations (Li et al., 2023). Therefore, precise characterization of PF is gaining significance to understand the pathogenesis of infection-related infertility and associated reproductive illnesses (Nagpal et al., 2024; Reis et al., 2022). Infertility continues to be a significant problem among TB endemic countries due to genital TB. The disease is mainly seen in the fallopian tube and endometrium, where granulomatous inflammation, fibrosis, formation of adhesions and tubal obstruction occur, leading to

reduced transport of ova, fertilization and implantation (Nagpal et al., 2024). Moreover, antimicrobial susceptibility surveillance plays a vital role in the choice of antimicrobial therapy, treatment outcomes, and antimicrobial stewardship initiatives for controlling the emergence of resistance, and should be performed (Unemo et al., 2024; World Health Organization, 2024). The present study was, therefore, conducted to assess the microbiological profile of aspirated peritoneal fluid from women with infertility undergoing a diagnostic laparoscopy and to observe the antimicrobial susceptibility of the isolated microorganisms and to find out the possible role of microbial infection in female infertility.

Materials and Methods

This observational analytical study was done over a period from December 2022 to December 2023 in the Department of Obstetrics and Gynaecology, Zanana Hospital, Jaipur, Rajasthan, India. In the study 80 women aged 22 to 40 years with infertility being evaluated by laparoscopy were enrolled. Peritoneal fluid aspirates were taken for microbial profile and susceptibility testing of the isolated organisms. Patients with recurrent pelvic or abdominal infection, inflammatory disease or malignancy, contraindications to laparoscopy or long-term antimicrobial therapy were excluded. The study received ethical clearance from the Institutional Ethics Committee of SMS Medical College, Jaipur and informed written consent was taken from all the participants.

Sample Collection, Laboratory Processing, and Statistical Analysis

A sample of the peritoneal fluid was aseptically taken, free fluid was aspirated from the pouch of Douglas or pelvic cavity, using a sterile syringe and sent immediately to the Microbiology laboratory for processing. A sample was taken from each specimen for cytological studies to determine cellular adequacy, inflammatory changes and atypical cells. Microbiological analysis was carried out by direct microscopy, Gram staining, culture on suitable media and identification of isolates by conventional biochemical assays. To enhance the sensitivity of the diagnosis, molecular techniques, such as polymerase chain reaction (PCR) were used to detect *Mycobacterium tuberculosis* and *Chlamydia trachomatis*. Susceptibility testing of significant isolates was performed following the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines and reported as susceptible, intermediate, or resistant. The data were inserted into the Microsoft Excel and analyzed through descriptive statistics. Categorical variables were presented as frequencies and percentages and p value < 0.05 was considered statistically significant.

Observations & Results

The results of this study offer a comprehensive view of the distribution of infertility cases, peritoneal cytology, peritoneal culture results, and the sensitivity and resistance patterns of drugs used to treat detected pathogens in a cohort of 80 infertile women who visited Zanana Hospital, Jaipur.

Age Distribution of Infertility Cases

The distribution of cases based on age revealed that infertility was most prevalent in the age group of 25-35 years (Table 1). Out of the 80 cases, 59 women (73.75%) belonged to this age category. This trend highlights the significant burden of infertility faced by women in their prime reproductive years. The least affected age group was women under 25 years, with only 5 cases (6.25%). Women over 35 years accounted for 16 cases (20%).

Table 1: Distribution of cases according to age

Age Group (Years)	N	Percentage
<25	5	6.25
25-35	59	73.75
>35	16	20
Total	80	100

Types of Infertility

The analysis of infertility types revealed that primary infertility was more common than secondary infertility among the study population (Table 2). Out of the 80 cases, 46 (57.5%) were cases of primary infertility, where women had never conceived before. In contrast, 34 cases (42.5%) were classified as secondary infertility, where women who had previously conceived were now facing difficulties in achieving a subsequent pregnancy.

Table 2: Distribution of cases according to infertility type

Types of Infertility	N	Percentage
Primary	46	57.5
Secondary	34	42.5
Total	80	100

Peritoneal Culture Results

Peritoneal culture results provided further insight into the microbiological profile of the infertile women in this study (Table 3). Among the 80 cases, the majority (58.75%) showed no detectable pathogens, indicating that in these cases, infertility may be attributed to factors other than infections, such as hormonal imbalances, structural abnormalities, or unexplained infertility.

However, among the cases with detectable pathogens, *Mycobacterium tuberculosis* was the most prevalent, found in 21 cases (26.25%). This finding highlights the significant role of tuberculosis as a contributing factor to infertility in this population. Genital tuberculosis is a well-known cause of infertility, particularly in regions where tuberculosis is endemic, as it can lead to scarring and damage to the fallopian tubes and endometrium, impairing fertility.

Neisseria gonorrhoeae was detected in 7 cases (8.75%), reflecting its role as a sexually transmitted infection that can cause pelvic inflammatory disease and subsequent infertility if left untreated. The presence of *Chlamydia trachomatis* in 5 cases (6.25%) also points to the importance of screening and treating sexually transmitted infections to prevent long-term reproductive health complications.

Table 03: Distribution of cases according to Peritoneal Culture

Peritoneal Culture	N	Percentage
Chlamydia trachomatis	5	6.25
Mycobacterium tuberculosis	21	26.25
Neisseria gonorrhoeae	7	8.75
Not Detected	47	58.75
Total	80	100

Drug Sensitivity and Resistance Patterns of Isolated Pathogens

Antimicrobial susceptibility testing revealed that *Mycobacterium tuberculosis* showed the highest sensitivity to Rifampicin (76.19%) and Isoniazid (71.43%), while marked resistance was observed against Kanamycin (76.19%), Amikacin (66.67%), Ethionamide (61.90%), and Bedaquiline (61.90%). *Neisseria gonorrhoeae* demonstrated maximum sensitivity to Ceftriaxone and Kanamycin (85.71% each), whereas high resistance was noted to Penicillin and Tetracycline (85.71% each), followed by Ciprofloxacin and Erythromycin (71.43%). Similarly, *Chlamydia trachomatis* exhibited the highest

sensitivity to Azithromycin, Doxycycline, and Ofloxacin (80% each), while resistance was most pronounced against Clindamycin (80%), Clarithromycin (60%), and Tetracycline (60%). These findings highlight the importance of antimicrobial susceptibility testing for the effective management of infection-associated infertility.

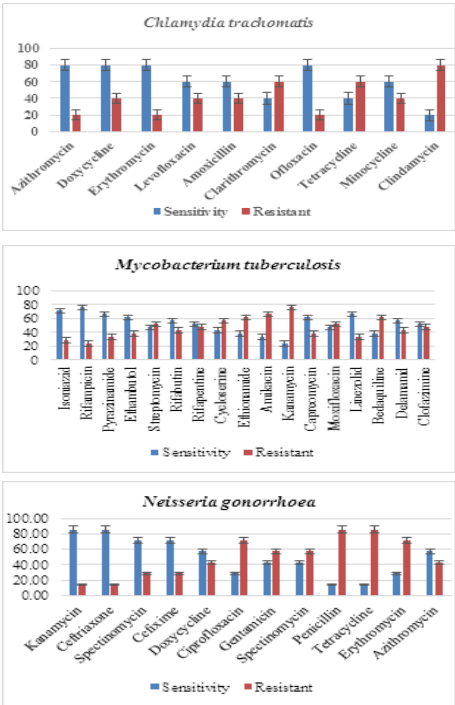


Figure 1 Drug Sensitivity and Resistance Patterns for *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae* & *Chlamydia trachomatis*

DISCUSSION

The present study aimed at assessing the role of microbiota of aspirated peritoneal fluid and the sensitivity patterns of the organisms isolated in infertile women undergoing diagnostic laparoscopy. The results indicate the possible role of infectious disease in female infertility and the necessity of microbiological examination in the assessment of a woman's reproductive health. The majority of participants were in the age group of 25-35 years (73.75%), representing the prime reproductive age when couples tend to seek fertility evaluations. Primary infertility (57.5%) was more common than secondary infertility (42.5%) which is similar to previous reports that showed high prevalence of primary infertility among women in tertiary infertility centers (Kumar & Singh, 2022; Gautam & Sharma, 2021). A significant number of samples identified as having inflammatory changes were found, 45% IAC-1, 41.25% IAC-2B. The results indicate inflammation of the pelvic cavity, either active or past. Adhesions, fibrosis and reduced reproductive function have been linked with chronic pelvic inflammation and can have negative consequences on fertility. In accordance, Reis et al. (2022) noted a role for inflammatory alterations in peritoneum affecting fertilisation and implantation, and Sharma and Mittal (2020) pointed to the negative effects of chronic pelvic inflammation on female reproductive function. Pathogenic microorganisms were found in 58.75% of the samples, though no pathogens were found under the microscope. The most common organism isolated was *Mycobacterium tuberculosis* (26.25%), thus genital tuberculosis is a significant cause of infertility in TB endemic areas and is still an important issue. Genital tuberculosis can affect a woman's fertility due to damage to the tubes, fibrosis and involvement of the endometrium. The same has been observed by Jindal et al. (2015) who found that genital tuberculosis is a significant cause of tubal factor infertility in India. Nagpal et al. (2024) have recently shown that diagnosis too late in life can lead to irreversible damage of the reproductive tract. The presence of both sexually transmitted pathogens, *Neisseria gonorrhoeae* (8.75%) and *Chlamydia trachomatis* (6.25%) further underpins the role of sexually transmitted infections in reproductive dysfunctions. Both pathogens are well-recognised causes of pelvic inflammatory disease and tubal factor infertility, and are frequently asymptomatic, so even though infected, these infections can remain undiagnosed until the onset of

infertility. It was reported by Grad and Wiesenfeld (2017) that gonococcal infections have the potential to ascend to the upper genital tract and lead to scarring and infertility. Likewise, Harb et al. (2019) showed that chronic infection by *C. trachomatis* is a significant cause of reproductive complications, due to the chronic inflammatory and tissue-destructive process it causes. Based on the antimicrobial susceptibility testing, the highest sensitivity of the isolates of *M. tuberculosis* was seen with Rifampicin and Isoniazid, with significant resistance seen with Kanamycin and Amikacin. Their findings are similar to the reported emergence of TB antimicrobial resistance (Frieden et al., 2023; Mathema et al., 2023). Similarly, *N. gonorrhoeae* showed high susceptibility to Ceftriaxone, Kanamycin and high resistance to Penicillin and Tetracycline. The same pattern of resistance is observed world-wide, and Ceftriaxone remains the recommended first line treatment (Unemo & Shafer, 2021). Azithromycin, Doxycycline, and Ofloxacin were found to be the most effective drugs; resistance was seen with Clarithromycin, Tetracycline, and Clindamycin. Resistance of *C. trachomatis* is relatively uncommon but emerging patterns should continue to be monitored to ensure the efficacy of treatment (Pym et al., 2023). The study has some limitations: it was conducted in one centre and the number of patients was relatively small, so the results may not be applicable to other centres. In addition, infertility is a polyetiologic disease and the presence of microorganisms does not mean a causal relationship has been proven. More comprehensive and larger multicentric studies using state of the art molecular diagnostic tools are required to elucidate the contribution of microbial infection to female infertility. The study overall reveals a significant presence of *M. tuberculosis*, *N. gonorrhoeae* and *C. trachomatis* in the peritoneal fluid samples collected from infertile females. The results lead to the suggestion that microbiological screening should be part of the assessment of infertility and underscore the need for early diagnosis and appropriate antimicrobial therapy to enhance reproductive health.

CONCLUSION

The present study revealed high incidence of microbial pathogen in the peritoneal fluid of infertile woman; *Mycobacterium tuberculosis* was found as the most common followed by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Based on the results, infectious pathogens might be one of the important factors involved in the pathogenesis of infertility and microbiological assessment should be considered as a valuable tool in the evaluation of infertility. These observed antimicrobial resistance trends also underscore the importance of susceptibility-based treatment and continued monitoring. The timely diagnosis and treatment of reproductive tract infections can help to achieve better reproductive outcomes and better care of fertility.

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