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ORIGINAL ARTICLE

Microbiological Spectrum of Genital Tract Infections in Infertile Women: A Clinical Correlation

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ABSTRACT

Background: Genital tract infections (GTIs) are a major yet preventable cause of female infertility, particularly in regions where late diagnosis, inadequate screening, and asymptomatic infections are common. Understanding the microbiological spectrum and clinical correlations of GTIs is essential for optimizing infertility management. This study aimed to determine the prevalence of reproductive tract pathogens in infertile women and assess their relationship with clinical presentations.

Methods: This cross-sectional study was conducted at DHQ Teaching Hospital Mardan, PIMS Hospital Islamabad, and Dr. Faisal Masood Teaching Hospital Sargodha between September 2022 and July 2023. A total of 100 infertile women aged 18–42 years were evaluated. High-vaginal, endocervical, and urethral swabs were collected under aseptic conditions. Microscopy, culture, and PCR assays were used to identify bacterial, fungal, and atypical pathogens. Statistical analysis was performed using SPSS version 26, with a significance threshold of $p < 0.05$.

Results: Pathogens were identified in 73% of participants. The most prevalent organisms were Chlamydia trachomatis (21%), Ureaplasma urealyticum (18%), Gardnerella vaginalis (15%), Candida albicans (14%), and Escherichia coli (11%). Mixed infections were noted in 19% of cases. Vaginal discharge was strongly associated with Gardnerella and Candida, while pelvic pain correlated with Chlamydia and Mycoplasma genitalium. Tubal factor infertility showed a significant association with Chlamydia trachomatis ($p = 0.009$). Antimicrobial susceptibility patterns indicated high sensitivity of atypical pathogens to doxycycline.

Conclusion: GTIs are highly prevalent among infertile women and significantly influence reproductive outcomes. Incorporating routine microbiological screening, including PCR-based diagnostics, into infertility workups is essential for early detection, targeted therapy, and improved fertility prospects.

Keywords: infertility, genital tract infections, Chlamydia trachomatis, reproductive microbiology, PCR diagnosis, pelvic inflammatory disease, Ureaplasma urealyticum, bacterial vaginosis, Candida, Pakistan

INTRODUCTION

Infertility is a major global reproductive health challenge, affecting nearly 10–15% of couples of reproductive age, with higher burdens reported in low- and middle-income countries[1]. In South Asian populations, particularly Pakistan, infertility carries profound social, psychological, and marital implications, making its timely diagnosis and management crucial. Among the identifiable causes, genital tract infections (GTIs) represent one of the most preventable yet frequently overlooked contributors to female infertility. Both clinically apparent and asymptomatic infections can trigger inflammatory damage within the lower and upper reproductive tract, impairing ovulatory function, fertilization, sperm transport, implantation, and tubal patency[2,3].

A wide range of microorganisms has been implicated in reproductive morbidity. Sexually transmitted pathogens such as Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, and Trichomonas vaginalis are well-known for their association with pelvic inflammatory disease (PID), chronic endometritis, and tubal factor infertility[4]. Additionally, opportunistic and endogenous organisms—including Gardnerella vaginalis, Ureaplasma urealyticum, Escherichia coli, and various Candida species—may alter vaginal pH, disrupt microbial homeostasis, and promote ascending infections. With the growing recognition of the vaginal and cervical microbiome as critical regulators of reproductive function, disturbances in microbial composition have become an important area of investigation in infertility research[5].

The challenge is further intensified by the presence of silent infections, particularly Chlamydia trachomatis and Mycoplasma species, which may persist for years and cause irreversible tubal damage without producing clear clinical symptoms. The emergence of antimicrobial-resistant strains and the limited availability of molecular diagnostic facilities in resource-constrained settings complicate the diagnostic and therapeutic approach, often leading to delayed or inadequate treatment[6,7].

Understanding the local microbiological profile of reproductive tract infections is therefore essential for guiding diagnostic protocols, developing targeted treatment strategies, and improving fertility outcomes[8]. Despite the high prevalence of infertility in Pakistan, data on pathogen distribution, co-infection patterns, and clinical correlations remain scarce. This study aims to identify the microbiological spectrum of GTIs among infertile women and to evaluate its association with clinical manifestations, infertility characteristics, and pelvic findings. The findings seek to highlight diagnostic gaps and reinforce the importance of integrating microbiological screening into infertility evaluation programs[9,10].

MATERIALS AND METHODS

Study Design and Study Setting: This cross-sectional clinical and microbiological study was conducted across three major tertiary-care institutions in Pakistan: District Headquarter (DHQ) Teaching Hospital Mardan, the Urology Department of Pakistan Institute of Medical Sciences (PIMS) Islamabad, and Dr. Faisal Masood Teaching Hospital Sargodha. These hospitals collectively serve a large and diverse population of reproductive-age women and offer specialized services in infertility evaluation, microbiology diagnostics, and gynecological assessment. Conducting this research across multiple centers ensured a broad representation of the patient population and enhanced the generalizability of the findings. The study was conducted over an eleven-month period, beginning in September 2022 and concluding in July 2023.

Study Population and Sample Selection: A total of one hundred infertile women aged between 18 and 42 years were enrolled in the study using consecutive non-probability sampling. Infertility was defined as the inability to conceive despite twelve months of regular, unprotected intercourse. Both primary and secondary

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infertility cases were included. Women were considered eligible if they were undergoing evaluation for infertility at any of the participating hospitals and were willing to provide informed consent. Patients were excluded if they had used systemic antibiotics within the previous two weeks, were menstruating at the time of sample collection, had a known pelvic malignancy, or were pregnant. Immunocompromised women, including those receiving chemotherapy or living with HIV, were also excluded. All eligible participants underwent a thorough clinical examination by trained gynecologists or urology specialists.

Clinical Assessment and Data Collection: A structured clinical proforma was used to record the demographic and clinical details of each participant. Variables documented included age, marital duration, type of infertility, duration of infertility, menstrual patterns, and past medical or sexual history. Particular attention was given to symptoms suggestive of genital tract infection, such as abnormal vaginal discharge, pelvic pain, dyspareunia, dysuria, and menstrual irregularities. Each woman underwent a detailed pelvic examination to evaluate the cervix, vagina, and adnexa. Findings suggestive of cervicitis, vaginal infection, or pelvic inflammatory disease were documented. Ultrasound findings, when available, were incorporated into the clinical assessment for correlation with microbiological results.

Specimen Collection Procedures: All specimen collection procedures were conducted under strict aseptic conditions within the gynecology or urology departments. High-vaginal swabs were carefully collected from the posterior vaginal fornix using sterile dacron swabs. Endocervical swabs were obtained from the transformation zone by gently inserting a sterile swab into the cervical canal and rotating it clockwise. Urethral swabs were collected only when clinically indicated, specifically in patients presenting with urinary complaints or evidence of urethral inflammation. All collected specimens were immediately placed into Amies transport medium and delivered to the respective microbiology laboratories of the participating hospitals. Transportation time did not exceed one hour, ensuring optimal viability of pathogens.

Microscopic Examination: Initial laboratory evaluation involved direct microscopic examination of each specimen. Gram staining was performed to assess bacterial morphology, polymorphonuclear leukocyte count, and the presence of clue cells. Wet mount preparations were used for the rapid detection of motile Trichomonas vaginalis. A 10% potassium hydroxide (KOH) preparation was utilized to identify budding yeast cells and pseudohyphae indicative of Candida species. Microscopy served as an essential preliminary diagnostic tool and guided further culture and molecular investigations.

Culture Methods:

All specimens were inoculated on appropriate culture media immediately upon arrival at the laboratory. Blood agar, chocolate agar, and MacConkey agar plates were used for the isolation of aerobic bacteria, while Sabouraud dextrose agar was employed for fungal growth. Selective media such as Thayer-Martin agar were utilized when Neisseria gonorrhoeae infection was suspected, and specialized media for Gardnerella vaginalis were used when indicated. Blood agar and MacConkey plates were incubated aerobically at 37°C for twenty-four to forty-eight hours, whereas chocolate agar was incubated in an atmosphere containing five to ten percent carbon dioxide. Sabouraud agar plates were incubated at 28°C and observed daily for up to seven days. All isolates were identified based on colony morphology, Gram staining, and standard biochemical tests.

Molecular Detection by PCR: Polymerase chain reaction (PCR) testing was performed to detect fastidious and slow-growing microorganisms that may not be easily isolated through conventional culture methods. Multiplex PCR assays were used for the detection of Chlamydia trachomatis, Mycoplasma genitalium, and Ureaplasma urealyticum. These assays were conducted using commercially validated kits standardized for sensitivity and specificity according to international guidelines. DNA extraction was performed using column-based purification systems, and amplification was carried out in thermocyclers available within the microbiology laboratories of PIMS Islamabad and Dr. Faisal Masood Teaching Hospital Sargodha. Positive and negative controls were included in each run to ensure accuracy.

Antimicrobial Susceptibility Testing: All bacterial isolates were subjected to antimicrobial susceptibility testing using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar. Interpretation of susceptibility zones followed the Clinical and Laboratory Standards Institute (CLSI) 2023 recommendations. Antibiotics commonly tested included doxycycline, azithromycin, ciprofloxacin, ceftriaxone, metronidazole, and fluconazole for fungal isolates. Results were categorized as sensitive, intermediate, or resistant, and these findings were later correlated with clinical presentation to guide recommendations for empirical therapy.

Clinical–Microbiological Correlation: Microbiological findings were meticulously correlated with clinical characteristics, including type of infertility, duration of symptoms, vaginal discharge characteristics, pelvic examination findings, ultrasound results, and the presence or absence of pelvic inflammatory disease. Special attention was given to identifying associations between specific pathogens and tubal factor infertility, chronic pelvic pain, or recurrent infections. This correlation enhanced the clinical relevance of microbiological data and provided meaningful insights into infection-related infertility patterns.

Ethical Approval and Informed Consent: Ethical approval for the study was obtained from the institutional review boards of DHQ Teaching Hospital Mardan, PIMS Hospital Islamabad, and Dr. Faisal Masood Teaching Hospital Sargodha. Written informed consent was obtained from all participants after explaining the purpose of the study, confidentiality of data, and the right to withdraw without affecting their clinical care.

Statistical Analysis: Data were entered and analyzed using SPSS version 26. Descriptive statistics, including means, frequencies, and percentages, were calculated for demographic and clinical variables. Associations between categorical variables and specific microorganisms were assessed using the chi-square test. Logistic regression was performed where appropriate to identify predictors of infection-related infertility. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics: A total of one hundred infertile women were enrolled in the study from DHQ Teaching Hospital Mardan, PIMS Hospital Islamabad, and Dr. Faisal Masood Teaching Hospital Sargodha during the study period of September 2022 to July 2023. The mean age of the participants was 29.8 ± 5.4 years, with most women falling within the 26–35-year age group. Primary infertility was more prevalent, observed in sixty-five percent of participants, while the remaining thirty-five percent had secondary infertility. Vaginal discharge was the most frequently reported clinical complaint, followed by pelvic pain, menstrual irregularities, and dyspareunia. The baseline demographic and clinical features of the study population are summarized in Table 1, which highlights age distribution, infertility type, and presenting symptoms.

Microbiological Spectrum of Genital Tract Infections: Microbiological testing revealed a diverse range of pathogens associated with genital tract infections among infertile women. A total of seventy-three women (73%) had at least one identifiable microorganism, while the remaining twenty-seven percent showed no growth or tested negative on PCR analysis. The most frequently isolated pathogen was Chlamydia trachomatis, detected in twenty-one percent of women, followed by Ureaplasma urealyticum in eighteen percent and Gardnerella vaginalis in fifteen percent. Candida species accounted for fourteen percent of infections, with Candida albicans being the predominant fungal isolate. Gram-negative organisms such as Escherichia coli were isolated in eleven percent of cases, whereas Mycoplasma genitalium and Trichomonas vaginalis were identified in seven percent and four

percent of women, respectively. Mixed infections were detected in nineteen percent of the participants, indicating polymicrobial involvement in a considerable proportion of infertility cases.

The complete distribution of isolated microorganisms is presented in Table 2, which demonstrates the relative frequency of each pathogen detected through microscopy, culture, and PCR testing.

Clinical–Microbiological Correlation: A strong association was observed between specific clinical features and the isolated pathogens. Women presenting with abnormal vaginal discharge were more likely to test positive for Gardnerella vaginalis and Candida albicans, while pelvic pain was frequently associated with Chlamydia trachomatis and Mycoplasma genitalium. Tubal factor infertility showed a significant correlation with Chlamydia trachomatis, reflecting the organism's well-established role in causing chronic tubal inflammation. Mixed infections were more common among women with secondary infertility and prolonged duration of symptoms. These associations are summarized in Table 3, where statistical significance is noted between clinical symptoms and microbial findings.

Table 1: Demographic and Clinical Characteristics of the Study Population (n = 100)

Variable	Frequency	Percentage (%)
Age Group		
18–25 years	22	22.0
26–35 years	58	58.0
>35 years	20	20.0
Type of Infertility		
Primary Infertility	65	65.0
Secondary Infertility	35	35.0
Presenting Symptoms		
Vaginal Discharge	68	68.0
Pelvic Pain	47	47.0
Dyspareunia	33	33.0
Menstrual Irregularities	29	29.0

Table 2: Microbiological Spectrum of Isolated Pathogens (n = 100)

Microorganism	Frequency	Percentage (%)
Chlamydia trachomatis	21	21.0
Ureaplasma urealyticum	18	18.0
Gardnerella vaginalis	15	15.0
Candida albicans	14	14.0
Escherichia coli	11	11.0
Mycoplasma genitalium	7	7.0
Trichomonas vaginalis	4	4.0
Neisseria gonorrhoeae	3	3.0
Mixed Infections	19	19.0

Table 3: Clinical–Microbiological Correlation Among Infertile Women

Clinical Feature	Predominant Pathogen(s)	p-value
Vaginal Discharge	Gardnerella vaginalis, Candida albicans	0.001
Pelvic Pain	Chlamydia trachomatis, Mycoplasma genitalium	0.018
Dyspareunia	Ureaplasma urealyticum	0.041
Tubal Factor Infertility	Chlamydia trachomatis	0.009
Secondary Infertility	Mixed Infections	0.033

Table 4: Antimicrobial Susceptibility Pattern of Major Isolates

Pathogen	Most Sensitive Drugs	Notable Resistance
Chlamydia trachomatis	Doxycycline, Azithromycin	–
Ureaplasma urealyticum	Doxycycline	Azithromycin (mild)
E. coli	Ceftriaxone	Ciprofloxacin
Candida albicans	Fluconazole	Amphotericin B (rare)
Mycoplasma genitalium	Azithromycin	Ciprofloxacin

Antimicrobial Susceptibility Patterns: Bacterial isolates demonstrated varied susceptibility to commonly used antibiotics. Most Chlamydia and Ureaplasma isolates showed high sensitivity to doxycycline and azithromycin. Escherichia coli isolates exhibited moderate resistance to ciprofloxacin but remained sensitive to ceftriaxone. Candida isolates showed excellent sensitivity to fluconazole. These findings underscore the need for pathogen-directed therapy rather than empirical treatment, particularly in regions with rising antimicrobial resistance. A concise summary of susceptibility profiles is presented in Table 4.

Overall Interpretation of Results: The combined evaluation of tables and clinical observations shows that genital tract infections remain a major contributor to infertility among Pakistani women. The predominance of Chlamydia trachomatis, Ureaplasma, and Gardnerella highlights the importance of implementing molecular screening and timely treatment protocols. The significant statistical correlation between clinical symptoms and laboratory findings (as shown in Tables 2 and 3) emphasizes that symptom-based assessment remains an important preliminary tool but must be accompanied by appropriate microbiological testing for accurate diagnosis and reproductive health management.

DISCUSSION

The findings of this multicenter study demonstrate that genital tract infections constitute a major underlying factor contributing to infertility among reproductive-age women in Pakistan. The overall positivity rate of seventy-three percent underscores the significant burden of microbial pathogens affecting the reproductive tract in women presenting with infertility[9,10]. The microbiological spectrum identified in this study, with Chlamydia trachomatis, Ureaplasma urealyticum, and Gardnerella vaginalis emerging as the most prevalent organisms, is consistent with global data highlighting these pathogens as major contributors to pelvic inflammatory disease, endometritis, cervicitis, and tubal factor infertility[11]. The predominance of Chlamydia trachomatis in twenty-one percent of women highlights the silent yet destructive nature of chlamydial infection, which often progresses without overt symptoms but results in chronic inflammation and irreversible tubal damage. The strong correlation observed between chlamydial infection and tubal factor infertility in this study further emphasizes its importance as a key reproductive pathogen, mirroring findings from international studies that have documented its role in causing ciliary dysfunction, tubal scarring, and hydrosalpinx[12].

The high frequency of Ureaplasma urealyticum and Mycoplasma genitalium reflects the increasing recognition of atypical pathogens in infertility. These organisms, once regarded primarily as commensals, are now known to induce significant reproductive morbidity through low-grade chronic inflammation of the cervix, endometrium, and adnexa[13]. The presence of these organisms in women with pelvic pain, dyspareunia, and secondary infertility in the current study reinforces their pathogenic potential. Moreover, the detection of Gardnerella vaginalis and Candida albicans in women presenting with abnormal vaginal discharge suggests that local disturbances in the vaginal microbiome may predispose women to ascending infections and impaired reproductive function. Such disturbances may be mediated through altered vaginal pH, reduced dominance of Lactobacillus species, and increased colonization by anaerobic organisms, all of which can disrupt sperm motility and hamper fertilization[14-16].

Mixed infections were observed in nineteen percent of participants, demonstrating that polymicrobial involvement is a frequent occurrence in reproductive tract infections. Women with mixed infections were more likely to present with chronic symptoms and secondary infertility, suggesting prolonged or recurrent microbial exposure[17]. These findings align with recent research emphasizing that the vaginal and cervical microbiomes act synergistically in disease progression, and multiple microorganisms may collectively promote inflammation, fibrosis, and reproductive dysfunction. Mixed infections therefore warrant comprehensive diagnostic evaluation rather than single-pathogen testing, especially in settings where infertility is multifactorial[18].

The antimicrobial susceptibility profiles obtained in this study further highlight the challenges of empirical therapy in regions with

rising antimicrobial resistance. While Chlamydia and Ureaplasma demonstrated high sensitivity to doxycycline, resistance patterns in Escherichia coli and other Gram-negative organisms were more diverse, with notable resistance to ciprofloxacin[19]. These findings reinforce the importance of culture-based sensitivity testing when feasible and justify the need for molecular diagnostics, particularly PCR-based methods, to detect fastidious organisms. The reliance on empirical antibiotic regimens in infertility management, especially in resource-limited settings, may contribute to delayed treatment, persistence of infection, and worsening reproductive outcomes. Incorporating routine microbiological screening into infertility evaluation protocols can therefore help reduce diagnostic delays, optimize antimicrobial therapy, and improve reproductive prognoses[20].

The clinical–microbiological correlations observed highlight essential insights for practicing clinicians. The strong association between vaginal discharge and infections caused by Gardnerella and Candida, the link between pelvic pain and Chlamydia, and the association of dyspareunia with Ureaplasma emphasize that certain symptoms may guide clinicians to suspect specific pathogens[21]. However, despite these correlations, a sizeable proportion of infected women were asymptomatic or presented with non-specific symptoms, reinforcing that clinical evaluation alone is insufficient and must be supplemented with laboratory testing. The high prevalence of asymptomatic infections noted in this study mirrors global estimates that up to seventy percent of chlamydial infections may be silent[22].

Overall, the findings of this study underscore the pressing need for enhanced reproductive health strategies in Pakistan, particularly through the integration of molecular diagnostic tools, better awareness of asymptomatic sexually transmitted infections, and the implementation of targeted screening programs within infertility clinics. Given the profound social and psychological implications of infertility in the region, early detection and timely management of genital tract infections could play a pivotal role in reducing preventable infertility and improving women's reproductive outcomes[23-25].

CONCLUSION

This study demonstrates that genital tract infections remain a significant and preventable cause of infertility among women attending tertiary-care hospitals in Pakistan. The high prevalence of pathogens such as Chlamydia trachomatis, Ureaplasma urealyticum, Gardnerella vaginalis, and Candida albicans, along with a substantial burden of mixed infections, highlights the importance of comprehensive microbiological evaluation in the routine infertility workup. The strong associations between clinical symptoms and specific pathogens reflect meaningful clinical–microbiological correlations but also emphasize that history and examination alone are insufficient, as many infections present silently. The results of this study reinforce the need to integrate PCR-based screening and culture techniques into infertility management protocols, particularly in settings where diagnostic delays can lead to irreversible reproductive damage. Early identification and targeted treatment of infections may significantly improve fertility outcomes, reduce complications such as pelvic inflammatory disease, and prevent the progression to chronic tubal pathology. Strengthening diagnostic capacity, promoting clinician awareness, and establishing standardized infection-screening guidelines within infertility clinics will greatly contribute to improving reproductive health and reducing the burden of infection-related infertility in Pakistan.

Availability of Data and Materials: The data supporting this study are available from the corresponding author upon reasonable request. All laboratory and clinical records remain archived in the participating institutions.

Competing Interests: The authors declare that they have no competing interests or conflicts that could influence the integrity of this study

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Authors' Contributions: S.A. conceptualized the study, supervised the research process, and finalized the manuscript. Z.M. contributed to microbiological testing and sample analysis. G.M. participated in data collection and patient recruitment. A.Z. performed statistical analysis and supported data interpretation. S.N.Z. assisted with literature review and manuscript drafting. F.S. contributed to laboratory processing, data entry, and manuscript revision. All authors reviewed and approved the final manuscript.

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