# Bacteriospermia in Men Among Infertile Couples in Nepalese Population

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# Abstract

Infection of male urogenital tract or male accessory glands is considered as one of the important causes of male infertility, and results in the presence of bacteria in semen affecting fertility potential of men. It is important to know the composition of bacteria in semen to understand the etiology of urogenital infections and its association with infertility. This study aims to know the rate of infection in semen of infertile men, and the association of seminal bacteria with semen parameters related to fertility potential. A cross-sectional study was carried out from June 2021 to July 2022, in which 213 semen samples were collected from the male partners of couples consulting for fertility problems in an infertility center of Nepal. All the samples were processed following WHO guidelines, 2021. Analysis of semen parameters was done immediately after the liquefaction of collected samples. Microbiological assessment was also done for identification of bacteria in semen by conventional method, which showed 25.4% of samples had bacteriospermia. *S. aureus* and *Corynebacterium* were predominant bacteria in semen. The volume of semen was significantly associated with bacteriospermia. The concentration of sperms, percentage of total sperm motility, sperms with normal forms and vitality were found to be less in semen with bacteria compared to those without bacteria, which was statistically insignificant. This study provides a baseline data on bacterial infection in semen of infertile men in Nepal.


# Introduction

Infertility can be defined as the inability to achieve pregnancy after a year of regular unprotected sexual intercourse [1, 2]. Globally about 15% of the couples are infertile, about 20% of the infertility cases are due to male factor alone and 30–40% of the cases are due to combination of both male and female factors [3, 4], and similar rate was reported in Nepal too [5, 6]. The number of infertile couples seeking medical treatment has been increasing year by year, not only in global scenario but also in Nepal [5].

Male infertility may be affected by various factors including age of patient, hormonal imbalance, testicular temperature, exposure to toxic chemicals and environmental pollutants, predisposing disease, infection, etc. Infections of the genitourinary tract account for about 15% of male infertility cases [4, 7]. Various sites of the male genitourinary system, such as the prostate, the epididymis, the testis and the urethra may be infected by bacteria [4]. Infection of male urogenital tract or male accessory glands result in the presence of bacteria in semen. The presence of bacteria in concentrations greater than $10^3$ bacteria/ml ejaculate is clinically regarded as a sign of an active infection and is called bacteriospermia [8]. Bacteriospermia is usually the result of acute or chronic bacterial infections and has a negative impact on sperm concentration, motility, sperm morphology and sperm DNA fragmentation [9, 10], affecting male fertility [11].

Studies done in various countries showed the association of bacteria with sperm abnormalities [12–15]. However, only few studies related to male infertility have been done in Nepal. Most of the studies in Nepal are confined to male infertility in relation to semen parameters only [5, 16–18]. Therefore, it is important to understand the bacterial species composition of seminal fluids to better understand the etiology and

pathogenesis of urogenital tract infections and the associations between urogenital infections and infertility.

# Materials And Methods

A cross-sectional study was carried out at Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal and Creator's IVF Nepal Pvt. Ltd., Lalitpur, Nepal. The study was approved by the ethical review board of the Nepal Health Research Council (NHRC), Kathmandu, Nepal (Approval No. 874/2019). All the patients were provided with the written informed consent form and included in the study only after obtaining their signatures. Information on the demographics of subjects were also collected. The subjects not willing to sign the consent, and under antibiotic therapy, for any disease within the past week were not included in the study. The age of patients included in the study was between 20–55 years (mean age 35.8 ± 5.8 years). Majority of the patients (62.4%) were under the age group 30–39 years, out of which 68.1% had primary subfertility and 31.9% had secondary subfertility.

In a cross-sectional study conducted at Creator's IVF Nepal Pvt. Ltd., Lalitpur, Nepal, semen samples were collected from 213 male patients among the couples undergoing fertility consultation and semen analysis, irrespective of the type of infertility, cause of infertility and age of the patient. Semen samples collected in the infertility center by masturbation with abstinence of 2–5 days were only included in the study. Samples were collected in a sterile leak-proof container following the World Health Organization (WHO) criteria for semen sample collection for microbiological analysis [8]. Semen samples were processed after liquefaction of samples at 37 $^{o}$C for routine semen analysis at the andrology laboratory of Creator's IVF Nepal Pvt. Ltd. according to WHO guidelines, 2021 [8]. Semen parameters included volume, sperm count, concentration, total motility, morphology and vitality. Microbiological assessment of samples was done, following WHO criteria [8], within 3 h of sample collection in the laboratory of the Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal. Samples were inoculated on MacConkey agar, blood agar and chocolate agar and incubated at 37 $^{o}$C for 24 h. Inoculated plates with MacConkey agar and blood agar were incubated aerobically and with chocolate agar in $CO_2$ enriched environment in a candle jar. Bacteriospermia has considered if the growth of bacteria in an agar plate was greater than $10^3$ bacteria/mL [19, 20]. The isolates obtained were identified on the basis of their cultural, morphological and biochemical characteristics.

Statistical Package for the Social Sciences (SPSS version 21.0) was used to calculate frequencies, rates and to determine the association between the variables. The frequency of samples on the basis of semen parameters was calculated. The rate of bacteriospermia was determined and expressed as a percentage. Mann Whitney U test was used to evaluate apparent differences for significance at 95% confidence level. The association of bacteriospermia with different variables was tested. Results were considered significant if the *p* value was less than 0.05.

# Results

# Assessment of basic semen parameters

Analysis of four basic semen parameters was done in all the samples obtained irrespective of bacteriospermia. According to WHO reference range (2021) [8], sperm concentration in semen was found to be normal in 86.9% of samples, total motility of sperms was found to be normal in 72.6% of samples, sperms with normal morphology was found in 56.7% of samples, whereas only 39.3% of samples had normal vitality (Fig. 1). Only 26.3% of the samples analyzed were normal with respect to the four tested characteristics of semen and 73.7% had at least one abnormality. The most common abnormality among the four semen parameters analyzed was sperm vitality.

# Microbiological Assessment

Microbiological analysis showed that 25.4% (54/213) of semen samples were found to have bacteriospermia. Only one type of bacteria was present in 87.03% (47/54) samples whereas two types of bacteria were present in 12.96% (7/54) of samples with bacteriospermia. Altogether 59 bacterial isolates were obtained from 54 samples, out of which 10 genera of bacteria were identified, whereas two isolates could not be identified by the conventional microbiological method. The most predominant bacteria were *Staphylococcus aureus* (35.6%), followed by *Corynebacterium* 25.4% (Fig. 2).

# Association Of Bacteriospermia With Semen Parameters

The mean values of the semen parameters (ejaculate volume, sperm concentration, total motility, sperms with normal morphology and sperm vitality) were compared between the semen of men with and without bacteriospermia. The presence of bacteria in semen samples depicted negative association with the volume of ejaculate ($p < 0.05$). The sperm concentration, total motility, morphology and vitality of the samples tends to be lower in men with bacteriospermia than in those without bacteriospermia, however the association was statistically insignificant with p-values greater than 0.05 (Table 1).

Table 1
Semen quality with respect to bacteriospermia

| Semen parameters | Mean value ± SD | | *p*-value |
|---|---|---|---|
| | Without bacteriospermia (Mean ± SD) | With bacteriospermia (Mean ± SD) | |
| Ejaculate volume (mL) | 2.84 ± 1.25 | 2.27 ± 1.15 | 0.001* |
| Sperm concentration (million/mL) | 62.69 ± 26.55 | 56.50 ± 28.77 | 0.132 |
| Total motility (%) | 45.47 ± 16.17 | 41.67 ± 17.62 | 0.109 |
| Sperms with normal morphology (%) | 4.69 ± 4.18 | 4.05 ± 2.58 | 0.529 |
| Sperm vitality (%) | 46.61 ± 16.76 | 43.78 ± 19.96 | 0.550 |
| *$p$-value < 0.05, statistically significant at 95% confidence interval | | | |

Comparison of mean of semen parameters (ejaculate volume, sperm concentration, total motility, sperms with normal forms and sperm vitality) among patients without bacteriospermia and with bacteriospermia. Ejaculate volume was significantly less in patients with bacteriospermia. All other semen parameters tested were less in patients with bacteriospermia, however statistical analysis resulted in *p*-value greater than 0.05 showing no significant association between semen parameters and bacteriospermia.

# Discussion

Bacterial infection of the genitourinary tract and semen as one of the causes of male infertility has remained controversial [21]. In the present study, an evaluation of the semen quality of men among the couples undergoing infertility treatment was done. The assessment of basic semen parameters, and culture of semen for the microbiological assessment were done for the determination of the effect of cultivable bacteria on the basic semen parameters. Based on the count, motility and morphology of sperms, half of the samples analyzed had at least one type of abnormality. Abnormality in semen based on the same seminal characteristics was reported to be 44% [22] and 66.2% [18] from two different hospitals of Nepal, which correlates our study with the semen abnormality trend of other studies among the Nepalese population. The most common characteristic in this study was teratozoopermia as with previous studies [14, 23]. However, asthenozoospermia [22, 24], and oligozoospermia [16] were also found to be the most common semen characteristics in Nepal.

The culture of semen in an aerobic environment resulted in the growth of bacteria in 25.8% of samples in the present study. A similar rate of bacteriospermia was obtained in various studies done in different

countries. The rate of bacteriospermia was 30% in Germany [15], 35% in Iran [25], and 35.3% in India [14], whereas a lower rate of bacteriospermia was also detected in different studies- 15% in Canada [26], 11.1% in Italy [27], and only 3.2% in a recent study in Germany [28]. The rate of seminal infection in infertile men close to the rate of our study has been reported form Nepal [23, 29]. The presence of bacteria in semen in a significant number may be an indicator of male accessory gland infection or male genital tract infection [30, 31]. Semen contamination may also be acquired by urinary tract organisms which have been shown to be associated with semen quality [30, 32].

*Staphylococcus* was the predominant genera obtained from semen samples from infertile men in this study. More specifically, *S. aureus* (32.5%) was more common than Coagulase negative staphylococci (CONS) (15%) in this study. The predominance of *S. aureus* was also revealed in other studies [33–37]. CONS was frequently isolated bacteria from the semen of infertile men with asymptomatic bacteriospermia [28]. However, *Staphylococcus* has been commonly isolated from infertile as well as fertile men, but the ability to form biofilm was greater in staphylococci isolated from infertile men than in healthy ones [38]. The biofilm forming ability of bacteria decreases sperm motility affecting fertility potential of men. *S. aureus* has also been reported to produce a surface protein, Sperm immobilization factor (SIF) protein capable of immobilizing spermatozoa [39]. An in vitro study has also shown that coincubation of spermatozoa with *S. aureus* significantly decreased the motility of sperms [40]. *Corynebacterium* was the second predominant bacteria in our study, and a similar result was obtained in a study of microbiota of semen of healthy and infertile men [38]. Corynebacteria isolated from infertile men were able to reduce cytokine levels and increase the level of biofilm formation supporting the negative influence of the bacteria in infertile men affecting fertility potential [38].

The presence of bacteria in semen is considered to be an important factor that may negatively affect the process of spermatogenesis and sperm functions which may consequently increase fertility problems in men [2]. However, the influence of bacteria on semen characteristics was controversial as several studies did not find any significant association between the bacteriospermia and semen parameters [7, 26]. Presence of bacteria in semen showed a significantly negative effect on semen volume. Bacteriospermia had a negative influence on sperm concentration, total motility, morphology and vitality of spermatozoa in our study, but was not significant statistically. Bacterial presence in semen had a negative effect on seminal volume [34], motility [21, 25, 26, 34], morphology [25, 26, 34] and sperm concentration [21, 25, 26, 34, 41]. Contrarily, no significant effect of bacteriospermia on semen parameters including semen volume [14, 42], concentration [14, 42], motility [14, 42], vitality [14, 41, 42] and morphology [14, 41, 43] was observed in previous studies.

# Conclusions

Bacteriospermia in infertile men studied in Nepal showed one-fourth the study population had bacteriospermia, and *S. aureus* was the predominant bacteria. The study could show the presence of cultivable aerobic bacteria in semen of infertile male patients in Nepalese population, but the actual microbiota could be understood by metagenomic analysis of semen samples.

# Abbreviations

CONS
Coagulase negative *Staphylococcus*
DNA
Deoxyribonucleic acid
NHRC
Nepal Health Research Council
SIF
Sperm immobilization factor
SPSS
Statistical Package for Social Sciences
WHO
World Health Organization

# Declarations


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### Competing interest

The authors declare no competing or financial interests.

# Ethics approval

This study was reviewed and approved by Nepal Health Research Council (NHRC), Nepal (Reg. no.: 874/2019).

### Consent to participate

Written informed consent was obtained from all individual participants included in the study.

### Consent for publication

Not applicable

### Availability of data

All relevant data are presented within the article as the main text, tables, or figures. Data can be made available upon reasonable request to the corresponding author.

### Code availability

Not applicable

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44. Detailed figure and table legends

# Figures

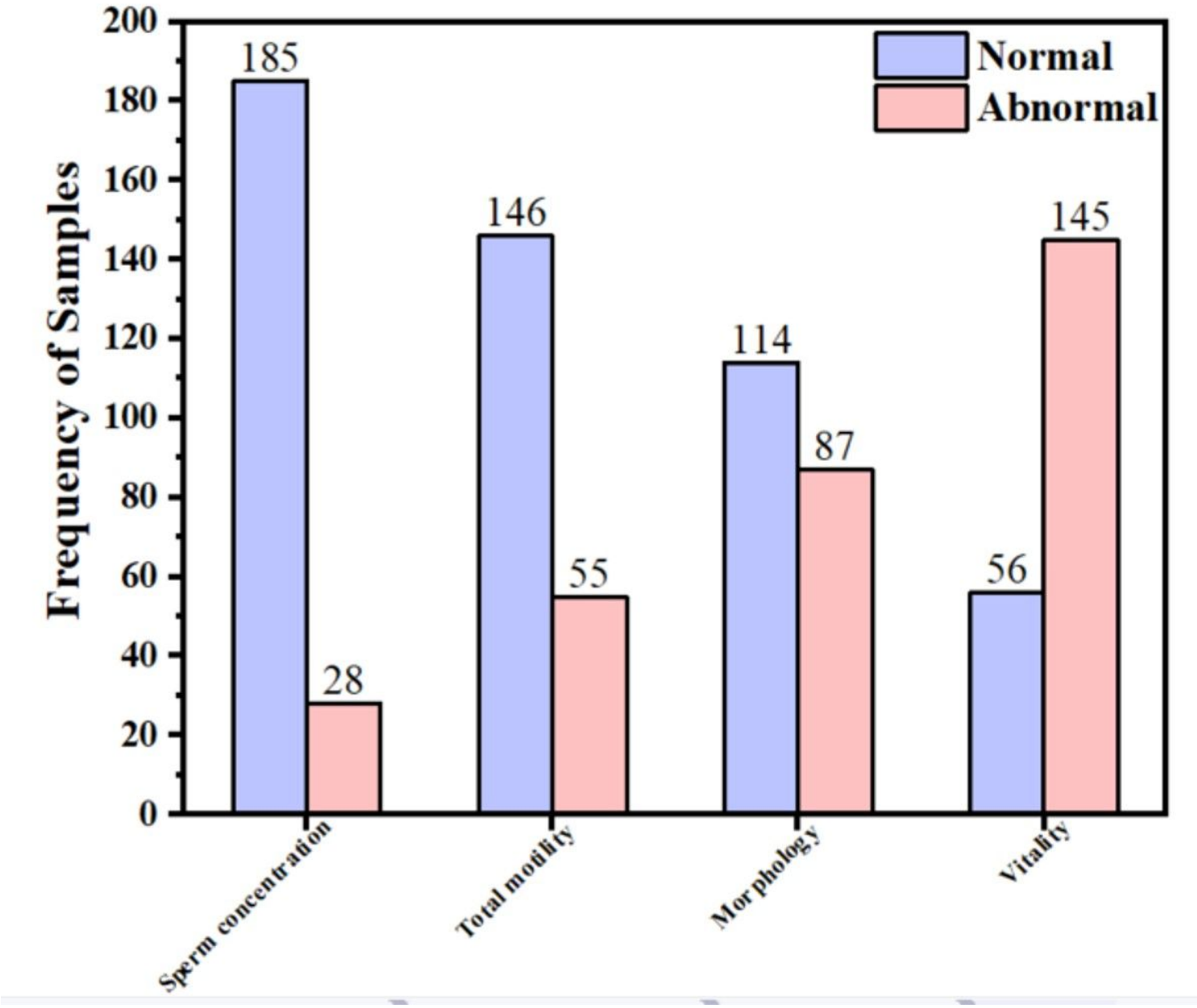
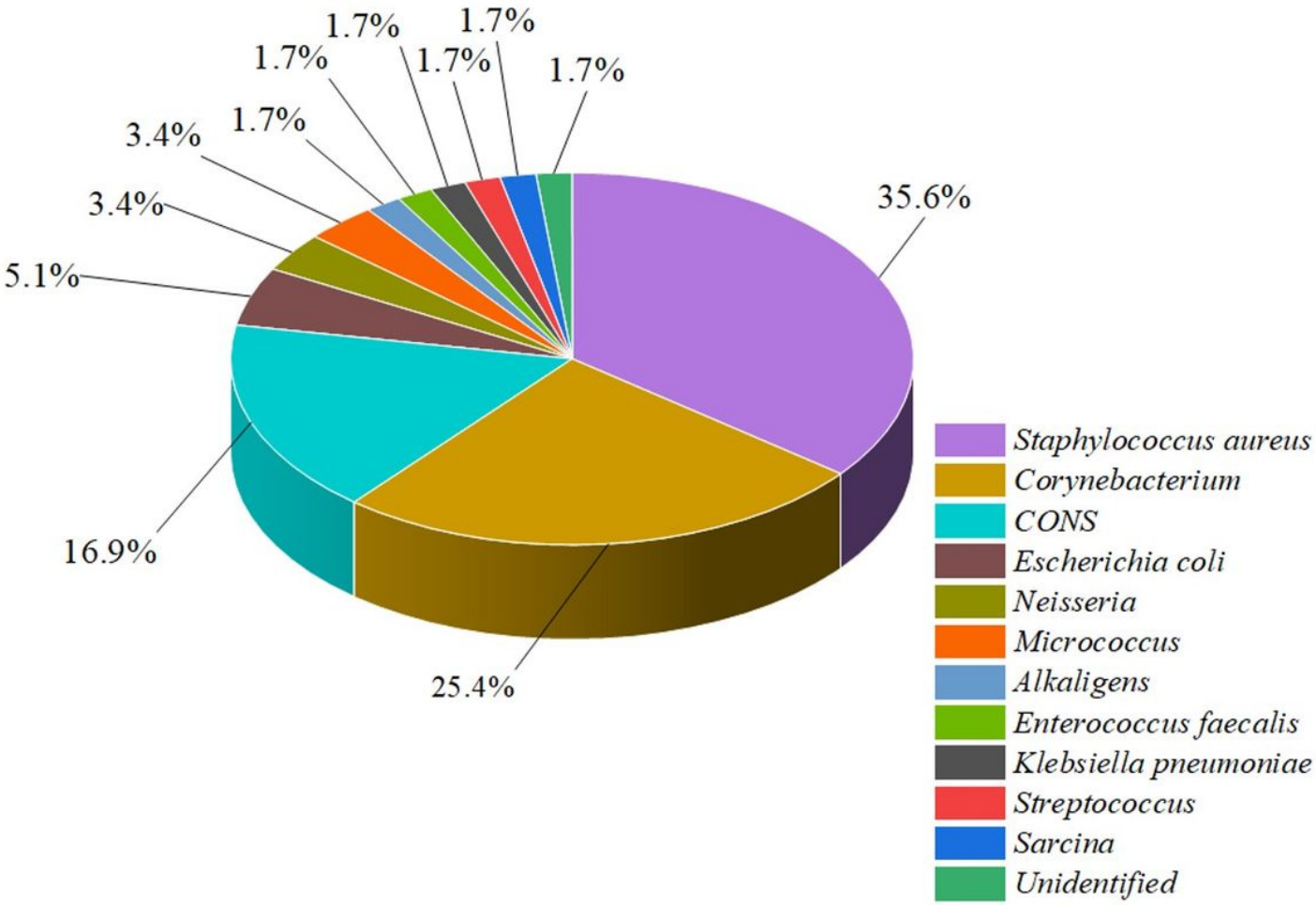



**Figure 1**

Frequency of semen samples with normal (blue bar) and abnormal (pink bar) values of sperm concentration (million/mL), percentage of total motile sperms, percentage of sperms with normal morphology and percentage of vital sperms according to WHO, 2021 (WHO reference range for normal

semen characteristics: sperm concentration≥16million/mL, total motility≥42%, sperms with normal forms≥4% and vitality≥56%).




## Figure 2

Percentages of bacteria isolated from semen samples. Majority of isolated bacteria were identified as *Staphylococcus aureus.*