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Bacterial Isolates Associated with Infertility among Males Attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State

Akpa, E. N.[1]; Elom, M. O.[2]; Usanga V. U.[1]; Kalu, M. E.[1]; *Agumah, N. B.[3]

[1]*Department of Medical Laboratory Science, Faculty of Health Science and Technology, Ebonyi State University, Nigeria.*
[2]*Department of Applied Biology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.*
[3]*Department of Medical Laboratory Science, Faculty of Health and Allied Sciences, Alex Ekwueme Federal University, Nigeria*

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***Corresponding Author:** Agumah, N. B.

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Abstract **Original Research**

Male infertility is a major public health concern, with bacterial infections increasingly recognized as contributors to impaired semen quality. Male infertility arises from a combination of genetic, environmental, and infectious factors, with bacterial infections posing particular concern due to their capacity to damage sperm cells and the male reproductive tract. Infertility is commonly categorized as either primary or secondary. Primary infertility refers to the inability to conceive or achieve impregnation, whereas secondary infertility describes the inability to conceive following a previous successful pregnancyThe emergence of antimicrobial resistance among semen-associated pathogens further complicates effective management. This study investigated the distribution of bacterial isolates associated with male infertility among patients attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State. A total of 270 semen samples were collected and analyzed using standard microbiological techniques. Bacterial growth was detected in 229 (84.8%) samples, while 41 (15.2%) showed no growth. The predominant bacterial isolates were *Staphylococcus aureus* (29.0%) and *Escherichia coli* (14.5%), with *Klebsiella pneumoniae* and *Streptococcus pneumoniae* each accounting for 8.6%. Although the study does not establish a causal relationship between bacteriospermia and infertility, it provides important descriptive evidence of the microbiological profile of semen isolates in this setting. Overall, the findings support the incorporation of routine microbiological evaluation and susceptibility testing as part of infertility work-up where feasible.

Keywords: Male infertility, bacteriospermia, antimicrobial resistance, semen microbiology, Staphylococcus aureus.

Background

Infertility is defined as the inability to achieve pregnancy after 12 months or more of regular, unprotected sexual intercourse. It affects approximately 15% of couples in the United States and an estimated 180 million couples worldwide [1]. Infertility represents a widespread global health concern, with male-related factors accounting for

about one in five infertile couples. It is estimated that approximately 1 in 20 men experience some form of fertility impairment, often characterized by reduced sperm count [2]. Male infertility arises from a combination of genetic, environmental, and infectious factors, with bacterial infections posing particular concern due to their capacity to damage sperm cells and the male reproductive tract. Infertility is commonly categorized as either primary or secondary. Primary infertility refers to the inability to conceive or achieve impregnation, whereas secondary infertility describes the inability to conceive following a previous successful pregnancy [3]. Although infertility is not life-threatening, it is regarded as a major life-altering condition due to its profound psychological, emotional, and social consequences [4].

Numerous studies have documented the presence of various microorganisms in seminal fluid [5-8]. In industrialized nations, commonly isolated bacterial pathogens include members of the *Enterobacteriaceae* family, *Chlamydia*, *Ureaplasma*, and certain Gram-positive bacteria [9]. In several regions, bacterial infections have been strongly associated with oligospermia and azoospermia, which are major causes of male infertility [5].

Bacterial infections may impair male fertility through inflammation and tissue damage that disrupt spermatogenesis and testosterone production [10]. Certain pathogens adversely affect sperm development and may induce sperm apoptosis, further compromising fertility [11,12]. Functional sperm cells are essential for successful reproduction; however, bacterial infections can cause significant sperm damage, including chromosomal fragmentation, membrane alterations, acrosomal injury, and mitochondrial dysfunction [10]. The integrity of sperm DNA and chromatin is critical for male fertility, and evidence indicates that DNA damage negatively affects reproductive outcomes [13]. Notably, *Chlamydia* infections have been shown to induce sperm chromosomal breakage [14]. Sertoli cells, Leydig cells, and spermatogonial stem cells play vital roles in normal spermatogenesis. Sertoli cells support sperm development by providing nutrients, growth factors, and energy, and their adequate number is essential for sustained spermatogenesis [15]. These cells also serve as key mediators of hormonal signaling, and disturbances in their metabolic functions can disrupt spermatogenesis and lead to infertility [16]. Although Sertoli cells help maintain testicular homeostasis by clearing apoptotic germ cells, they may become infected through phagocytosis of infected spermatogonia. Leydig cells are responsible for steroid hormone synthesis, particularly testosterone, which is essential for spermatogenesis, sexual development, and male reproductive function. Inflammation or apoptosis of Leydig cells can impair testosterone production, thereby adversely affecting male fertility [17]. Moreover, close interactions between Leydig cells and testicular macrophages facilitate the spread of infection within the testes [18]. Dysfunctions in these cell types caused by bacterial infections can further compromise spermatogenesis. Inflammatory cytokines released in response to infections caused by *Escherichia coli*, *Staphylococcus aureus*, *Mycoplasma* species, and *Pseudomonas aeruginosa* have also been shown to negatively affect sperm production [19].

The burden of male infertility associated with bacterial infections is likely to increase if adequate preventive and therapeutic measures are not implemented. Historically, infertility received limited attention in many African countries due to high fertility rates [8]. Data from the World Fertility Survey and Demographic and Health Survey indicate that infertility rates have remained stable or declined in some African countries, including Ghana, Kenya, Senegal, Cameroon, Sudan, and Nigeria [20,21].

In Nigeria, male factors contribute to approximately 40–50% of infertility cases, with regional variations in prevalence and causative factors [20,21]. Studies conducted across different regions of the country have reported varying prevalence rates. In Kano, Northern Nigeria, a prevalence of 40.8% male factor

infertility was observed among 500 men investigated, with isolates including *Staphylococcus aureus*, *Escherichia coli*, *Candida* species, and *Streptococcus* species [22]. In mid-western Nigeria, approximately 50% of evaluated couples were affected by infertility [23]. Male factor infertility accounted for 42.4% of cases in southwestern Nigeria [24], while infertility constituted about 40% of gynecological consultations in Maiduguri, northeastern Nigeria [25]. Prevalence rates of 46% were reported in Ile-Ife [26], and 55–93% in Enugu, eastern Nigeria [22].

Despite its significant impact, male infertility remains under-researched globally, particularly in developing regions where access to diagnostic and treatment facilities is limited [27]. Factors such as genetic abnormalities, hormonal imbalances, lifestyle practices, environmental toxins, varicocele, and bacterial infections have been implicated. Among these, bacterial infections represent a significant yet underreported contributor to male infertility, especially in sub-Saharan Africa. In Nigeria, infertility is further complicated by socio-cultural stigmatization, limited healthcare access, and a high prevalence of sexually transmitted infections. Consequently, research focusing on bacterial infections and male infertility is crucial for improving reproductive health outcomes. Addressing this issue will not only benefit affected individuals but also contribute to broader public health strategies and medical advancements [22].

Materials and Methods

Study Area

The study was carried out in Alex Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA) Ebonyi State, Nigeria. Abakaliki the capital city of Ebonyi State is located in south eastern Nigeria. The state has a total land mass of 5.530km^3 and a population of 3,242.5 million people [28]. The inhabitant of this area are predominantly farmers and traders, while the rest are civil and public servants. They are major producers of rice, ram, cassava, and palm oil. Ebonyi state has other natural resources such as lead, zinc, limestone, crude oil and natural gas with few large-scale commercial mining sites.

Study Design

The study is a cross-sectional study of infertile men who attend Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State. The research involved collection of semen samples from the above patients for analysis, culture and susceptibility.

Study Population and Sample Size

The study population were males who are currently in marriage and attend Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State. A total of 270 men were recruited for the study and the sample size was determined using the following formula described by [30].

$$n = \frac{Z^2Pq}{d^2}$$

where n is the desired sample size, Z is the standard variation, usually set at 1.96 (which corresponds to 95% confidence interval), p is the proportion in the target population estimated to have a particular characteristic (21 %) which is the highest reported among all authors [29], q is 1.0-p. d is the degree of accuracy desired, which is set at 0.05. therefore, the minimum sample size is

$$n = \frac{(1.96)^2\ (0.21)\ (0.79)}{(0.05)^2}$$

$$= 245$$

With the uncertainty about the true prevalence of syphilis, a 10 % attrition rate was anticipated, therefore adjusting the minimum sample size to 270.

Inclusion and Exclusion Criteria

Selection of participants was restricted to infertile men who attend Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi state. Participants who were on antibiotics before the start

of the study or who did not give consent to the study were excluded.

Sample and Data Collection

Semen samples were obtained by masturbation into sterile, leak-proof plastic containers. Participants were adequately counseled and instructed on proper sample collection and provided with a designated private room within the laboratory. Participants residing close to the hospital were permitted to collect samples at home, provided the samples were delivered to the laboratory within 15–20 minutes after ejaculation.

Ethical Consideration

Ethical approval for the study was obtained from the Health Research and Ethics Committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State (Approval No. NHREC/16/06/25/624).

Sample Assessment

Semen samples were evaluated for liquefaction time, volume, colour, pH, viscosity, sperm motility, viability/vitality, morphology, sperm count, bacterial culture, and antimicrobial susceptibility in order to determine the association of bacterial isolates with infertility.

Liquefaction

Liquefaction time was assessed within 30 minutes post-ejaculation, in accordance with WHO guidelines [31,32]. The time of ejaculation was recorded, and samples were maintained at room temperature to preserve physiological conditions. Liquefaction was observed and recorded within the stipulated timeframe.

Volume, Colour, and pH

Following complete liquefaction, semen volume was measured using a sterile 10 mL syringe. Semen colour was recorded using a standard semen colour chart, while pH was determined using pH indicator paper immediately after volume measurement. All parameters were recorded at room temperature within the recommended post-ejaculation period [31].

Viscosity

Semen viscosity was assessed after liquefaction using the pipette-drop method. Liquefied semen was aspirated into a wide-bore pipette and allowed to drop by gravity. A thread length of less than 2 cm was considered normal, while a thread length of 2 cm or more, or difficulty in drop formation, indicated increased viscosity [31].

Sperm Motility

Sperm motility was evaluated within 60 minutes post-ejaculation [33]. A drop of liquefied semen was placed on a clean, grease-free glass slide, covered with a coverslip, and examined microscopically using a ×40 objective.

Sperm Viability/Vitality

Sperm viability was assessed using the eosin staining method as described [34]. Equal volumes of semen and 0.5% eosin solution were mixed on a glass slide. After two minutes, the preparation was examined microscopically. Viable spermatozoa remained unstained, whereas non-viable spermatozoa stained red.

Sperm Morphology

Sperm morphology was evaluated using the carbon fuchsin–methylene blue staining technique [35]. Thin smears of liquefied semen were prepared, fixed in 95% ethanol for 10 minutes, washed with sodium bicarbonate formalin solution, and stained sequentially with diluted carbon fuchsin and Loeffler's methylene blue. The smears were examined under ×40 and ×100 objectives, and the proportion of normal and abnormal spermatozoa was calculated as follows:

Percentage of normal sperm= $\frac{\text{Number of normal cells counted}}{\text{Total sperm cells counted}} \times 100$

Sperm Count

Sperm concentration was determined using a Neubauer haemocytometer and white blood cell pipette technique [34]. Liquefied semen was diluted at a ratio of 1:20, mixed thoroughly, and loaded into the counting chamber. After settling for one minute, sperm cells were counted under ×40 objective. Sperm concentration was calculated using standard formulae, and total sperm count was obtained by multiplying sperm concentration by semen volume.

Culture Media Preparation

Culture media including mannitol salt agar, blood agar, chocolate agar, and Mueller–Hinton agar were prepared according to manufacturers' instructions. Media were weighed, dissolved in distilled water, autoclaved at 121°C for 15 minutes, cooled, poured into sterile Petri dishes, and allowed to solidify.

Bacteriological Analysis of Semen Samples

Semen samples were cultured on blood agar, MacConkey agar, mannitol salt agar, and chocolate agar. Bacterial isolates were identified based on colonial morphology, Gram staining, and biochemical characteristics.

Isolation and Identification of Bacterial Isolates

Well-mixed semen samples were inoculated onto blood agar and MacConkey agar within one hour of collection and incubated aerobically at 37°C for 24–48 hours. Chocolate agar plates were incubated in a 5% CO_2 candle jar under the same conditions [36].

Biochemical Identification and Characterization

Isolates were characterized using Gram staining and standard biochemical tests including catalase, coagulase, indole, motility, Kligler iron agar, oxidase, bacitracin, and optochin tests.

Catalase Test

The catalase test was performed by placing a colony on a clean glass slide and adding a drop of 3% hydrogen peroxide. Immediate bubbling indicated a positive result, while absence of bubbles indicated a negative reaction [37].

Coagulase Test

Coagulase activity was assessed using the slide method as described by [38] and [39]. A bacterial suspension was prepared on a slide using saline, followed by the addition of plasma. Immediate agglutination within 10 seconds indicated a positive result, while absence of clumping indicated a negative test.

Oxidase Test

The oxidase test was performed to detect the presence of cytochrome c oxidase based on colour change, as described by [37]. The filter paper method was employed. A small piece of filter paper was soaked in 1% Kovács oxidase reagent and allowed to dry. Using a sterile loop, a well-isolated colony from a fresh (18–24 h) culture was smeared onto the treated filter paper. The development of a dark purple colour within a few seconds indicated a positive result, while the absence of colour change indicated a negative reaction.

Urease Test

The urease test was conducted following the guidelines of the American Society for Microbiology [40]. Urease is a constitutively expressed enzyme that hydrolyses urea into carbon dioxide and ammonia: $(NH_2)2CO + H_2O \rightarrow CO_2 + 2NH_3$. Urease test media contained 2% urea and phenol red as a pH indicator. The production of ammonia increased the pH, resulting in a colour change from yellow (pH 6.8) to bright pink (pH 8.2). Stuart's urea broth, a

highly buffered medium, required substantial ammonia production to produce a colour change and was differential for *Proteus* species. Christensen's urea agar, with reduced buffering capacity, supported the growth of many Enterobacteriaceae, allowing observation of urease activity.

Citrate Test

The citrate utilization test was performed according to the American Society for Microbiology [40] as part of the IMViC test series for differentiating members of the Enterobacteriaceae. Simmons citrate agar was used, with citrate as the sole carbon source and ammonium dihydrogen phosphate as the sole nitrogen source. Utilization of citrate resulted in the production of alkaline carbonates and bicarbonates, leading to a colour change of the bromothymol blue indicator from green (neutral pH) to blue (alkaline pH >7.6), indicating a positive reaction.

Indole Test

The indole test was carried out to detect indole production from tryptophan metabolism, as described by [38 and 39]. Test isolates were inoculated into sterile tryptone water and incubated at 37°C for 24 h. Subsequently, 0.5 mL of Kovács reagent was added and allowed to stand for 10 minutes. The formation of a deep red ring indicated a positive result, while the absence of colour change indicated a negative reaction.

Kigler Iron Agar (KIA) Test

Kligler Iron Agar testing was performed as described by [41] to differentiate Gram-negative bacilli, particularly Enterobacteriaceae. The medium contained glucose, lactose, phenol red, ferrous sulfate, and sodium thiosulfate. Changes in colour and gas or hydrogen sulfide production were recorded following incubation.

Optochin Susceptibility Testing

Optochin susceptibility testing was conducted using the agar dilution method following the protocol of [42]. Bacterial suspensions prepared in 0.9% NaCl from overnight cultures on 5% sheep blood agar were adjusted to 0.5 McFarland standard. The suspensions were inoculated onto Mueller–Hinton agar supplemented with 3% lysed horse blood containing varying concentrations of optochin. Plates were incubated at 35°C for 20–24 h in a 5% CO_2 atmosphere. Minimum inhibitory concentrations (MICs) were recorded as the lowest concentration inhibiting visible growth.

Bacitracin Susceptibility Test

Bacitracin testing was performed as described by [43]. A standardized inoculum was streaked onto sheep blood agar plates, and bacitracin discs were applied. Plates were incubated aerobically at 35°C for 18–24 h. Zones of inhibition around the discs were measured and recorded.

Nutrient Broth Preparation

Nutrient broth was prepared as a general-purpose medium by dissolving peptone (5 g), beef extract (3 g), and sodium chloride (5 g) in 1,000 mL of distilled water. The pH was adjusted to 7.0–7.4. The medium was dispensed into appropriate containers and sterilized by autoclaving at 121°C for 15 minutes [44].

6.5% NaCl Broth Test

The 6.5% sodium chloride tolerance test was used to differentiate *Enterococcus* species from *Streptococcus* species [45]. Sodium chloride (6.5 g) was dissolved in 100 mL of nutrient or brain heart infusion broth. After sterilization, test organisms were inoculated and incubated at 35–37°C for 24–48 h. Turbidity indicated a positive result, while clear broth indicated a negative reaction.

Gram Staining

Gram staining was performed using the standard four-step differential staining technique as described by [46]. Smears were prepared, heat-fixed, stained with crystal violet, treated with iodine, decolorized with acetone, counterstained with neutral red, and examined under oil immersion (×100 objective).

RESULTS AND DISCUSSION

In this study *Staphylococcus aureus* is the most frequently isolated pathogen, accounted for 29 % (79 occurrences) of the cases. *Escherichia coli* follows with 14.5 % (39 occurrences), *Klebsiella pneumoniae* and *Streptococcus pneumoniae* were tied, each representing 8.6 % (23 occurrences). Other isolates such as *Pseudomonas aeruginosa* (5.9 %, 16 occurrences), *Enterococcus faecalis* (5.6 %, 15 occurrences), and *Proteus mirabilis* (4.5 %, 12 occurrences) contributed to a smaller but meaningful portion of the cases. Less frequent pathogens include Coagulase Negative *Staphylococcus* species (3.3 %, 9 occurrences), *Citrobacter frundii* (3.7 %, 10 occurrences), and *Streptococcus pyogenes* (0.7 %, 2 occurrences). *Streptococcus* species is the least frequent, occurring in only 0.4 % of cases (1 occurrence). The category No growth represents 15.2 % (41 occurrences) of cases where no bacterial growth was detected (Table 1).

Table 1: Bacterial Isolates Associated with Male Infertility in Alex Ekwueme Federal University Teaching Hospital, Abakaliki, (AE-FUTHA) Ebonyi State.

Bacterial Pathogen	Frequency	Percentage (%)
Staphylococcus aureus	79	29.0
Pseudomonas aeruginosa	16	5.9
Coagulase Negative *Staphylococcus* species	9	3.3
Escherichia coli	39	14.5
Streptococcus pyogenes	2	0.7
Klebsiella pneumonia	23	8.6
Enterococcus faecalis	15	5.6
Citrobacter freundii	10	3.7
Streptococcus pneumonaie	23	8.6
Proteus mirabilis	12	4.5
Streptococcus species	1	0.4
No growth	41	15.2
Total	**270**	**100.0**

Out of the 270 men, 229 (84.8 %) tested positive for bacterial infection, while 41 (15.2 %) had no bacterial growth. (Table 2).

Table 2: Prevalence of Bacterial Isolates in Infertile Men in Alex Ekwueme Federal University Teaching Hospital, Abakaliki, (AE-FUTHA) Ebonyi State.

Bacterial Infection	Frequency	Percentage (%)
Bacterial infected	229	84.8
No growth	41	15.2
Total	**270**	**100.0**

The findings of this study indicate that *Staphylococcus aureus* was the most prevalent bacterial isolate associated with male infertility, accounting for 79 (29.0%) of cases. This was followed by *Escherichia coli* with 39 (14.5%), while *Klebsiella pneumoniae* and *Streptococcus pneumoniae* showed equal prevalence at 23 (8.6%) each. Other isolates included *Pseudomonas aeruginosa* 16 (5.9%), *Enterococcus faecalis* 15 (5.6%), and *Proteus mirabilis* 12 (4.5%). No bacterial growth was observed in 41 (15.2%) of the 270 semen samples analyzed (Table 1). These findings are consistent with the report of [52], although variations in the order of prevalence were observed. Notwithstanding these differences, there was considerable overlap in the spectrum of dominant bacterial isolates, with both studies identifying *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Proteus mirabilis* as frequently associated with male infertility. The recurrent identification of these organisms across diverse populations reinforces their etiological relevance in infertility-related bacteriospermia.

Furthermore, the predominance of *Staphylococcus aureus* observed in this study aligns with reports by [53-55], all of whom identified this organism as the most common isolate in semen cultures of infertile men. These consistent findings suggest that *Staphylococcus aureus* warrants particular clinical attention during diagnostic evaluation and empirical management of suspected semen infections. Although earlier studies have reported similar bacterial profiles, the present study strengthens the practical implication that *Staphylococcus aureus* should be considered a primary pathogen in the differential diagnosis of bacteriospermia-associated infertility.

Of the 270 participants examined, 229 (84.8%) demonstrated bacterial growth, while 41 (15.2%) showed no evidence of infection (Table 2). This prevalence differs from the findings of [55], who reported bacteriospermia in 25.3% of samples analyzed. Despite this discrepancy in overall prevalence, both studies consistently identified *Staphylococcus aureus* as the predominant isolate, underscoring its recurrent association with male infertility.

This study demonstrates a high prevalence of bacteriospermia among infertile men attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki, with a diverse range of bacterial isolates.

Although the study does not establish a causal relationship between bacteriospermia and infertility, it provides important descriptive evidence of the microbiological profile of semen isolates in this setting. Overall, the findings support the incorporation of routine microbiological evaluation and susceptibility testing as part of infertility work-up where feasible. Further multicenter and longitudinal studies incorporating molecular diagnostics and fertility outcome measures are required to better elucidate the clinical significance of bacteriospermia and to inform standardized management guidelines.

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