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Prevalence of Bacterial Infection among Males with Fertility Problems Attending Federal Teaching Hospital Owerri

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ABSTRACT

Background: Bacterial infections of the male reproductive tract have been linked to male infertility. This study set out to determine the prevalence of bacterial growth and identify the bacterial species among males with fertility problems in Federal teaching hospital Owerri, Imo state, Nigeria.

Methodology: The study population incorporated 200 consenting male patients aged 18years and above. Semen samples were cultured on chocolate agar, mannitol salt agar, blood agar and MacConkey agar. Antibiotics sensitivity testing was conducted using kirby-bauer disk diffusion method.

Results: The semen culture results indicated that 62.5% of the study participants had bacterial growth. *Staphylococcus* species was mostly the organism isolated from the positive cultures (38.4%), followed by *Escherichia coli* (20.0%) and coagulase-negative *Staphylococcus* species (CoNS) (16.8%). The S. aureus isolated in this study showed high susceptibility to Vancomycin (97.3%) with moderate susceptibility to Levofloxacin (66.7%) and Ceftriaxone (64.6%). High resistance to Penicillin and Augmentin was also observed.

Conclusion: The study demonstrated that bacterial infection is prevalent in males attending fertility clinic, with *S. aureus* being the frequent isolate. The multidrug resistance observed emphasise the need for semen culture and antibiotics sensitivity testing before commencement of treatment.

Keywords: Semen, male infertility, *Staphylococcus aureus*, bacteria, antibiotics susceptibility.

INTRODUCTION

Male infertility as defined by the World Health Organization (WHO) is a disease of the male reproductive system which prevents the male to impregnate a fertile female for a minimum of at least 1 year of regular unprotected sexual intercourse [1].

A reduction in male fertility may be attributed to many factors, such as testicular failure, urogenital abnormalities, immunologic problems, varicocele, genetic abnormalities, endocrine disturbances, systemic diseases, cancer, genital tract infections, environmental agents, distorted lifestyle and gonadotoxic factor exposure [1, 2].

Different parts of the male reproductive tract are affected by infections, like the epididymis, testes, and male accessory sex glands. The spermatozoa produced are also affected by urogenital infections at different levels of their maturation, development, and transport [2].

About 8% to 35% of male infertility cases are due to bacterial infections of the male reproductive tract [3]. Diverse bacteria such

as Staphylococcus aureus, Ureaplasma urealyticum, Mycoplasma hominis, Chlamydia trachomatis, and Escherichia coli, Streptococcus spp., Klebsiella spp. and Candida spp. which are known as facultative infections have been known to impede sperm function in vitro [4, 5]. They reduce fertility by damaging sperm DNA, blocking reproductive tracts, and physically impairing sperm motility through direct agglutination [3].

In Africa, infertility is mostly a public health concern due to negative social impact like isolation, disinheritance, stigmatization or even divorce in some cases [6]. Inorder to provide current baseline data and reference for further research on male fertility, this study set out to determine the prevalence of bacterial growth and identify the bacterial species among males with fertility problems in Federal teaching hospital Owerri, Imo state, Nigeria.

MATERIALS & METHODS

Study Area

The study was conducted at the Federal Teaching Hospital Owerri (FTHO), a prominent tertiary healthcare facility located in Owerri, capital of Imo state, South-East of Nigeria. Owerri lies between latitude 5°10¹N to 5°57¹N and longitude 7°28¹E to 5°35¹E and covered an area of about 24.88 km² within Nigeria's rainforest zone.

Study Design

The study is a cross-sectional, descriptive hospital-based study conducted among male patients with fertility problems presenting at the Infertility Clinics of Federal Teaching Hospital Owerri (FTHO).

Study Population

The study population incorporated 200 consenting male patients aged 18years and above who were referred for semen analysis due to primary, secondary infertility or other provisional diagnosis of issues relating to male sexual organs, and met the inclusion criteria of the study.

Sample Size Determination

A consecutive sampling method was used to recruit all eligible male patients who meet the inclusion criteria and gave consent to participate during the study period.

Cochran's formula for determining sample size in a cross-sectional study was used in this study.

N = Z^2 Pq / d^2

N = minimum sample size

Z = Normal standard deviation usually set at 1.96 which corresponds to 95% confidence interval.

P = proportion or the target population amongst males attending fertility clinic in Nigerian tertiary health institution. Umar et al. [7]. in Sokoto state reported that the prevalence of male infertility was 7.3%.

q= Complement of the prevalence (P- 1)

d=Degree of accuracy desired usually set at 0.05.

Hence N = (1.96) ^2 X (0.073) X (0.927) / (0.05) ^2

N = 104.

However, a sample size of 200 was used in consideration of an anticipated response rate of 80% (0.8). This was calculated by dividing the original calculated sample size (N) by the anticipated response rate as follows:

NS = N / 0.8. Substituting in the above formula

NS = 104

0.8 = 130.

The minimum sample size was then summed up to 200

Inclusion criteria:

Male patients attending fertility clinic at FTHO who had failed to establish pregnancies after 1year or more.

Those aged 18years and above.

Those that gave consent.
Those that abstained from antibiotics for ≥2 weeks

Exclusion criteria:
Those who did not give their consent were excluded from the study.
Semen specimens collected without masturbation.
Those that has recent urogenital trauma/surgery.

Ethical Consideration and Informed consent
Ethical approval was obtained from the Research Ethics Committee of FTHO. Informed written consent was obtained from all participants. Data confidentiality was maintained by assigning anonymous codes.

Method of Data Collection
Data were collected through qquestionnaire using interviewer-administered technique. The data was collected by the researcher and other research assistants after informed verbal explanation and written consent was obtained from the participants. The interview was done with the participants preferred language (either English or Igbo), with the researcher or research assistant aid. Each questionnaire took about 15 minutes to administer.

Patient Preparation and Sample Collection.
Semen samples were collected from males with fertility problems attending fertility clinic at federal teaching hospital Owerri during the study period, who gave consent and met the inclusion criteria. Prior to providing a sample for analysis, each patient was provided with clear instructions for collecting the semen sample. Samples were produced through masturbation and collected in a sterile cup at specific sample collection room within the laboratory. A minimum abstinence period of 3 days on sexual intercourse, alcohol consumption and smoking was required. None of the patients had taken prior antibiotics. After collection of each sample, it was placed in an incubator at 37ºc for 40 minutes to allow liquefaction before being analysed.

Laboratory Analysis
Semen culture
Culture of the homogenized semen samples on chocolate agar, mannitol salt agar, blood agar and MacConkey agar was done in laboratory within 3 hours of specimen collection as per WHO guidelines aseptically. Cultures were incubated at 37°C for 24-48hrs. Bacterial growth was examined, and isolates were identified using colony morphology, Gram staining, and standard biochemical tests.

Antibiotic Susceptibility Testing (AST)
AST was conducted using kirby-bauer disk diffusion method following clinical laboratory standards of institute (CLSI) guidelines.

Statistical Analysis
SPSS (v25) was used for descriptive and inferential statistical analysis. Frequency distribution tables, percentages, charts and graphs were employed to assess trends.

RESULT
CHARACTERISTICS OF STUDY PARTICIPANTS.
The ages of the participants ranged from 18-64years with an average age of 38.5years. For the age distribution, 95 (47.5%) were less than 40 years and 105 (52.5%) were above 40years. Fifty-three (26.5%) of the participants have had fertility issues for 1year, 39(19.5%) for 2years, 78(39%) for 3-5years while 30(15%) for more than 5years (Table 1).

TABLE 1: CHARACTERISTICS OF STUDY PARTICIPANTS.

VARIABLE	SUB-VARIABLE	NUMBER OF PARTICIPANTS	PERCENTAGE (%)
AGE	<40years	95	47.5

	>40years	105	52.5
	Total	**200**	**100**
DURATION OF INFERTILITY	1year	53	26.5
	2years	39	19.5
	3-5years	78	39
	>5years	30	15
	Total	**200**	**100**

PREVALENCE OF BACTERIAL GROWTH AND THE DIFFERENT BACTERIAL SPECIES IDENTIFIED AMONG THE PARTICIPANTS

Out of the 200 semen samples cultured, bacterial growth was observed in 125 (62.5%) as shown in Table 2. Forty-eight (38.4%) were *Staphylococcus* species, 25 (20.0%) were *Escherichia coli,* 21 (16.8%) were coagulase negative *Staphylococcus* species, 9(7.2%) were *Streptococcus species*, 3 (2.4%) were *Pseudomonas aeuroginosa* and 9(15.2%) were mixed growths. Figure 1. Of the 19 mixed growths, 11 (57.9%) were mixed growth of *Staphylococcus* and *Streptococcus species*, 6 (31.6%) were mixed growth of *Staphylococcus spp* and *Escherichia coli* while 2 (10.5%) were mixed growth of *Escherichia coli* and *Streptococcus species.* Figure 2

TABLE 2: PREVALENCE OF BACTERIAL GROWTH AMONG THE PARTICPANTS.

CULTURE	FREQUENCY	PERCENTAGE (%)
No growth	75	37.5%
Growth observed	125	62.5%
Total	**200**	**100%**

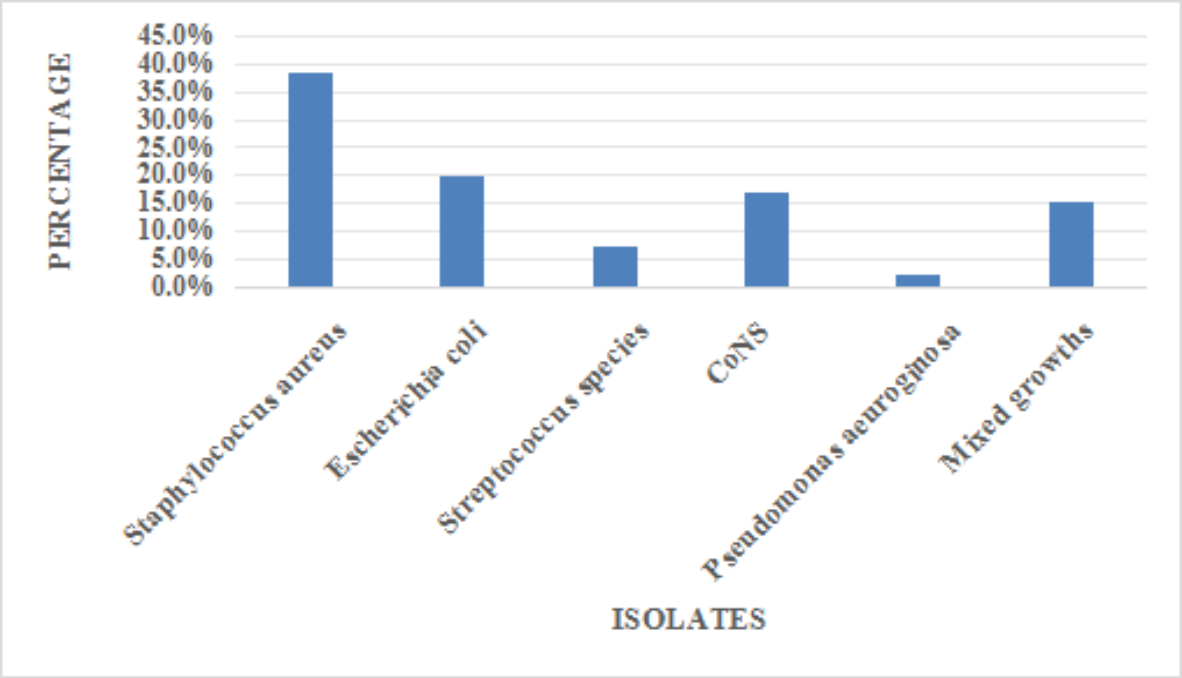

Figure 1: The bacterial species identified among the study participants.

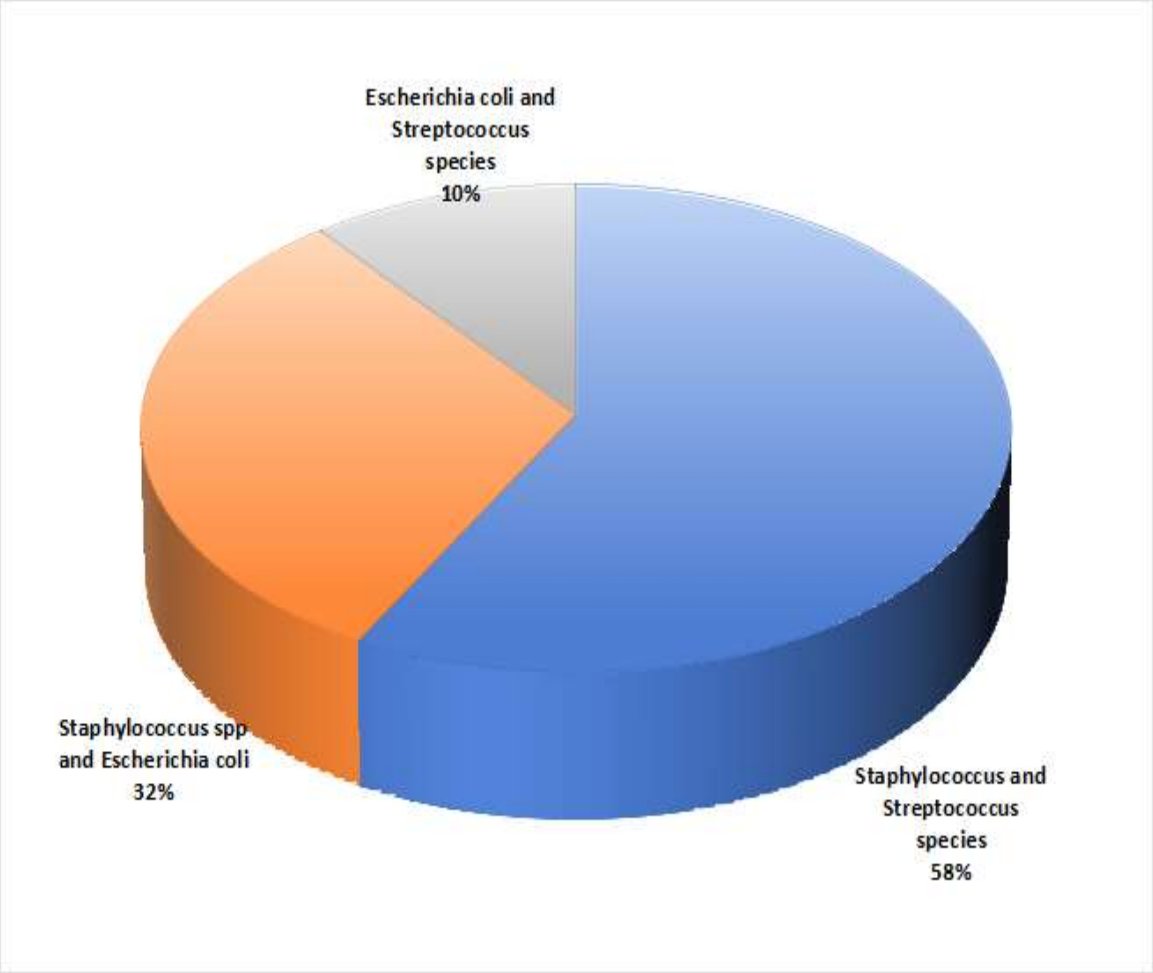

Figure 2: The bacterial species in the mixed growth identified among the study participants.

ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *STAPHYLOCOCCUS AUREUS* AMONG THE STUDY PARTICIPANTS.

Staphylococcus aureus is the most prevalent organism isolated. Its antibiotic susceptibility profile showed that Vancomycin had the highest percentage sensitivity (97.9%) followed by Levofloxacin (66.7%), Ceftriaxone (64.6%) and Gentamicin (35.4%). The lowest was Augmentin (12.5%) followed by Penicillin (27.1%). Table 3

TABLE 3: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF STAPHYLOCOCCUS AUREUS ISOLATES

Antibiotic	Sensitive n (%)	Resistant n (%)
Vancomycin	47(97.9)	1(2.1)
Penicillin	13(27.1)	35(72.9)
Levofloxacin	32(66.7)	16(33.3)
Augmentin	6(12.5)	42(87.5)
Ceftriaxone	31(64.6)	17(35.4)
Gentamycin	17(35.4)	31(64.6)
Azithromycin	15(31.3)	33(68.7)
Imipenem	15(31.3)	33(68.7)

DISCUSSION

The age distribution in this study revealed that 52.5% of the participants were above 40 years while 47.5% were below 40 years. This aligns with reports that advancement in age reduces male fertility which may be

why there were more participants above 40years. Aging in males facilitates increased DNA fragmentation and reduced testosterone levels all of which leads to impairment of spermatogenesis [8, 9]. However, the substantial proportion of partcipants below 40 years indicates that infertility is also a significant problem among younger men in this setting. Unlike the older men, infertility in men under 40 is frequently related to various factors including infections, lifestyle factors, and occupational exposures [10]. The most common duration of infertility in patients presenting for care was 3–5 years (39%), and 15% reported infertility of more than 5 years. The long delay before attending a clinic may be explained by the sociocultural barriers, the stigma and the lack of awareness that prevent men from seeking fertility help [11].

The semen culture results indicated that 62.5% of the study participants had bacterial growth in their semen samples. The prevalence of bacterial growth in semen of infertile men varies by study. Gholami et al. [12] reported a lower rate of 12% whereas a previous study by Isaiah et al. [13] reported a rate of 65.7% which is in agreement with the findings of this current study. This high prevalence of positive semen cultures is consistent with infectious etiologies being a significant contributor to male infertility in Nigeria and similar sub-Saharan African settings [14]. Staphylococcus species was mostly the organism isolated from the positive cultures (38.4%), followed by Escherichia coli (20.0%) and coagulase-negative Staphylococcus species (CoNS) (16.8%). Similar study in Port-Harcourt by Ohanu et al [15] and in Iran by Ali et al [16] also recorded *Staphylococcus species* as the most isolated bacteria from infertile men followed by *Escherichia coli.* S. aureus is a well-known pathogen in male reproductive tract infections, capable of causing urethritis, prostatitis, and epididymo-orchitis, which can impair sperm quality and fertility [17]. In contrast to the findings of this study, Ndukwu and Ikpeama [18] reported *Escherichia coli* as the most isolated organism in semen. The presence of *Escherichia coli* in semen also negatively affects male fertility. It attaches to sperm cells and causes them to clump together thereby immoblising them [19]. It also alters sperm DNA and morphology by triggering oxidative stress and inflammation [19].

The prevalence rate of coagulase-negative Staphylococcus species (CoNS) (16.8%) observed in this study is similar to the 14.28% reported by Katayon et al. [20] in Iran, but lower than the 71.4% prevalence observed by Volz et al. [21] among their study participants in Germany. CoNS is commonly thought to be less virulent. However, new evidence shows that CoNS may be opportunistic pathogens of the male genitourinary tract, especially in patients with co-morbidities, immunosuppression, or development of biofilms on reproductive tissues [22, 23]. Their presence in semen may contribute to subclinical inflammation, subtle sperm damage, and biofilm-related infertility thereby warranting clinical attention [22, 24].

The S. aureus isolated in this study showed high susceptibility to Vancomycin (97.3%) with moderate susceptibility to Levofloxacin (66.7%) and Ceftriaxone (64.6%). They demonstrated high resistance to Penicillin and Augmentin. The high susceptibility of S. aureus to Vancomycin noted in this study (97.3%) aligns with global reports on S. aureus susceptibility to Vancomycin [5, 25, 26, 27, 28, 29]. However, the 2.1% resistance noted in our study is a concern and may indicate emerging VISA (Vancomycin-intermediate *Staphylococcus aureus*) and VRSA (Vancomycin-resistant *Staphylococcus aureus*) strains. Reports of emergence of VISA/VRSA in clinical isolates of *Staphylococcus aureus* from different parts of Nigeria abound. Similar to our findings, Ogefere and Igweh [30] reported a 4.5% VRSA isolates in Benin and Ebede et al. [31] reported 6% VRSA isolates in Enugu. Olufunmiso et al. [32] reported a higher prevalence of 39.5% VRSA isolates in

semen samples of patients attending three teaching hospitals in Ogun State, Nigeria. Likewise, a study in Ethopia recorded a higher prevalence of 14.1%.in clinical isolates [33]. However, a systematic review and meta-analysis by Shariati et al. [34] reported a global VRSA and VISA prevalence of 1.5% and 1.7% respectively. Misuse and overuse of vancomycin have been linked to the emergence of VISA and VRSA.

The moderate susceptibility to Levofloxacin and Ceftriaxone noted in this study could be as a result of Methicillin-Resistant S. aureus (MRSA). Both antibiotics have been known to show excellent bactericidal activity and high sensitivity rates to various bacteria but not to MRSA [35, 36, 37]. The Penicillin resistance may be linked to β-lactamase production [38].

CONCLUSION

The study demonstrated that bacterial infection is prevalent in males attending fertility clinic, with *S. aureus* being the frequent isolate. The multidrug resistance observed emphasize the need for semen culture and antibiotics sensitivity testing before commencement of treatment.

Declaration by Authors
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