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ORIGINAL ARTICLE

Bacteriological Evaluation of Semen Samples among Male Patients Attending a Tertiary Hospital in North-Central, Nigeria

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ABSTRACT
Bacterial pathogen in semen is a productive health disorder emerging as a global serious medical and social issues that has much trauma, emotional instability and psychological stress of the affected individuals, this can be accessed by the quality and quantity of sperm cells as well as its structure. This research was carried out to detect bacteria pathogen in semen samples among male patients attending Bwari General Hospital Abuja. Semen samples from fifty (50) men were collected in sterile bottles for chemical and bacteriological analysis. A loopful of semen samples was inoculated immediately onto blood, chocolate and MacConkey agar immediately with the aid of a sterile wireloop for microbiological examination while the remainder was allowed to liquefy at 37^{o}C for thirty minutes and was examined macroscopically for the following; appearance, volume, viscosity; and microscopically for the following; leucocytes, motility, sperm count and sperm morphology. The morphological sperm characteristics were studied by simple microscopy. Samples were cultured and bacteria isolates were molecularly characterized. Bacteria growth was positive for 37/50(74%) samples and negative for 13/50(26%) samples. The patients'demographical study was taken, statistically the age group between 41-50 years have the lowest prevalence rate of the infection. The younger age group and farmers have the highest rate of infection as compared to other occupation. Enterococcus faecalis, Lysinibacillus macrolides and Bacillus fusiformis with GenBank Accession number FJ378657.2, KX129780.1 and AY548954.1 respectively were molecularly identified. These organisms were previously mistaken for Staphylococcus aureus, Escherichia coli and Proteus species respectively. These bacteria showed a drastic effect on semen quality which could lead to infertility. Routine check up and personal hygiene is highly recommended as a control measure.
Keywords: Bacterial pathogen, Semen, Sperm count, WBCs

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INTRODUCTION

Bacterial Infection in semen is an underlining cause of poor semen quality and quantity faced by males all over the world resulting in infertility and other infections [1]. It is thought to be responsible for up to 15% of case of male infertility. According to clinical data, as many as 60% of patients treated with assisted reproductive technology (ART) has suffered local inflammation of bacterial infection [2].The direct relationship between acute or chronic inflammations and infections of the male urogenital tract and subsequent development of infertility is actively debated; however bacterial infection in semen remains a major cause of death, disability, social and economic disorder for millions of people throughout the world [3]. According to the national institute of health (2010), about one third of cases of infertility among married couples are caused by the male factor, another one-third is caused by the female factor. In the remaining one-third, either male and female factors or no apparent cause is detected. In other words, in approximately 40% of infertile couples, the male factor is either the sole or a contributing cause of infertility. Several studies have revealed the role of sperm parameters such as low concentration, poor

motility, and morphological abnormalities in the infertility situation in males. These factors are sometimes associated with the presence of bacterial Infection.

Semen, also known as seminal fluid, is an organic bodily fluid created to contain spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of male or hermaphroditic animals and can fertilize the female ovum. Semen is produced and originates from the seminal vesicle, which is located in the pelvis [4].The process that results in the discharge of semen from the urethral orifice are called ejaculation. In humans, seminal fluid contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose are elements of seminal fluid which promote the survival of spermatozoa, and provide a medium through which they can move or "swim". Semen fluid is made mostly of water, plasma and mucus (a lubricating substance). It contains 5 to 25 calories, and is made-up of small amounts of essential nutrients including: Calcium. In the sexually mature human male, sperm cells are produced by the testes (singular, testis); they constitute only about 2 to 5 percent of the total semen volume. As sperm travel through the male reproductive tract, they are bathed in fluids produced and secreted by the various tubules and glands of the reproductive system. After emerging from the testes, sperm are stored in the epididymis, in which secretions of potassium, sodium, and glyceryl phosphorylcholine (an energy source for sperm) are contributed to the sperm cells. Sperm mature in the epididymis [5]. Sperm bacterial contamination is quite frequent and can contribute to the deterioration of the sperm quality of infertile. The Bacteria responsible for semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse. The most frequently isolated microorganisms in male patients with genital tracts infection or semen contamination is Escherichia coli [6].

Bacterial in semen was considered a major cause of infertility in the semen of asymptomatic infertile men, we have, primary and secondary infertility. Primary infertility is denoted for those women who have not been conceived previously. In secondary infertility, there is at least one conception but fails to repeat. Bacterial in Semen of the male genitourinary tract account for up to 15% of cases of male infertility. The bacteria responsible for semen contaminations generally originate from the urinary tract of patients but can be transmitted by the partner via sexual intercourse [7].

Bacterial infection of the male genitourinary tract account for up to 15% of cases of male infertility where acute and chronic infection and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process causing qualitative and sperm alterations [8]. Infertility on the other hand is the inability of a sexually active, non-contraceptive couple to achieve pregnancy in one year [9]. Infertility been a multifarious condition requiring multidisciplinary involvement is caused by a variety of factors. It is estimated that approximately 15% of couples worldwide suffer from infertility [3], however male and female factors are equally responsible for these condition with 30 – 40% causes each and the remaining 20% is ascribed to idiopathic reasons. The aetiology of about 55% of infertility due to male factor is controversial. However, bacterial infection is the underlying cause in most cases [10]. Various factors that may lead to decrease of reproductive potentials of males include; genetic or acquired conditions, urogenital abnormalities, varicocele, genetic disease, endocrine disturbance, testicular failure, immunological problems, cancer, systemic infection, genital tract infection, altered daily routine and exposure to gonadotoxic or ecological agents [8]. Amongst these, acute and chronic urogenital tract infection (U.T.I) pose a prominent factor to infertility causing obstruction of the male reproductive system, impairment of sperm cell function [11], testicular damage, epididymitis, and orchitis [12].

The world health organization (WHO) defines infection of the semen as the presence of greater than 1000 bacteria per MI of semen. However, the presence of very high number of white blood cells in semen also suggests the presence of infection. Studies suggests that sperm motility is obstructed by different genera of bacterial like *Escherichia coli, Mycoplasma hominis, Staphylococcus aureus, chlamydia trachomatis, Neisseriagonorrhoea, proteus mirabilis.* Among others [13]. However, Escherichia coli, and Enterococci are the main micro –organisms with the most detrimental influence on sperm motility and morphology [11]. *E.coli* is the most frequently involved in non-sexually transmitted epididymitis and also the prevalent in 65-70% of total cases of acute prostatitis. Genital ureaplasma and mycoplasma are also microorganisms that colonise male urethra and contaminate the semen during ejaculation with its effect on semen quality and fertilizing potential [14]. Along with urogenital infections an unhealthy lifestyle is also an important factor contributing to male infertility [12].

The W.H.O defines infertility as a biological inability to achieve conception on one year or after one year of unprotected coital exposure [15].On a world-wide scale, 50-80 million people suffer from infertility [3]. The World Health Organization [9], estimated approximately 8-10% of couples suffers from this problem.

The aetiology of about 55% of infertility due to male factor is controversial, however, bacterial infection is the underlying cause in the most cases [10]. Recent studies have shown that the presence of bacteria in semen samples may compromise the sperm quality [1]. The bacteria responsible for semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse [7]. The impact on fertility of a few bacteria such as chlamydia trachomatis, *Neisseria gonorrhoeae* and *Ureaplasmaurealyticum* has been well established. *Neisseria gonorrhoeae* is also known to be involved in damage to the reproductive tract and has been recognized.

Several studies have reported that the semen quality and thus male fertility is declining over past decades in several countries all over the world. However, different geographical areas vary in sperm count and semen quality. Studiesalso reveal that men from western countries-Denmark, Germany and Norway have poor semen quality with a higher risk of testicular cancer in comparison to eastern countries [16]. Fertility decline has been also reported in eastern European countries-Bulgaria, the Czech Republic, Hungary, Poland and Russia as well as in Africa [3]. A decline in fertility, with a reduction in sperm counts is also reported in India [16]. A recent report on the status of infertility in India states that almost 50% of infertility is related to reproductive anomalies or disorders in the male. However, in Nigeria, decrease in fertility has been recorded in Enugu, Ife, Benin,Kano,Ebonyi and even Lagos [17]. This reduction in fertility from past few decades suggests altered lifestyle, pollution, chemical based foods, lack of nutrition, stress, deskbound work attributes to some extent. [8]. The most commonly attributed factor is increasing industrialization and civilization that exposes hazardous chemicals, pesticides and electro-magnetic waves in the environment that also affect other factors contributing to reproductive disorders. Many cases of idiopathic infertility have a genetic basis such as chromosomal aberration, micro deletion and mutation [8]. Male genital tract infections are an important and correctable aetiology of male infertility. Up to 12% of male infertility cases are caused by male genital tract infections, including prostatitis, epididymis and orchitis [7]. Some studies showed that bacteria, yeasts and protozoa may interact directly with sperm. These interactions result in attachment between bacteria and sperm, agglutination phenomena and morphological alterations to sperm [12]. Among these species, there are pathogens which cause genito-urinary infections both in men and women, such as *E. coli, E. faecalis, U. urealyticum* and *Candida spp*[13]. These organisms are present in the human urogenital tract and may be responsible for damage. Most of these phenomena have been observed in vitro experimental studies. Bacterial infection poses a serious threat to sperm resulting in abnormalities which is a critical factor in male infertility such as:low sperm count, poor sperm motility, abnormal sperm shape [15].

Risk factors associated with bacterial infection responsible for male infertility include: varicocele, aging,sexually transmitted diseases, environmental factors like occupational or other long-term exposure to certain types of toxins and chemicals (such as herbicides and pesticides) may reduce sperm count by either affecting testicular function or altering hormone systems. Estrogen-like and hormone-disrupting chemicals such as bisphenol A, phthalates and organochlorines are particular potential concerns. Chronic exposure to heavy metals such as lead, cadmium, or arsenic may affect men who have long-term and intense occupational exposure to these chemicals [18]. Lifestyle Factors, testicular overheating, Cocaine or heavy marijuana,heavy drinking, smoking,obesity,bicycling[18].

Seminal tracts are ducts through which sperm move from the testicles to the outside part of the body. If an infectious process exists at any point along this route, sperm are forced to cross the infected area which can damage them and affect their ability to fertilise the egg [12]. Seminal tract infections affect fertility in several ways: Blockage of seminal tracts may prevent sperm from being released during orgasm (Azoospermia) or cause oligospermia or low sperm count in semen, when attached to sperm, they may decrease their motility (Asthenozoospermia) and prevent them from moving to the egg,they may cause changes to sperm morphology (Teratospermia), they may increase sperm DNA fragmentation index, which compromises embryo implantation and pregnancy development. They favour the production of antisperm antibodies and therefore reduce chances of fertilization [19].

Seminal tract infections seldom show symptoms, it is common that they remain for a long time without being identified and generate consequences that may lead to infertility. In some cases,seminal tract infections may cause: abnormal semen, irritation, itching, painful urination, urethral discharge and infertility.Seminal tract Infection may be caused by the presence of harmful microorganisms in the prostate, seminal vesicles, the vas deferens, the epididymis and the testicles. These microorganisms tend to be transmitted during unprotected sexual intercourse. Any sexually active male may develop a seminal tract infection; however, the risk is proportional to the number of sexual partners he has or has had.

Treatment of bacterial Infection in semen is by antibiotic susceptibility analysis result. General measures such as proper sanitation practices, personal hygiene are also necessary aspects of prevention. Avoidance of multiple sexual partners is a major aspect of prevention of sexual transmitted infections as most of

these bacterial are sexually transmitted. Scientist on their part should develop strains of antimicrobial to vaccinate individuals against S.T.I.

This study therefore, aims at assessing the level of this pathogenic bacteria in semen samples among males attending Bwari hospital ascertaining the level of infertility as a result of these infections. Thereafter, semen samples were processed for bacteriological analysis and examined to evaluate sperm concentration and motility. The morphological sperm characteristics were studied by simple microscopy.

MATERIAL AND METHODS

POPULATION SIZE.

The research was limited to male between the ages of 20 - 60 years.

The sample of this research was collected and conducted on 50 patients in Bwarigeneral hospital.

ETHICAL CONSIDERATION

Ethical approval was granted by the Research and Ethics Committee of the Ministry of Health with reference number FHREC/2022/01/93/28.06.22 and Human Services, FCT, Hospital management also granted ethical approval with reference number FCTA/HHSS/HMB/GEN/78411.The Medical Director of Bwari General Hospital also gave approval. Due to the sensitive nature of the subject, the purpose of the study was explained to the patients involved and confidentially as assured. They were assured of the anonymity of their identity and the confidentially of their responses. Participants were assured that participation in the study was voluntary and they were free to withdraw at any point in the study.

COLLECTION OF SAMPLES

Patients were instructed to wash their hands, penis and scrotum with soap and water before ejaculation to avoid possible contamination from urine and external genitalia. Samples were then collected by masturbation (non coitus) after 3-5 days of sexual abstinence and fast from antibiotics. These samples will be collected in labelled sterile containers properly closed and kept at body temperature ($37^{o}C$) preferably in their pockets and report to the laboratory on or before 15 minutes of production.

METHODOLOGY

A loopful of semen samples was collected immediately and inoculated onto blood, chocolate and MacConkey agar with the aid of a wire loop for microbiological examination while the remainder was allowed to liquefy at $37^{o}C$ for thirty minutes and was examined for the following;

APPEARANCE

Semen samples were examined immediately after liquefaction. Normal semen is usually thick and viscous when ejaculated but become liquefied usually within 20 minutes due to a fibrinolysin in the fluid. A normal sample has homogenous gray opalescent appearance. It may appear less opaque if the sperm concentration is very low or brown when red blood cells are present.

VOLUME

The volume of was measured by decanting the whole sample aseptically into a graduated centrifuge tube and level was recorded in ml.

Normal volume = 2ml [9].

SEMEN VISCOSITY

The viscosity of the sample was determined with the aid of Pasteur pipette. A drop of semen was allowed to fall back to the sample and the length of the thread was observed. A normal sample leaves the pipette as small discrete drops while in abnormal case, the drop forms a thread greater than 2cm long [9].

WBC (LEUKOCYTES)

A drop of each semen samples was placed on a clean glass slid, covered with cover slip and examined microscopically using 40x objectives for the presence of white blood cells [9].

SEMEN MOTILITY

A drop (10-15ul) of well mixed liquefied semen was placed on a slide and covered with a 20-20mm cover slip. It was then viewed under the microscope using 40x objectives. The microscopic field was scanned systemically and the motility of each spermatozoa encountered was graded a, b, c and d (WHO 2010). Where a = Rapid progressive motility, b = slow or sluggish motility, c = non progressive motility, d = immobility.

PERFORMING A SPERM COUNT

Each semen sample was diluted in replicate 1:20 in a solution of sodium bicarbonate formaldehyde and filled in an improved neubauer counting chamber. The 1ml square at the four corners of the ruled area was counted and the number of spermatozoa per ml was counted using 40x objectives. Only intact spermatozoa with head and tail were counted.

The number of spermatozoa per ml of semen was calculated as;

= number of cells counted x 100,000

Where dilution factor is 20 and the dept is 0.1
Normal sperm count = 20x106 spermatozoa/ml or more [9].

SEMEN CULTURE

Semen samples will be inoculated using a wire loop on sterile blood agar, Chocolate and MacConkey agar plate and incubated for 24hrs at 37o C in normal air with 5% CO_2.
Each plate was examined for evidence of growth and the isolates (microorganism) identified by standard biochemical test.

IDENTIFICATION OF THE ORGANISMS

The morphological characteristics of the growth on blood, MacConkey or nutrient media were observed for the following characteristics;
I.Elevationi.e raised or depressed
II. Colour
III. Edge of colonies i.e. smooth or rough
IV. Size of colony i.e. tiny, moderate or large
V. Colony consistency i.e. specifying whether it swarms

GRAM STAINING

A smear of the organism was made on a clean, grease free slide by using a sterilized wire loop to pick a colony from the culture media and emulsified in a drop of normal saline on a slide. The smear was then heat fixed through the bunsenburner flame and placed on a staining rack in preparation for staining. Methyl violet, lugol's iodine, acetone and safranin stains were used for the staining accordingly. The smear was stained first with the primary stain (methyl violet) for about 30 sec., poured off and again lugol's iodine was added for 30 sec, washed off with distilled water and decolorize with acetone for another 30 sec. This was followed by counter staining with the secondary stain safranin for 60 sec, washed off and allow to dry. This was examined microscopically using 100x objectives with oil emersion. On observation, those found to retain the primary stain colour (violet) were confirmed as gram positive and those with pink colour as gram negative.

CATALASE TEST

Here a drop of hydrogen peroxide was placed on a clean grease free slide and a colony of the test organism was immersed and observations was made for the appearance of bubbles.
A positive result indicated by the appearance of bubbles that is catalase positive, which is implicative of staphylococcus spp. However, absence of bubbles that is catalase negative is seen with streptococcus spp.

MOLECULAR ANALYSIS

DNA Extraction, Gel Electrophoresis and Sequencing Procedure for Isolates from Semen culture.

Genomic DNA was extracted from the culture received using the Quick DNA™ fungal/bacterial Miniperp kit (symo, research, catalogue No D6005). The 16S target region was Amplified using onetaq® quick -load 2x master mix.(NEB, catalogue No M0486) with the primer presented in table 1. The PCR product were run on gel and gel extracted with the zymoclean™Gel DNA recovery kit (zymoresearch, catalogue No D4001). The extracted fragments were sequenced in forward and reverse direction (Nimagen.Brilliant Dye™ Terminator cycle sequence kit V3.1, BRD3100/1000 and purified (zymo research zR-96 DNA sequence clean up kit catalogue No. D4050). The purified fragments were analyzed on the ABI 3500x Genetic Analyzer (Applied Biosystems, Thermofisher scientific) for reaction for every sample. CLC bio main work bench v7.6 was used to analyze the ab1file generated by the AB3500XL genetic analyzer and result were obtained by a blast search (NCB1).

Table 1: The primers sequence.

16S -27F- represents forward primer
16S- 1492R- represent the backward primer

Name of primer	Target	Sequence 5'to 3'
16S -27F	16S rDNA Sequence	AGAGTTGATCMTGGCTCAG
16S- 1492R	16S rDNA Sequence	CGGTTACCTTGTTACGACTT

STATISTICAL ANALYSIS

The researcher adapts simple percentage techniques method (%).
In analyzing the data which will facilitate easy understanding of the findings. The percentage scores in each research question will be calculated based on the number of respondents.
Percentage formula=percentage.
%=F/N*100/1.
Where F =Total number of frequencies.
N=Total number of respondents.

Semen analysis and culture was carried out for 50 men who visited the hospital laboratory. Table 1 shows that out of the 50 semen samples collected, 70%(35) was found to have normal colour of greyish white fluid while the remaining 30% was found to have abnormal colour in the following order 12%(6), 4%(2), 14%(7) for brownish, bloody and yellowish colours respectively. Eighty two percent (82%) of the male with normal semen colour were found to have normal cell morphology. The prevalence of abnormal white blood cells (WBCs), oligospermia and Azospermia was higher in semen samples with abnormal colour ranging
100%. Table 2 shows 37% (30/42) active progression for semen samples with normal liquefaction after 30 minutes, 50%(1/2) and 33%(2/6) respectively for threadlike and watery semen samples respectively. Table 3 represents the isolates which was initially reported as *Staphylococcus aureus, Escherichia coli* and *Proteus species* after the normal gram staining and biochemical test were molecularly identified as *Enterococcus faecalis*,*Lysinibacillus macrolides* and *Bacillus fusiformis* with GenBank Accession number FJ378657.2, KX129780.1 and AY548954.1 respectively.
The demographical study of the population shows that the male patients between the ages of 21-30 years and farmers have a higher prevalence of bacterial infection of semen ranging 47 and 41% respectively. The overall prevalence of infertility cases observed among the study population is 12%(6/50), it is distributed in the following ascending order; 0%, 10%, 13%, and 17% for students, Civil servants, Business men and Farmers respectively. The overall prevalence of infected cases was seen to be 38%(19/50), demographically distributed among occupation of the study population in the following order; 2%, 3%, 6% and 8% for students, Civil servants, Business men and Farmers respectively. The blast results correspond to the similarity between the sequence queried and the biological sequences within the NCBI database.

Table 1: Showing the Colour, Volume and Microscopy of the Semen samples

S/N	Sample color	No.of Samples	%of Samples	Volume	% Oligospermia	% Azospermia	%Normozoospermia	%Dead cells	WBC >5/HPF in %
1.	Grayish white Fluid	35	70	3.0	2/35(6)	1/35(3)	29/35(82)	3/35(9)	15/35 (43%)
2.	Brownish Fluid	6	12	1.2	1/6(17)	2/6(33)	1/6(17)	2/6(33)	6/6(100)
3.	Bloody Fluid	2	4	1.0	0/2(0)	1/2(50)	0/2(0)	1/2(50)	0/2(0)
4.	Yellowish Fluid	7	14	1.1	4/7(57)	3/7(43)	0/7(0)	0/7(0)	7/7(100)

Table 2: Showing the Consistency, Motility and Sperm count of the Semen samples

S/N		At collection	Liquefaction after 30min (%)	AP A50%	SP B15(%)	NM (%)	Above 20million cells (NC)	Below 20million cells (OC)	No cells Azospermia
1.	Viscous	42/50(84)	40/42(95)	30/42(71)	33/42(79)	2/42(5)	30/42(72)	11/42(26)	1/42(2)
2.	Thread-like	2/50(4)	0/2(0)	0/2(0)	1/2(50)	1/2(50)	0/2(0)	1/2(50)	1/2(50)
3.	Watery	6/50(12)	0/6(0)	1/6(16)	4/6(67)	2/6(33)	1/6(12)	2/6(33)	3/6(50)

KEY
AP = Active progression; A50% = Above 50%; SP = Sluggish progression
B15% = Below 15%; NM = non-Motile; (NC)= Normal cells
(OC) = Oligospermia; WBC = White Blood Cell

Table 3: Showing the Bacteria Isolate and Blast result from the Semen Culture Blast Result

S/N	Isolates	Initial Identification	Percentage ID	Molecular Identification	GenBank Accession
1	Isolate 1	*Staphylococcus aureus*	83.92%	*Enterococcus faecalis*	FJ378657.2
2	Isolate 2	*Escherichia coli*	98.53%	*Lysinibacillusmacroides*	KX129780.1
3	Isolate 3	*Proteus species*	84.32%	*Bacillus fusiformis*	AY548954.1

Table 4: Demographic distribution of Bacterial Infection of semen among different occupation

S/n	Occupation	Number of Infected cases	% Overall prevalence	% Infertility cases
1	Student	2/7	10(2/19)	0(0/7)
2	Civil servant	3/10	16(3/19)	10(2/10)
3	Business men	6/15	32(6/19)	13(2/15)
4	Farmers	8/18	42(8/19)	17(3/18)
n=50	Total	19/50	38(19/50)	12(6/50)

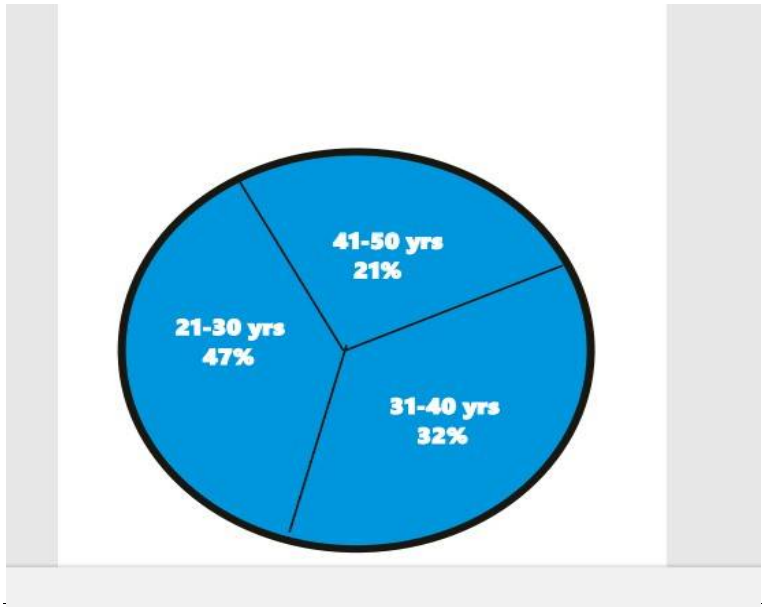

Figure 1: Demographic distribution of Infection among different age group.
Among age group 47% is the highest between young people while 21 % is lowest between old people.

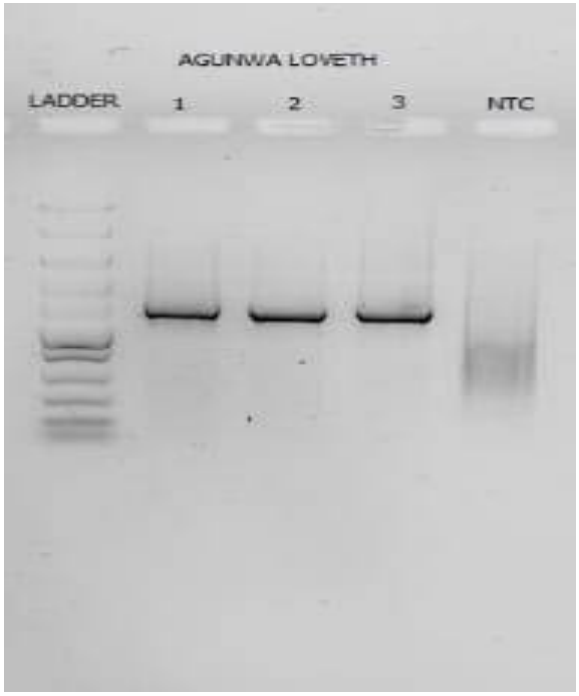

Plate 1: GEL ELECTROPHORESIS RESULT OF SEMEN CULTURE ISOLATES.

FINAL SEQUENCE.

>No 1. ***Staphaureus – Enterococcus feaecalis*****.**

AGAGTTTGTTCATGGTTCAGGATCGACCACGGCCGGCGTGCTTAATTCTTGCAAGTTCGATGGCTTCTGTCCTCC
TGGGTTGCACTCAACTCAATAGAGTAGTGCGGTCGGGGTGAGTAACaCGTGGGTAACCTTGCCCATCAGAGAGGG
ATAACGGGAACCCGGGTCAGAATGCGGCATAGCATCTTATTTGGCAGGGCATAAACAGTGAAAGGCGCTTTCGTC
TGTCGCTTTTGACACCCGCGGACCATTATATAGTAGGGACGGTAGGGGCTCACCAAGGCCGGAGAGGTTAGCTGT
CGTGAAGGGGTGATCGGCCACGCGGGGTGAGAGACTGGTCCCAGACTCTTAAGGGAGGCAACAGTAGGGAATCTT
CGGCAAGGTAGCAAAGTTTAATTGAGCACCACGGCGGAAGTCAAGAAGCTTTTAGGATGGTAAAATTTGTTGTT
AGAGAAGACCAAAAATTTTAGTATATGTTAGTCCCTTGACGGTATACTTACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGCTGCGCAGGCGGTGTA
TTAAGTCGGAATTGTGAAAACCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGCCTTGAGTGCAGAAGA
GGAGAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAATTCGGAGGAACACCAGTGGCGAAGGCGGCTGTCTG
GTCTGTAACTGACGCTGAGGCTGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGA

>No 2. ***E.coli – Lysinibacillus macrolides*****.**

TTAGAGTTTTGAATCATGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGAGAAG
GAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTACCCTATAGTTTGGGATAACT
CCGGGAAACCGGGGCTAATACCGAATAATCTATGTCACCTCATGGTGCACATACTGAAAGACGGTTTCGGCTGTC
GCTATAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGAC
CTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACATCCTACGGGAGGCAGCAGTAGGGAATCTT
CCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTA
AGGGAAGCAACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCT
TTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTTGAGTGCAGAAGAG
GAAAGTGGAATTCGCAAGTGTAGCGGTGAAATGCGTAGAGATTGTGGAGGAACACGCAGTGGCGAAGGCGACTT
TCTGGTCTGTACACACTGACCGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGTCGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCC
CG

>No. 3 ***Proteus – Bacillus fusiformis.***

AGAGTTTGTTCATGGTTCAGCAcGAACTCAGAcGACGTGCcTaATACATGCAAGTCGAGCGAACAGAAAAGGAGcT
TGCTCCTTTgaCGTTATCGGcGGACGAGGTGAGTgACGcGTGAGCAACgTACCgTATAGTTTGGGATGacTCCGGGA
AACCGGGGAAATGCTAGAATAATCTCTTATAaTTCATGAAGAcAcAATGGAAGaCGGTTTcGGCtGTcgctATAGGA
AGGTCCCCcGAcGCATTAAcTAGTTGGtGAGGTAACGGcTCacCAAGGCGacGAATCGTAACCTGCTTGAGAGGGTg
ATCCACCCCCcTGGGAcTGAgaCaCGGcCCCgacTGTGTGTGTCTCTACGGGAGGCAGCAGTAGGGAATTTTCCACA
ATGGGCGAAAGCCTGATGGAACCCATCCGCGTGAGTGAAGAAAGTTTTTGGATTGTAAAAGTATATTGTAAGGG
AAGGACAAGTACAGTAGTAATTGCCTGTACCTTGACGGTTCCTTATTAAAAAACCACGGCTAAGTTCGTGCCAAC
CACCGCGGTTAATCGTAGGGGGCCAACGTTTTCTGGAATTATTGGGCGTAAAACGCCCGCCGGCGGTTCTTTCAA
TTTGAAGGGAAAATCCCCGGATCCACCGTGGAGGGTCCATGGAAACTGGGCGGCTTGAGTTCAGAAGAGGGAAG
GGGAATTTCCAGGGGAACGGGGAAAAGCGTAGGGATTTGGAGGGACACCCGTGGCGAAGGCCGCCCCCTGGTCCG
TAACTGACCCTGAGGCTGAAGTAACGTTGGGAGCAACAGGGATTAGATTCCCTGGTAGTTCCCGCCGTAAACGAT
GAGTGAAAGTAGAGGTGCCTCCACCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCCTG

DISCUSSION

This study shows that the overall prevalence of bacterial infection in semen sample among men who visited the Tertiary Hospital of Bwari, North-Central Abuja is 38%; 12% of the infected males were experiencing infertility cases. The result of this study shows a lower prevalence when compared to the previous studies by some researchers [17; 20;21] who reported 80.3% infected rate atIfe in Osun state, 83.7% in Ebonyi South East Nigeria, and an infection rate of 69.9% in Lagos. Higher infection rate was also recorded in Mexico South Americaan infection rate of 66% [22].

Infected Semen samples with abnormal appearance and consistency has higher prevalence of Oligospermia and Azospermia, whereas semen without bacterial infection, normal appearance, consistency and normal white blood cell count were found to be normospermia. However, statistics from [23] indicates that the rate of bacterial infection in semen was significantly low in other African countries such as Burkina Faso (14.8%) Algeria (45.8%) Ethiopia (5.6%) Kenya (19%). The differences in the rate of pathogen observe in this study and affirmation studies could be due to increase in disease resistant to

antibiotics increase in harmful environmental exposure and radiation, Irregular lifestyle addiction to alcohol and smoking.

The isolates *Enterococcus faecalis*, *Lysinibacillus macrolides* and *Bacillus fusiformis* with GenBank Accession number FJ378657.2, KX129780.1 and AY548954.1 respectively were mistaken for *Staphylococcus aureus, Escherichia coli* and *Proteus species respectively.* This result differs from the findings of [24] who recorded Escherichia coli, Staphylococcus species and Proteus mirabilis to be the predominant isolates in semen culture.

This study has revealed *Enterococcus faecalis* to be associated with semen infection. This organism was previously known as an opportunistic pathogen associated with nosocomial infection, endodontic infection and among the gut microbiota [25; 26; 27]. *Lysinibacillus macrolides* has been previously discovered to be a promising endophytic bacterium, vaginal microbiota, soil dwelling human pathgogen[28; 29;30]. In this study, *Lysinibacillus macrolides* has been discovered to be associated with semen pathogen. *Bacillus fusiformis* has been observed in ulcero-membranous angina, hospital gangrene, noma, appendicitis, diphtheria, foetid bronchitis, gangrenous laryngitis, pyorrhea alveolaris, brain abscess and in healthy mouth. Recently, it was observed to have antifungal activity against different fungi [31]. *Bacillus fusiformis* isolated in this study, was found in the semen. Semen sample is meant to be sterile, any bacteria isolated from a semen sample aseptically collected in a sterile bottle is an infection. The fact that *Enterococcus faecalis*, *Lysinibacillus macrolides* and *Bacillus fusiformis* were isolated for the first time in these semen sample means that they are opportunistic pathogen. This is subject to review.

In the distribution of bacterial infection among age group recorded among the study population,age group 21-30 has the highest prevalence rate of infection of 47%; age group 41-50 shows the least prevalence rate of infection which is 21%. This means that the spread of these bacteria is more common among the sexually active age group.This agrees with the findings of [17 and 18] from southeast Nigeria that the middle age group (sexually active males) are more prone to sexually transmitted infection than other age group.

Farmers in the study area were discovered to have the highest prevalence rate of bacterial infection which is 42%. Other occupation showed less prevalence rate of infection. This agrees with the findings of[21] who recorded 26% in Lagos. This shows that the level of education, exposure, poor personal hygiene and lack of knowledge on the use of contraceptive could be the major predisposing factor to contacting infections. This could lead to fertility disorder. A low prevalence rate was recorded among civil servant (16%) which agrees with the findings of Ikechebelu [32].

CONCLUSION

Based on the findings of this research, the prevailing bacterial isolates in the study area are *Enterococcus faecalis*,*Lysinibacillus macrolides* and *Bacillus fusiformis.* This is the first time these organism a are associated with semen infection. The level of pathogenic bacteria in semen sample among males was high. The distribution of contaminating species among different age group indicated that *Lysinibacillus macroides* was the most prevalent and commonly occurring etiological agent between younger males(21-30yrs) while *Bacillus fusiformis* was the least prevalent among older males were the least infected among all age group in the research area. The distribution of Bacteria species across different occupation indicated that farmers have the highest distribution of pathogen as compare to civil servant who showed the lowest distribution. Bacterial infection indicated a decrease in sperm quality which is responsible for reduction of sperm motility sperm morphology, testicular damage and induction of decapitation in spermatozoa.

RECOMMENDATIONS

In order to curb the menace of bacterial infection and reduction in sperm quality which affect reproductive health in males, National health program should emphasis early diagnosis and treatment of genital tract infection since long-term infection may result to fertility disorder. Individuals should avoid multiple sexual partners and exposure to conditions which pre-expose them to infections. More qualitative research needs to be done in the field of gender difference to ascertain the genuine cause of infertility and adequate control measures. Personal hygiene and environmental sanitation should be encouraged. Routine medical check-up is highly recommended for men as most men rarely show symptoms to early infection.

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