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***Chlamydia trachomatis* Antibodies and Other Bacterial Isolates Associated with Adult Males Attending Infertility Clinics in Calabar, Nigeria**Idiong, V.M.¹, Idiong, M.E.², Ibeneme, E.O.^{3,4*}, Epoke, J.³, Utsalo, S.J.³

Department of Medical Microbiology and Parasitology, University of Calabar Teaching Hospital, Calabar-Nigeria¹, Department of Obstetrics and Gynaecology, University of Calabar Teaching Hospital, Calabar-Nigeria², Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, University of Calabar, Calabar-Nigeria³, Dr. Lawrence Henshaw Memorial Specialist Hospital Reference Laboratory, Calabar, Nigeria⁴

Author for Correspondence*: +234-803-372-5219 / ibeneme.eo@gmail.com

Abstract

Male infertility cases are a major concern in infertility clinics. This study sought to determine the seroprevalence of *Chlamydia trachomatis* antibodies (CTA) and isolate bacterial agents associated with men attending infertility clinics in Calabar. Blood and semen samples were obtained from 172 men between 27 and 46 years of age. The prevalence of CTA was investigated using the rapid screening kits – immuno-comb and Bivalent *Chlamydia trachomatis* IgG kits. Semen samples were immediately analyzed and cultured on blood agar (BA), chocolate agar (CHOC) and cysteine lactose electrolyte-deficient agar (CLED). The BA and CHOC culture plates were incubated anaerobically in 5% CO₂, and CLED plate aerobically, at 37°C for 24 to 48 hours. Bacterial isolates were identified with standard biochemical tests. Out of 172 participants examined, 134 (77.9%) were primary infertility cases, 38 (22.1%) were secondary infertility cases, while 78 (45.3%) participants had oligospermia. Overall prevalence of *Chlamydia trachomatis* infection (CTI) was 13% (22/172), while 52 (30%) had microbial growth from the culture of their semen. Out of 78 oligospermic individuals, 14 (17.9%) had significant antibody titre (signifying CTI); only 8.5% (8/94) of the normospermia individuals had significant antibody titre, although this difference was not statistically significant ($p>0.05$). Out of the participants with CTI, 6 (4.5%) were primary infertility cases, while 16 (42.1%) were secondary infertility cases; 63.6% (14/22) were oligospermic, while 36.4% (8/22) were normospermic. Microbial growth was detected

in the semen culture of 52 (30%) of all participants in the study; 50% of which was *Staphylococcus aureus*, and the least was *Neisseria gonorrhoeae* (4%). Most of the participants with primary (70.1%), secondary infertility (68.4%) and oligospermia (74.4%) did not have microbial growth from their semen culture. Serological tests to detect *Chlamydia trachomatis* antibodies need to be routinely carried out on intending fathers so that prompt and proper treatment could commence.

Keywords: Oligospermia, bacterial infections, *Chlamydia trachomatis*, antibodies, male infertility, Calabar.

Introduction

Infertility, although not life-threatening, could radically transform a person's life and lead to significant psychological trauma (Uadia and Emokpae, 2015). According to World Health Organization (WHO), infertility is the inability of couples to achieve conception despite regular unprotected sexual intercourse for one year in the absence of knowledge of reproductive pathology; in males, it refers to the inability of a man to impregnate a woman after 12 months regular unprotected sexual intercourse in the absence of knowledge of reproductive pathology (Elhoussein *et al.*, 2019; Uadia and Emokpae, 2015).

In 1991, WHO estimated that about 8-12% of couples worldwide experience some form of infertility during their reproductive years, which affects 50-80 million couples, with 20-35 million living in Africa and 3-4 million living in Nigeria (Uadia and Emokpae, 2015). A more recent

estimate put infertility at 15% of couples worldwide. Of all infertility cases among couples, half of the reproductive problems are caused by the male counterpart (Kim *et al.*, 2012). In the past, socio-cultural beliefs in Africa have enabled blame of fertility problems on the female gender; even though such blames are shared by both male and female genders (Umar *et al.*, 2020). Male-factor is responsible for 40–50% of all infertility cases in Nigeria, although it varies with different regions (Emokpae *et al.*, 2005; Idrisa *et al.*, 2003; Ikechebelu *et al.*, 2003).

Sexually transmitted diseases are of critical concern to clinicians and fertility experts in the field of reproductive health and medicine. Estimates reveal that 15% of male infertility is as a result of genital tract infection (Sellami *et al.*, 2014). Males of reproductive age who reported having repeated episodes of penile discharge, painful urination, genital ulcers and testicular pain were more likely to be infertile (Uadia and Emokpae, 2015). Men are capable of harbouring sub-clinical infections in their genital tract over a long period of time and many pathogens which cause sexually-transmitted infection, such as *C. trachomatis* have been detected in semen samples of asymptomatic men (Sellami *et al.*, 2014). The World Health Organization (WHO) reported that *C. trachomatis* infection is the most common sexually-transmitted bacterial infection worldwide, and affects more than 90 million people (Samplaski *et al.*, 2014; Sellami *et al.*, 2014): its role in male infertility is controversial with some authors associating it with poor semen quality (Sellami *et al.*, 2014; Gdoura *et al.*, 2001) and others claiming no association (Gdoura *et al.*, 2007; De Barbeyrac *et al.*, 2006; Motrich *et al.*, 2006). Some studies reveal a relationship between *C. trachomatis* infection and decreased sperm concentration, motility, altered pH and reduction of volume of the ejaculate (Pajovic *et al.*, 2013; La Vignera *et al.*, 2011; Mazzoli *et al.*, 2010).

Other bacteria which have been isolated from semen samples in infertility cases include *Mycoplasma* species and *Ureaplasma* species (Sellami *et al.*, 2014; Al-Marzoqi *et al.*, 2012), *Neisseria gonorrhoeae* (Sellami *et al.*, 2014; Olajubu *et al.*, 2013; Al-Marzoqi *et al.*, 2012; Wazir *et al.*, 2010), *Enterococcus* species (Al-Saadi and Abd, 2019; Vilvanathan *et al.*, 2015),

Staphylococcus species, *Streptococcus* species and *Pseudomonas* species (Nwadioha *et al.*, 2016; Omosigho *et al.*, 2015; Olajubu *et al.*, 2013), Enterobacteriales (Vilvanathan *et al.*, 2015; Al-Marzoqi *et al.*, 2012; Wazir *et al.*, 2010; Kosamat and Adebolu, 2005), and *Candida* spp (Nwadioha *et al.*, 2016; Olajubu *et al.*, 2013; Al-Marzoqi *et al.*, 2012). Fertility-grade sperm production often requires a healthy urogenital tract; microbial infections most often lead to inflammatory processes in the genital tract, which may lead to male infertility, although the mechanisms remain poorly understood (Stojanov *et al.*, 2018). This study sought to establish a relationship between bacterial infection and male infertility in men attending infertility clinics in Calabar, Nigeria.

Materials and methods

Study area

This study was carried out in both government and private hospitals and laboratories in Calabar Municipality. These included the Gynaecology Clinic and Surgical Out-Patient Department (SOPD) of the University of Calabar Teaching Hospital, Victoria Itam Clinic as well as Meditech Laboratories, Alfa Laboratories, NOSAM Laboratories, SUFAT Laboratories, all located in Calabar Metropolis.

Study subjects

This study was carried out on 172 adult males aged 29 to 45 years with infertility problems and who were attending urology and gynaecology clinics, SOPD and the above-mentioned laboratories for investigations. The consent of each of the participants was sought before investigations and prior to sampling. Ethical clearance for this study was obtained from the Cross River State Medical Research Ethics Committee and University of Calabar Teaching Hospital Ethical Committee.

Collection of samples

Semen and blood samples were collected from patients in the clinics and laboratories and immediately brought to the laboratory for analyses and culture with minimal delay. Blood samples were used to screen for the presence of *Chlamydia trachomatis* antibodies. Approximately two milliliters of venous blood was collected from each participant, observing all aseptic measures. The collected blood sample was transferred into clean

dry test tube for analysis. The blood was allowed to stand at room temperature for about 20 minutes to clot before centrifuging at 3,000 revolutions per minute for 5 minutes. After centrifugation, the serum was separated into clean sample bottles using a clean Pasteur pipette. The serum was then stored at 4°C for not more than seven days if immediate analysis was not possible. If storage was to exceed seven days, the serum was frozen at -20°C. For analysis, the serum was thawed, centrifuged and the supernatant used for analysis.

Laboratory screening for *Chlamydia trachomatis* antibodies

Screening for *Chlamydia trachomatis* antibodies was conducted using the rapid screening kit – Immunocomb *Chlamydia trachomatis* Bivalent IgG kit (ORGENICS Inverness Medical Professional Diagnostics, Yavne, Israel) according to the manufacturer's instructions. Participants whose samples showed positive test results were noted to have significant *C. trachomatis* antibodies.

Semen analysis and culture

Semen analysis was carried out on all the study participants. Participants were given clean, dry, leak proof universal containers and requested to collect semen through masturbation after three to four days of sexual abstinence. Semen specimens were examined immediately on arrival. The volume and the consistency of the samples were measured. The percentages of motile, sluggish and dead cells were evaluated by placing one drop of well-mixed liquefied semen on a slide and covered with a cover slip. The sample was focused using X10 objective first and then X40 objectives was used to examine several fields to assess motility. This was done to counting 100 spermatozoa and noting, how many were actively or sluggishly motile and non-motile, out of 100. The percentages were recorded.

Sperm count was performed by diluting the semen 1:20 with sodium bicarbonate formalin diluting fluid and the mixture well mixed. A Pasteur pipette was then used to fill an Improved Neubauer Ruled counting chamber (Weber Scientific Ltd., Sussex, England) with well-mixed diluted semen and then allowed for three

to five minutes for spermatozoa to settle. The X100 objective lens was used for counting. The condenser iris was sufficiently closed to give good contrast. The number of spermatozoa in an area of two square millimeters, i.e. four large squares, was counted. The number of sperm cells in one milliliter of fluid was calculated by multiplying the number counted by 50,000 or 100,000, when 2 large areas were counted; this gives the number of sperm cells/mL and categorization of fertility status of each semen sample (WHO, 2010).

Semen samples were cultured on blood agar (BA), chocolate agar (CHOC) and cysteine-lactose electrolyte deficient (CLED) agar. This was achieved using sterile wire loop. Each semen sample was mixed well and a loopful was transferred to each plate and streaked. Blood and chocolate agar plates were incubated aerobically using canister and candle to generate 5% CO₂ needed by some bacteria to grow. Cysteine-lactose electrolyte deficient agar was incubated aerobically for 24 to 48 hours, if there was no bacterial growth during the first 24 hours.

Identification of bacterial organisms

Identification of organisms was not based on one test, alone, but on a composite picture. Attention was paid to their appearance, which includes shape, size, consistency, colour, pigmentation, opacity, elevation, haemolysis on blood agar and lactose fermenting ability on CLED. In addition, biochemical tests were carried out specifically to identify a particular organism.

Analysis of data

The data obtained during the course of the study were analyzed using descriptive statistics to evaluate the role of *Chlamydia trachomatis* infections in oligospermia, to compare the chlamydial antibody titres of oligospermic individuals with normal sperm count (normospermic) individuals and to evaluate the role of bacterial infections in oligospermia. The results were also analyzed statistically using chi-square technique to compare the level of *Chlamydia* antibody titres of oligospermic men with those of non-oligospermic men.

Results

Table 1: Comparison of normospermia and oligospermia among participants across age groups

Age	No. Examined (n = 172)	No. (%) of Normospermic participants (n = 94)	No. (%) of Oligospermic participants (n = 78)
21-30	26	18 (19.1)	8 (30.8)
31-40	112	58 (61.7)	54 (48.2)
41-50	34	18 (19.1)	16 (47.1)
Total	172	94 (54.7)	78 (45.3)

Overall prevalence of oligospermia in the study participants was 45.3% (78/172), while normospermic individuals made up 54.7% (94/172) of all study participants. The prevalence of normospermia and oligospermia among participants across age groups (Table 1) showed that the age group 31-40 years had the highest number of normospermic individuals (61.7%). The lowest rate of normospermia (19.1%) occurred in individuals between the ages of 31 and 40, and 41 and 50 years of age. Oligospermia was more prevalent in participants between the ages of 31 and 40 years (48.2%) and was found to be lowest in individuals between the ages of 21 and 30 (30.8%).

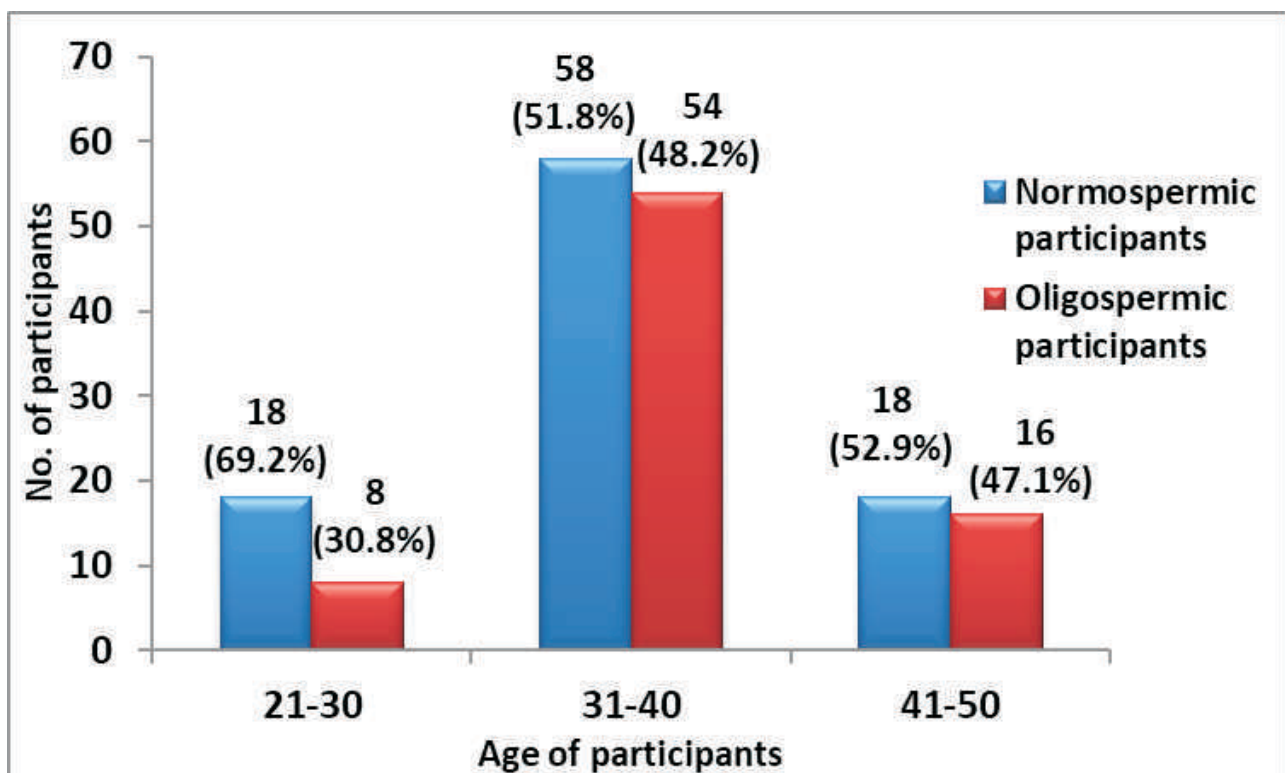


Figure 1: Comparison of normospermia and oligospermia among participants within age groups

The prevalence of normospermia and oligospermia based on age of participants (Figure 1) showed that in the age group 21-30, the prevalence of normospermia (18/26; 69.2%) was far higher than oligospermia (8/26; 30.8%); the difference between prevalence of normospermia and oligospermia among this age group was statistically significant ($p < 0.05$). The lowest in difference between prevalence of normospermia and oligospermia among the three age groups was in 31-40 (normospermia – 51.8%; oligospermia – 48.2%).

Table 2: Degree of infertility and oligospermia among participants in relation to age

Age	No. (%) Examined (n = 172)	Degree of infertility		No. (%) of Oligospermic participants (n = 78)
		No. (%) of participants with primary infertility (n = 134)	No. (%) of participants with secondary infertility (n = 38)	
21-30	26 (15.1)	26 (19.4)	0 (0)	8 (10.3)
31-40	112 (65.1)	84 (62.7)	28 (73.7)	54 (69.2)
41-50	34 (19.8)	24 (17.9)	10 (26.3)	16 (20.5)
Total	172	134 (77.9)	38 (22.1)	78 (45.3)

Overall prevalence of primary and secondary infertility was 77.9% (134/172) and 22.1% (38/172), respectively. The prevalence of primary infertility was highest in age group 31-40 (62.7%; 84/134) and almost similar in age groups 21-30 and 41-50 (19.4% and 17.9% respectively). Almost two out of three participants with secondary infertility were in the age group 31-40 (28/38; 73.7%). Fifty-four (69.2%) participants with oligospermia were in the age category 31-40; oligospermia was twice as prevalent in the age category, 41-50 (20.5%; 16/78), as in the age category, 31-40 (10.3%; 8/78) (Table 2).

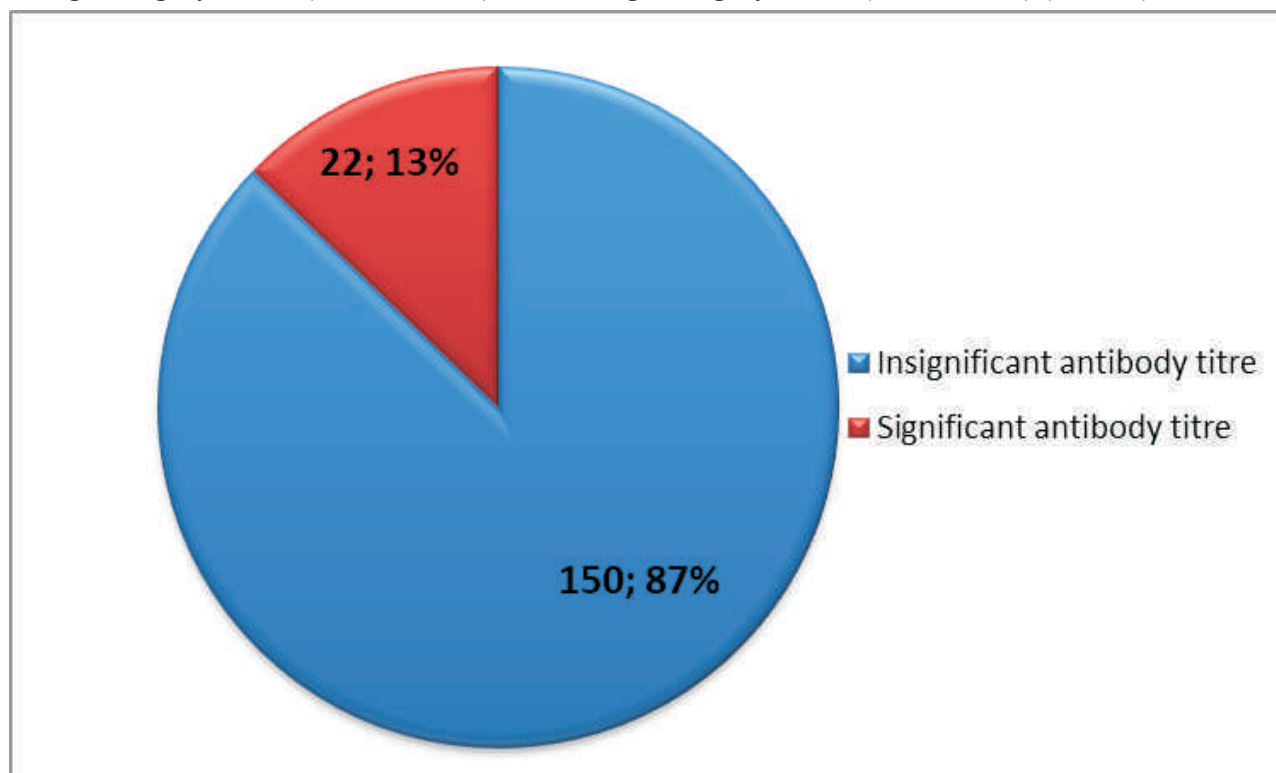


Figure 2: Seroprevalence of chlamydial antibodies in participants

The seroprevalence of chlamydial antibodies among participants studied showed that 22 out of 172 (13.0%) had significant chlamydial antibody titre (CAT), while 150 (87.2%) had insignificant CAT (Figure 2).

Table 3: Degree of infertility and oligospermia among participants in relation to chlamydial antibody titre

Chlamydial antibody titre	No. (%) Examined (n = 172)	Degree of infertility		No. (%) of Oligospermic participants (n = 78)
		No. (%) of participants with primary infertility (n = 134)	No. (%) of participants with secondary infertility (n = 38)	
Significant	22 (12.8)	6 (4.5)	16 (42.1)	14 (17.9)
Insignificant	150 (87.2)	128 (95.5)	22 (57.9)	64 (82.1)

The prevalence of primary infertility was higher in participants with insignificant CAT (95.5%; 128/134) than in participants with significant CAT (4.5%; 6/134). A higher percentage of participants with secondary infertility had insignificant CAT (57.9%; 22/38); 16 (42.1%) participants had significant CAT (Table 3).

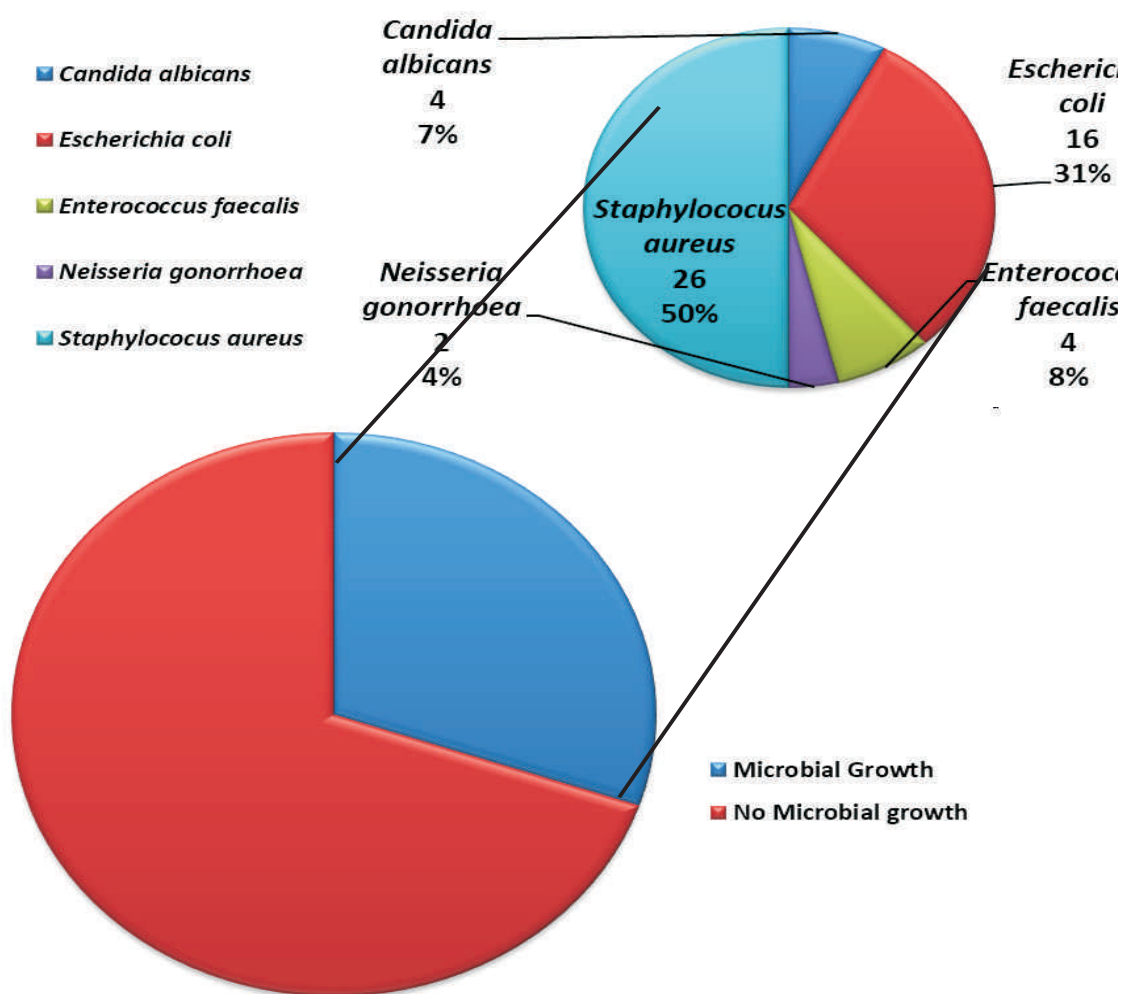


Figure 3: Microbial growth status and bacterial isolates from culture of semen from participants

Culture of semen from oligospermic and normospermic individuals revealed that of 172 semen samples, 52 (30%) had microbial growth, while 120 (70%) had no bacterial growth after 24 hours of incubation. Characterization of the bacterial agents isolated from studied participants revealed that of the 52 samples with bacterial growth, *Staphylococcus aureus* was the most prevalent isolate (26/52; 50%), followed by *Escherichia coli* (16/52; 30.8%). The least prevalent isolate was *Neisseria gonorrhoea* (2/52; 3.8%) (Figure 3).

Table 4: Degree of infertility and oligospermia among participants in relation to microbial growth status from semen samples

Semen microbial growth status	No. (%) Examined (n = 172)	Degree of infertility		No. (%) of Oligospermic participants (n = 78)
		No. (%) of participants with primary infertility (n = 134)	No. (%) of participants with secondary infertility (n = 38)	
Microbial growth	52 (30.2)	40 (29.9)	12 (31.6)	20 (25.6)
No microbial growth	120 (69.8)	94 (70.1)	26 (68.4)	58 (74.4)

The prevalence of primary infertility was higher in participants without microbial growth from culture of their semen (70.1%; 94/134) than in participants with microbial growth (29.9%; 40/134). A higher percentage of participants with secondary infertility had no microbial growth from culture of their semen (68.4%; 26/38); 12 (31.6%) participants with secondary infertility had microbial growth (Table 4).

Discussion

Among study participants, overall prevalence of oligospermia was 45.3%, and normospermia, 54.7%. Slightly higher rates of 51.5% and 85.1% were reported by Olajubu *et al.* (2013), in Sagamu, and Kosamat and Adebolu (2005), in South-West Nigeria, respectively. Similar findings of 45.3% were reported by Nwabuisi and Onile (2001), in Ilorin, 43% by Nwadioha *et al.* (2016), in Makurdi, 40.8% by Emokpae *et al.* (2009), in Kano, 42% by Al-Moushaly *et al.* (2013), in Romania, 43.5% by Vilvanathan *et al.* (2015), in India and 46.7% by Wazir *et al.* (2010), in Pakistan. Reasonably lower prevalence rates of 22.3% were reported by Umar *et al.* (2020), in Sokoto, 22.8% by Omo-Aghoja *et al.* (2017), in Benin, 28.6% by Omosigho *et al.* (2015), in Bida, Emokpae *et al.* (2007), in Kano, 13% by Gdoura *et al.* (2001), in Tunisia, and 15.9% by Elhussein *et al.* (2019), in Sudan. Oligospermia, a condition which may result from autoimmune natural sperm agglutinating antibodies in the serum of sub-fertile men which decreases the chances of fertilization (Uadia and Emokpae, 2015; Nagy *et al.*, 1989). Environmental toxins such as chemicals (from pesticides), air pollutants (such as sulphur dioxide, nitrogen dioxide, carbon monoxide) and toxic, heavy metals (such as lead, arsenic, mercury, aluminum, cadmium) may act in many stages in sperm development, causing abnormal sperm count, motility, and morphology, and consequently, oligospermia. Detrimental lifestyle habits (such as smoking, substance abuse), unhealthy nutrition, hormonal abnormalities, and genetic factors (such as production of anti-sperm

antibodies) could also give rise to oligospermia (Uadia and Emokpae, 2015).

Our study established the prevalence of chlamydial infection in 12.8% in males. This is a little lower in prevalence compared with rates of 15.2% and 19.5% in studies conducted by Sellami *et al.* (2014) in Tunisia, and Osazuwa *et al.* (2014) in Tanzania, respectively. Al-Moushaly (2013) reported a prevalence rate of 5% in Romania. Also, in this study, the number of participants with secondary infertility had significantly higher chlamydial antibody titre (CAT) than those with primary infertility.

C. trachomatis infection is the most prevalent sexually-transmitted infection in the world and commonly causes pathologies such as urethritis, epididymitis and prostatitis in men (Samplaski *et al.*, 2014). The primary site of infection is the penile urethra, and retrograde infection of the epididymis and testis, which follows the primary infection (Samplaski *et al.*, 2014; Emokpae *et al.*, 2005). In addition to the possible obstruction of the epididymis caused by chlamydial infection, the epididymis loses its crucial role in sperm maturation, negatively impacting sperm function (Samplaski *et al.*, 2014). Evidence from a recent study suggests that prostatitis caused by *Chlamydia*, had reduced sperm concentration, motility, and morphology and that a strong correlation exist between anti-*C. trachomatis* IgA and low sperm concentration (Mazzoli *et al.*, 2010). A study in Sweden involving 244 couples treated for infertility, reported that one in every five men tested positive for antibodies to

Chlamydia; a high number of the patients with antibodies had an ongoing infection (Uadia and Emokpae, 2015). Evidence from electron microscopy clearly demonstrate that *C. trachomatis* attach to spermatozoa (Samplaski *et al.*, 2014), both on the surface and in the nucleus (Vigil *et al.*, 2002); although, its role in male infertility is not clear. Co-infection with other microorganisms may further impair semen parameters (Samplaski *et al.*, 2014).

Thirty percent (52/176) of all semen specimen, in this study, yielded microorganisms as follows: *Staphylococcus aureus*, 50%; *Escherichia coli*, 30.8%; *Enterococcus faecalis*, 7.7%; *Candida albicans*, 7.7%, and *Neisseria gonorrhoea*, 3.8%. This is far higher than the microbial prevalence rates of 7% reported by Nwabuisi and Onile (2001), in Ilorin. Omosigho *et al.* (2015), Olajubu *et al.* (2013), Sellami *et al.* (2014) and Vilvanathan *et al.* (2015) reported slightly higher rates of 39.1%, 32.4%, 31.4%, and 35.3%, in Bida, Sagamu, Tunisia, and India, respectively. Far higher rates of 47%, 72.1%, 50.3% and 90% were reported in Makurdi (Nwadioha *et al.*, 2016), in Ogun State (Kosamat and Adebolu, 2005), in Pakistan (Wazir *et al.*, 2010) and in Iran (A-Saadi and Abd, 2019), respectively. Overwhelming evidence suggest that *Staphylococcus aureus*, followed by *Escherichia coli*, including *Candida* species, are the dominant microorganisms isolated from semen culture of infertile men (Nwadioha *et al.*, 2016; Kosamat and Adebolu, 2015; Olajubu *et al.*, 2013; Al-Marzoqi *et al.*, 2012; Emokpae *et al.*, 2009). Other studies also reported *Enterococcus* isolation from semen of infertile men (Al-Saadi and Abd, 2019; Vilvanathan *et al.*, 2015). Microbial infection of the seminal fluid has been observed to be the major cause of male infertility (Uadia and Emokpae, 2015). These infections of seminal fluid often led to a decrease in the number of sperm cells and the suppression of their capacity to result in pregnancy. Stephen *et al.* (1989) observed in earlier studies that the viability and structural integrity of the spermatozoa lie on its mobility. The deleterious effects of pathogenic micro-organisms on sperm cells' reproductive potential, in the form of deterioration of spermatogenesis, impairment of sperm functions, and obstruction of the seminal tract (Sanocka-Maciejewska *et al.*, 2005), have been documented. All five factors which contribute to overall sperm quality, such as

sperm motility, speed, density, morphology (shape and size) and liquefaction are all affected by STIs (Emokae *et al.*, 2005). Olajubu *et al.* (2013) commented that bacterial infection could be responsible for decline in motility of sperm cells and sperm density. Bacterial infection role may result in change in the chemical properties of semen, inflammation of the sperm ducts and impeding of sperm motility. All these lacks of or inadequate motility may result in agglutination (Orji *et al.*, 2007).

Cross-infection from multiple sex partners might play a role in some individuals having multiple microorganisms from culture of their semen. Sexual preferences may equally influence the nature and types of microorganisms isolated from semen. For instance, anal sex may result in the transmission of bacteria which are normal flora of the intestine (Onemu and Ibeh, 2001).

Previously, *Staphylococcus aureus* was regarded as a contaminant when isolated from seminal fluid, but has now gained acceptance as a pathogen to be reckoned with on the basis of its coagulase-producing capability (Uadia and Emokpae, 2015).

Conclusion

Findings from this study make it obvious that assessing semen quality remains one of the most important steps in infertility interventions. Male factor contributes significantly to infertility, but are easily treatable causes. A thorough investigation of the male partner at the initial clinic visit, followed by proper and adequate treatment, is vital to prompt assisted conception. Abnormal sperm indices in this study are high: bacterial infection is also significant with *Staphylococcus aureus* and *Escherichia coli* proving that non-traditional, infertility-associated bacteria should no longer be overlooked in the management of male-factor infertility.

Intentional steps should be taken to create an environment of awareness regarding the issue of male-factor infertility and male partners should also be educated to be involved in the infertility investigation process, so as to reduce the negative social impact on the female partners. Fertility-based interventions should be focused at prevention, since surgical treatment of infertility and Assisted Reproductive Techniques (ARTs) are above the reach of most couples in

sub-Saharan Africa. Therefore, microbiologic investigation for infertility at regular periodic intervals is advised in this sub-region.

Conflict of interest

The authors declare no conflict of interest.

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