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Assessment of microbiological quality of semen of male patients with infertility at Murtala Muhammad specialist hospital kano, Nigeria

Abstract

The study was aimed to assess microbiological quality of male patients with infertility at Murtala Muhammad specialist hospital Kano, Nigeria. Two hundred (200) Semen specimens were collected from males with infertility attending the clinic and General out Patient Department of MMSH Kano. The seminal fluids were diluted with sterile saline, centrifuged and cultured on Nutrient agar, Blood agar, Chocolate agar and MacConkey agar then incubated aerobically and in 5% CO_2 at 37°C for 24 hours for the isolation of pathogenic microorganism. Isolates were identified based on Gram's staining, biochemical tests and API 20E Test. The result shows that out of the 200 samples examined 76 (38%) were found to be infected with a total of seventy six (76) isolates. *Staphylococcus aureus* was found to have the highest occurrence of 31 (40.79%), whereas the least was found to be Mycoplasma species, 1(1.32%). Other microorganisms encountered include; Coagulase negative Staphylococcus species 14(18.42%), *Escherichia coli* 11(14.47%), *Klebsiella pneumoniae* 6(7.89%), *Proteus mirabilis* 5(6.58%), *Pseudomonas aeruginosa* 3(3.95%), *Neisseria gonorrhoeae* 2(2.63%) and Candida species 3(3.95%). The result also shows that patients within the age range of 30-39 years were most infected with 35(40.1%) infected out of 93 examined. The highest number of isolated microorganisms was observed in samples with concentration of 0-20 x106/ml. The semen motility rate was reduced significantly in samples with pathogenic microorganisms.

Keywords: infertility, kano, microorganisms, semen analysis

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Introduction

Infertility is the inability of a sexually active, non-contraception couple to achieve spontaneous pregnancy in one year. About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility.[1] One in eight couples encounters problems in attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Both sexes are more or less equally involved in infertility problem.[2] Men either alone or along with their female partners, contribute to 40–45% cases of infertility.[3] Furthermore, infectious aetiology involving bacteria, virus, fungi, and protozoa contribute to 15% of male factor infertility.[4] Some of the microbes are acquired exogenously or endogenously and they could cause infertility in several ways: by damaging sperm motility, deterioration of spermatogenesis, altering the chemical composition of the seminal fluid by dysfunction of accessory sex glands, or by inducing autoimmune processes due to inflammation.[5]

Microbial infections of the genital tracts or semen are major causes of male infertility.[6,7] According to World Health Organization,[1] semen consists of concentrated suspension of spermatozoa and the fluid secreted by the accessory sex organs namely prostate gland, seminal vesicles, bulbourethral glands, and epididymides. The fluid secretion is about 90% of semen volume and dilutes the concentrated epididymal spermatozoa at ejaculation. Since the ejaculate is a mixture of secretions derived from the urogenital tract and the male accessory glands, seminal culture identifies the presence of germs in any section of the seminal tract.[8]

Male urogenital tract infections are one of the most important causes of male infertility worldwide. Askienazy-Elnhar,[9] reported that genital tract infection and inflammation have been associated to 8-35% of male infertility cases and male accessory sex glands infection is a major risk factor in infertility.[4] Studies have also shown that when the characteristics of semen infected and uninfected were compared, semen with micro-organisms had poor indices of fertility.[10] Infertility affecting couples around the World is both a medical as well as social problem particularly in Nigeria.[11] Evidences are being accumulating on the association of asymptomatic bacteriospermia and altered semen quality.[12] There is difference as to the influence of certain microbial infection on male infertility.[13] Urinary tract infections are common in men and clinicians working with infertility frequently encounter patients with these diseases.[1] More than 90% of male infertility cases is due to either low sperm count (oligospermia), no sperm at all (azoospermia) or poor seminal fluid quality or combination of the two and this claimed to the increase prevalence of sexually transmitted diseases (STDs) and urogenital infections alarmed since 1992.[14] Previous studies have identified *Staphylococcus aureus*, *E. coli*, *Citrobacter species*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Proteus mirabilis* with infertility in male partners of infertile couples.[5,7,15] The study was aimed to assess microbiological quality of male patients with infertility at Murtala Muhammad specialist hospital Kano, Nigeria.

Materials and methods

Study area

The study was conducted at Microbiology Department of Murtala Muhammad Specialist Hospital (MMSH), Kano. Kano state is located

in the North-west Nigeria with coordinates 110 30 N 80 30 E. It shares borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the West and Niger republic to the North. It has a total area of 20,131km^2 (7,777sqm) and population of 11,058,300.[16]

Study population

A total of 200 samples were collected from male patients who had either primary or secondary infertility cases.

Inclusion criteria for patients

Male patients with any kind of infertility attending MMSH were involved in the study.

Exclusion criteria

a. Male patients who refused to observed at least three days of sexual abstinence prior to the test.

b. Patients who refused to suspend any chemo antibiotic treatment for at least one week prior to sample collection

Ethical clearance

An approval (MOH/off/797/T.I/49) for the study was obtained from Research and Ethic committee Kano state ministry of health. The aim of the study was explained clearly to the clients and informed consent obtained before proceeding to the study.

Determination of sample size

Sample size for the study was determined from a standard formula for the calculation of minimum sample size.[17] Sample size was given by the formula.

$$N = (Z1-a)^2 (p) (1-p) / d^2$$

N = minimum sample size.

Z1--a = value of standard normal deviate which at 95% confidence interval has found to be 1.96.

P = the best estimate of prevalence obtained from literature review (12.3%).

d = difference between the true population rate and sample that can be tolerated, this is the absolute precision (in percentage) on either side of the population.

N = (1.96)2 (0.123) (1-0.123)/(0.05)2 = 165.758 as the minimum number of samples for the study.

Therefore, a total of 165.759 with 20% (33.152) of this subject will be added to the research for attrition, making a total of approximately 200 samples.

Sample collection

Semen specimens were collected from males with infertility attending the clinic and GOPD of MMSH Kano. The samples were collected from patients who have had 3-7 days of sexual abstinence from intercourse preferably by masturbation into a sterile clean wide-mouth container. Upon collection, samples were transferred without any delay to the Microbiology Department of MMSH in a nearly as possible to body temperature by placing the container inside a flask containing water at 33-37˚C. Time of collection to the time the samples were received in the laboratory was recorded which must not exceed 45 minutes. Furthermore, analyses of samples with SQA machine were conducted at Microbiology Department, Aminu Kano teaching hospital (AKTH), Kano.

Sterile collection of semen for microbiological analysis

Microbiological contaminations from non semen sources were avoided from patients by counselling them on passing urine before ejaculation, washing hand and penis with soap and rinse away the soap, drying hands and penis with a fresh disposable towel and finally ejaculating into a given sterile container.

Culture

Culture of seminal fluid samples was done in aseptic condition within one hour of collection in accordance with WHO.[1] The seminal fluids were diluted with sterile saline (1:10) and centrifuged at1500RPM for 15min. After removing the supernatant, the sediments were cultured using 10µl calibrated loops on Nutrient agar, Blood agar, Chocolate agar and MacConkey agar which were incubated aerobically and in 5% CO_2 at 37˚C for 24 hours for the isolation of pathogenic microorganism.[1] Seminoculture were considered positive when the number of colonies was ≥104 CFU/ml for Gram positive cocci and ≥105 CFU/ml for Gram negative.[18] Sabouraud dextrose agar slants were used for the isolation of yeasts and yeast-like fungi after 48 hours aerobic incubation at 37oC. Mycoplasmal agar enriched with 30% serum and supplemented with 100µg/ml ceftazidime was used for the isolation of Mycoplasma species which were declared negative at 96 hours incubation at 37˚C.[6]

Isolation and identification of isolates

Nutrient agar plates were prepared for the isolation of pure colonies from the primary plates. A colony was picked and streaked on nutrient agar plates and then incubated at 37˚C for 24 hours. Using a sterile wire loop, a colony from each plate was picked and prepare for Gram's staining and other biochemical tests, some of which include Catalase, Coagulase, DNAse, Triple iron agar, Oxidase and API 20E Test.[19]

Results

Isolation of microorganisms

The microbial isolates obtained from seminal fluids analyzed in laboratory are presented in Table 1. The result shows a total of seventy six (76) isolates were obtained, *Staphylococcus aureus* was found to have the highest occurrence of 31(40.79%), whereas the least was found to be Mycoplasma species, 1(1.32%). Other microorganisms encountered include; Coagulase negative Staphylococcus species 14 (18.42%), *Escherichia coli* 11(14.47%), *Klebsiella pneumoniae* 6(7.89%), *Proteus mirabilis* 5(6.58%), *Pseudomonas aeruginosa* 3(3.95%), *Neisseria gonorrhoeae* 2(2.63%) and Candida species 3(3.95%).

Microbial distribution in relation to age

The distribution of microorganisms in relation to patient's age is presented in Table 2. From the table, out of the 200 samples examined 76(38%) were found to be infected. The result also shows that patients within the age range of 30-39 years were most infected with 35(40.1%) infected out of 93 examined while those within the age range of 60-69 years were the least with only 1(1.3%) infected out of total of 4 samples.

Table 1 Various organisms isolated and their frequencies of occurrence

Isolates	Number (n)	Percentage (%)
Coagulase negative *Staphylococcus* species	14	18.42
Staphylococcus aureus	31	40.79
Escherichia coli	11	14.47
Klebsiella pneumoneae	6	7.89
Proteus mirabilis	5	6.58
Pseudomonas aeruginosa	3	3.95
Mycoplasma species	1	1.32
Neisseria gonorrhoae	2	2.63
Candida albicans	3	3.95
Total	76	100

Table 2 Frequency of occurrence of microorganisms in relation to age group

Age range (years)	Number examined (%)	Number infected (%)
20-29	24 (12.0%)	04 (5.3%)
30-39	93(46.5%)	35 (40.1%)
40-49	70 (35.0%)	32 (42.1%)
50-59	09 (4.5%)	04 (5.3%)
60-69	4 (2.0%)	01 (1.3%)

Distribution of semen parameters in relation to microbial isolates

The distribution of semen parameters among various microorganisms are shown in Table 3. The highest number of isolated microorganisms was observed in samples with concentration of 0-20 x106/ml. This was followed by those with concentration of 20-60x106`/ml while the least number of microorganisms was observed from samples with concentration >60 x106/ml. The table also shows the highest progressive motility been recorded in a few fraction of Bacteriospermic samples while majority shows poor to medium motility. Motility rate was reduced significantly in samples with pathogenic microorganisms.

Table 3 Distribution of semen parameters in relation to microbial isolates

Bacteria	Concentration (x106/ml)			Motility (%)			Morphology (%)		
	Good (> 60)	Medium (20-60)	Poor (0-20)	Good (> 50)	Medium (30-50)	Poor (0-30)	Good (>30)	Medium (20-30)	Poor (0-20)
S. aureus	3	12	16	4	8	19	6	5	20
CNSS	4	2	8	4	5	5	1	5	8
Escherichia coli	2	3	6	1	3	7	0	4	7
P. mirabilis	0	2	3	1	2	2	2	1	2
K. pneumoniae	1	3	2	0	2	4	2	2	2
P. aeruginosa	0	0	3	1	1	1	0	0	3
Mycoplasma	0	0	1	0	0	1	0	1	0
N. gonorrhoae	0	1	1	0	2	1	0	1	1
C. albicans	1	1	1	0	0	3	1	1	1

CNNS, Coagulase Negative Staphylococcus species.

Discussion

The study was aimed to assess microbiological quality of male patients with infertility at Murtala Muhammad specialist hospital Kano, Nigeria. The result shows a total of seventy six (76) isolates were obtained, *Staphylococcus aureus* was found to have the highest occurrence of 31(40.79%), whereas the least was found to be Mycoplasma species, 1(1.32%). The higher prevalence of *S. aureus* in the study agrees with previous.[5–7,20,21] Komolafe & Awoniyi[21] reasoned that, the isolation of *S. aureus* might be associated with body hygiene of the couples involved. Moreover, Charanchi et al.,[22] contends that, the higher occurrence of *S. aureus* in semen of patients with infertility could be attributed to its minimal growth requirements, high resistance to environmental factors and ability to colonize and establish infection. Conversely, the least occurrence of Mycoplasma species in this study disagrees with the work of Domes et al.[20] Coagulase-negative Staphylococcus species are known opportunistic pathogens which are usually involved in Nosocomial and human urinary infections. Since urinary tract always act as locus of infections for the seminal tract, the heterogeneity of microorganisms encountered are capable of causing classical infections of the urogenital tract[23] and according to Ibadin & Ibeh[5] male urogenital tract infections are one of the most important causes of male infertility worldwide.

Table 2 shows the distribution of microorganisms in relation to patient's age. From the table, out of the 200 samples examined 76(38%) were found to be infected. The result also shows that patients within the age range of 30-39 years were most infected with 35(40.1%) infected out of 93 examined while those within the age range of 60-69 years were the least with only 1 (1.3%) infected out of total of 4 samples. This is in agreement with the works of Komolafe & Awoniyi[21] where male partners of 20 to 49 years age range were found to be most

affected. This might be due to their involvement in high-risk sexual behaviour or polygamous relationships, or as a result of urogenital infections.[24] This implies that infertility and polymicrobial infections were common among young couples as shown in Table 2. Momoh et al.,[15] noted that many who are infected with several pathogens may lack symptoms and signs of infection but presence of polymicrobial infections is one of the reasons adduced for erectile dysfunction. According to Brogden et al.,[25] the presence of one microorganism can predispose the host to colonization or infection by another organism there by justifying the recognition of the significance of polymicrobial infections and the major types of microbial community interaction associated with human health and diseases.

The interrelationships of microorganism and semen parameters showed that the highest number of isolated microorganisms was observed in samples with concentration of 0-20x106/ml. This was followed by those with concentration of 20-60x106`/ml while the least number of microorganisms was observed from samples with concentration >60x106/ml. The table also shows the highest progressive motility been recorded in samples without pathogenic microorganisms. Motility rate was reduced significantly in samples with pathogenic microorganisms. This implies that the presence of pathogenic microorganisms exerts a negative effect on spermatozoa concentrations which supports earlier observations.[6] *S. aureus* occur in all ranges to spermatozoa concentrations. This may be related to its success as major human commensal and pathogen, partly due to some strain that have the ability to rapidly develop resistance to several antimicrobial agents.[26] This suggests that these microorganisms are strongly associated with deterioration of semen indices. Similarly, the presence of pathogenic microorganisms in seminal fluid as recorded in this study was associated with decline in total and progressive motility indices when compared with non-pathogenic microorganisms which agrees with Golshani et al.[27] This is suggestive that pathogenic microorganisms markedly affect the kinetic of characteristics of spermatozoa when present in semen.[6] For instance, *E. coli*, Coagulase Negative *Staphylococci* may play an important role in sperm impairment due to infertility, worldwide.[28] Their significant negative effect is towards sperm motility, morphology, and vitality.[28] Ekhaise & Richard[7] quoted Stephen et al.,[29] who equally reported that, the viability and structural integrity of the semen lies on its characteristic feature of mobility. It is therefore probable that the presence of *E. coli* in semen decreases sperm motility which is dependent upon sperm bacterial/semen ratio/ml.

Conclusion

In the study *Staphylococcus aureus* is the most prevalent microbes associated with infertility in male partners of infertile couples and the presence of *Escherichia coli* might be an indication of poor personal hygiene among couples. The result also shows that patients within the age range of 30-39 years were most infected. The interrelationships of microorganism and semen parameters showed that the highest number of isolated microorganisms was observed in samples with concentration of 0-20x106/ml.

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None.

Conflict of interest

The author declares that there is no conflict of interests involved in this study.

References

1. Patrick J Rowe, Frank H Comhaire, Timothy B Hargreave, et al. *World Health Organization (WHO) Manual for the Standardized Investigation and Diagnosis of the Infertile Couple*. England, Cambridge University Press; 2000.
2. Deka PK, Sarma S. Psychological aspects of infertility, *British Journal of Medical Practitioners*. 2010;3(3):a336.
3. Weng SI, Chiu M, In MF, et al. Bacterial communities in semen from men of infertile couples: metagenomic sequencing reveals relationships of seminal microbiota to semen quality. *PLoS ONE*. 2014;9(10):e110152.
4. Diemer T, Huwe P, Ludwig M, et al. Urogenital infection and sperm motility. *Andrologia*. 2003;35(5):283–287.
5. Ibadin O, Iben IN. Bacteriospermia and sperm quality in infertile male patient at University of Benin Teaching Hospital, Benin City, Nigeria. *Mal J Microbiol*. 2008;4(2):65–67.
6. Onemu SO, Ibeh IN. Studies on the significance of positive bacterial semen cultures in male fertility in Nigeria. *Int J Fertil Womens Med*. 2001;46(4):210–214.
7. Ekhaise FO, Richard FR. Common bacterial isolates associated with semen of men attending the fertility clinic of the University of Teaching Hospital (U.B.T.H), Benin City, Nigeria. *Afr J Microbial Res*. 2011;5(22):3805–3809.
8. Keck C, Gerber-Schafer C, Clad A, et al. Seminal tract infections: impact on male fertility and treatment options. *Hum Reprod Update*. 1998;4(6):891–903.
9. Askienazy-Elnhar. Male genital tract infection: the point of view of the bacteriologist. *Gynecol Obstet Fertil*. 2005;33(9):691–697.
10. Sanocka-Macleiewska D, Ciupinska M, Kurpis ZM. Bacterial infection and semen quality. *J Reprod Immunol*. 2005;67(1-2):51–56.
11. Okonofua FE. New reproductive technologies and infertility treatment in Africa. *Afr J Reprod Health*. 2003;7(1):7–11.
12. Moustafa MH, Sharma RK, Thornton KJ, et al. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. *Hum Reprod*. 2004;19(1):129–138.
13. Al-Marzoqi AH, Aboud MM, Sabri MA. Study of Bacterial infection associated with male infertility in Hillah city-Iraq. *Biology Agriculture and Healthcare*. 2012;2(9):10–17.
14. Wong WY, Thomas CM, Merkus JM, et al. Male factor subfertility: possible causes and impact of nutritional factors. *Fertil Steril*. 2000;73(3):435–442.
15. Momoh ARM, Odike MAC, Samuel SO, et al. Resistance Pattern Of Urinary Tract Infection Bacterial Isolates To Selected Quinolones. *Benin Journal of Post Graduate Medicine*. 2007;9(1):22–27.
16. *National Population Commission (NPC)*. National population census result. Abuja, Nigeria; 2006.
17. Nwabuisi EC, Onile BA. Male infertility among sexually transmitted disease clinic attendees in Ilorin, Nigeria. *Niger J Med*. 2001;10(2):68–71.
18. Enwaru CA, Iwalokam VN, Enwuru VN, et al. The effect of presence of Facultative bacteria species on semen and sperm quality of men seeking fertility care. *African journal of urology*. 2016;22(3):213–222.
19. Chessbrough M. *District laboratory practice in tropical countries*, 2nd edn, part 2. England, Cambridge university press; 2006:440 p.
20. Domes T, Lo KC, Grober ED, et al. The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. *Fertil Steril*. 2012;97(5):1050–1055.

21. Kampole OI, Awoniyi AO. Prevalence of microbial isolates associated with infertility in men attending clinics of OAUTHC, Ile-Ife. *International journal of microbiology research and reviews*. 2013;1(5):088–091.

22. Charanchi S, Kudi A, Tahir F. Antimicrobial sensitivity pattern of urogenital bacteria isolates among HIV-positive patients in the Federal Medical Center in Gombe. *The Internet J Infect Dis*. 2012;10(1):1–6.

23. Novy MJ, Wilkin SS, Eschenebach DA. Infection as a cause of infertility. *Globar Libr Women med*. 2008.

24. Sheikh AF and Mehdinejad M. Identification and determination of coagulase negative *Staphylococcus* specie and antimicrobial pattern of isolates from clinical specimens. *Afr J Microbial Res*. 2012;6(8):1669–1674.

25. Brogden KA, Guthmiller JM, Taylor CE. Human microbial infection. *Lancet*. 2015;365(9455):253–255.

26. Rossi BV, Abusief M, Missmer SA. Modifiable risk factor and infertility: What are the connections? *Am J Lifestyle Med*. 2014;10(4):220–231.

27. Gulshani I, Taylor CE, Ross MD. Effect Methotrexate on subsequent fertility in patients undergoing ART. *Fertility and Sterility*. 2006;92(2):515–519.

28. Rodin DM, Larone D, Goldstein M. Relationship between semen cultures, leukospermia, and semen analysis in men undergoing Fertility evaluation, *Fertil Steril*. 2003;3:1555–1558.

29. Hirsch AM, Hirsch SM. The effect of infertility on Marriage and self-concept. *J Obstet Gynecol Neonatal Nurs*. 1989;8(1):13–20.

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