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Assessment of Bacteria Present in Semen of Patients attending Hospitals in Makurdi

Ada Tersoo Raymond, Anefu Daniel Oche and Ali Eric Ahangba

Department of Biological Sciences, Benue State University, Makurdi, Nigeria.

RESEARCH ARTICLE

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Corresponding author
Ada Raymond Tersoo
tersooada@gmail.com

ABSTRACT

Bacterial infection in semen is a reproductive health disorder, emerging globally as a serious medical and social problem that results in much trauma, emotional instability and psychological stress of the affected individuals. This can be assessed by the quality and quantity of sperm cells as well as its structure. This research was carried out to detect bacterial infection in semen samples among males in some selected hospitals in Makurdi. The research was carried out from October 2017 to February 2018. Semen samples from 115 men were collected for bacteriological analysis and examined to evaluate sperm concentration and motility. The morphological sperm characteristics were studied by simple microscopy. Samples were cultured and bacteria species were identified by biochemical analysis. Bacterial growth was positive for 75 samples (65%) and negative for 40 samples (35%). Statistically, they was no significant difference between the distribution of the pathogen and the different age group (P>0.05). *Escherichia coli* is the bacteria species most isolated from the culture among the older age group. Farmers have the highest rate of infection as compared to other occupation.Others strains of bacterial such as, *Chlamydia trachomatis, Neisseria gonorrhoea, Staphylococcus* spp, *Klebsiella*spp, *Proteus mirabilis* were also identified from the study and showed a drastic effect on semen quality. Most of these bacteria were susceptible to Amoxyclave, ciproxin and ofloxacin. Hence is recommended for treatment.

Keywords: Semen, bacteria, morphological sperm

INTRODUCTION

Bacterial infection remains a major cause of death, disability, social and economic disorder for millions of people throughout the world [1].In East Nigeria, the estimated percentage of bacterial infection rose from 42.4% in 2003 to 55.9% in 2013 [2,3]. High cases have also been recorded in Ife, Lagos and Ebonyi[4]. Bacterial infection is defined as a proliferation of one of the harmful strains of bacterial either on or inside the body which could be cocci, bacilli, spiral or even irregular in shape[5]. It is a complex health situation which particularly alters the quality of life of its host. In 2010, around 19% and 23% of people particularly of reproductive age (20-44 years) were affected by primary and secondary bacterial infection [6]. Poverty, Poor personal hygiene, poor access to health care, human migration, and antibiotic resistance all contribute to

the expanding impact of this disease. Recent studies however indicate that bacterial infections may also affect the male reproductive function in different ways [7]. These pathogens may act either directly on the spermatozoa, indirectly on the seminal plasma or by forming anti-sperm antibodies [8]. Bacteriospermia and the recruitment of seminal leukocytes can potentially impair male fertility through the deterioration of spermatogenesis, impairment of sperm function, and genital tract dysfunction and/or obstruction [9]. Also, bacterial infections of the male genitourinary tract account for up to 15% of cases of male infertility where acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process causing qualitative and quantitative sperm alterations [8,10]. The bacteria responsible for semen contaminations generally originate from the urinary tract of patients but can be transmitted by the partner via sexual intercourse [11]. Bacterial infection in semen is an underlining cause of poor semen quality and quantity faced by males all over the world resulting in infertility (Sanocka*et al.*, 2005). According to [12], 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. However, in our society especially the south eastern part of Nigeria, it is customary for every case of infertile marriage to be blamed on the female partner due to ignorance and a lot of misconceptions about what a fertile man is. Once the man is able to have intercourse, ejaculate semen which he adjudges to be thick then the wife's inability to get pregnant must be a problem with her and not the man [3]. In an attempt to find the possible cause of male factor infertility, semen analysis quickly comes to mind. It is the first and perhaps the most important laboratory investigation of the male partner of an infertile couple due to the fact that a number of studies have revealed the role of sperm parameters such as low concentration, poor motility and morphological abnormalities in the infertility situation in males [13-15]. To this end, this study aims to assess the level of pathogenic micro-organism (bacteria) in semen samples among males in some selected hospitals in Makurdi.

EXPERIMENTAL PROCEDURES

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 coitius) after 3-5 days of sexual abstinence and fast from antibiotics. These samples were collected in labelled sterile containers properly closed and kept at body temperature (37°c) preferably in their pockets and report to the laboratory on or before 15 min of production.

Methodology

0.5ml of semen samples was collected immediately with the aid of a pastuer pipette for microbiological examination while the remainder was allowed to liquefy at 37°c for 30 min 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 min 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 semen was measured by decanting the whole sample aseptically into a graduated centrifuge tube and level was recorded in ml with normal volume =2ml [16].

Measurement of pH

The pH was determined by spreading a drop of the sample evenly into the pH Paper. After 30 sec, the colour of the impregnated zone was compared with the calibration strip.pH of normal semen =7.2-7.8[5].

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[5].

WBC (LEUKOCYTES)

A drop of each semen sample was placed on a clean glass slid, covered with cover slip and examined microscopically using 40x objectives for the presence of white blood cells [5].

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 [5].

Were 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 =$20x10^6$ spermatozoa/ml or more [5].

Semen culture

Semen samples were inoculated using a wire loop on sterile blood agar, macConkey agar and nutrient agar plate and incubated for 24hrs at 37°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 organism (biochemical test)

The morphological characteristics of the growth on blood, macConkey or nutrient media was observed based on the following characteristics;

i. Elevation raised or depressed
ii. Colour
iii. Edge of colonies smooth or rough
iv. Haemolysis on blood media/ chocolate media
v. Size of colony i.e tiny, moderate or large
vi. Colony consistency i.e specifying weither it swarms
vii. Fermentative ability i.e either lactose or non-lactose fermenting on macConkey media
viii. Hydrogen sulphide production

ix. Sliminess.

Most of the tiny colonies were most likely to be gram positive cocci, thus they were confirmed by gram staining. slimness was often implicative on *klebsiella*spp. Lactose fermenters were mostly coliforms including *Escherichia coli* and *Enterobacter* spp. *Pseudomonas* spp was often implicated by a fruity smell after at least 48 hrs of growth. *Proteus* spp was often found to exhibit swarmness.

Large or moderate sized colonies with unconfirmed identities underwent further biochemical analysis for identification in triple sugar iron test (TSI) and indole test. Pinpoint or tiny colonies also underwent further biochemical test (catalase and coagulase test) to identify *staphylococcus* spp.

Some of the identification procedures include:

Gram staining

The small or pinpoint colony isolated from the culture was suspected to be gram positive organisms. In other to confirm this, they were gram stained.

Here a smear of the organism was made on a clean, grease free slide by using a sterilised wire loop to pick a colony from the culture media and emulsifying in a drop of normal saline on a slide. The smear was then heat fixed through the bursen flame and placed on a staining rack in preparation for staining.

The reagent used for staining include; methyl violet, lugol iodine, acetone and safranin.

Here the smear was stained first with the primary stain (methyl violet) for about 30 sec, poured off and again lugol iodine was added for 30 sec, washed off with distilled water and decolorise with acetone for another 30 sec. This was followed by counterstaining with the secondary stain safranin for 60 sec, washed off and allow to dry. This was then examined microscopically using 100x objectives with oil emersion.

On observation, those found to retain the primary stain colour (violet) were confirmed as gram positive. If the gram positive organism appeared round, they are considered as cocci. Gram positive cocci were found either in chains as (*Streptococcus* sp*)* or in clusters as (*Staphylococcus* sp). Those found to appear reddish were considered as gram negative of which most were found to be rod-shaped (bacilli).

The gram negative bacilli identified underwent further biochemical test for proper identification. While the gram positive cocci (though their manner of occurrence of their colonies either in clusters or chains) was not enough for identification underwent catalase test for confirmation.

Catalase test

Here a drop of hydrogen perioxide was placed on a clean grease free slide and a pick of a colony was immersed and observations were made for the appearance of bubbles.

A positive result was indicated by the appearance of bubbles i.e catalase positive, which is implicative of *staphylococcus* spp. However, absence of bubbles i.e catalase negative is seen with *streptococcus* spp.

Triple sugar iron test (TSI)

The triple sugar iron test is another test biochemical test carried out on gram negative bacilli that seemed to appear morphologically large or moderate in size on the culture media.

The colonies to be cultured in the TSI medium were picked using a sterilised wire loop and inoculated on the TSI medium. These medium was then covered tightly with sterile cotton wool and incubated at 37°c for 24 hrs.

On observation after 24 hrs, the butt and the slant were noted for alkaline (red) or acidic (yellow) reactions. Some even shoved an elevated clear zone which was indicated as the production of gas (hydrogen sulphide).

The result of the TSI test i.e TSI positive or negative, or the production of hydrogen sulphide of the cultured organism were then compared to a chart provided by the company that produced the media.

Proteus sp was indicated as TSI positive on the chart.

Indole test

This is also used in the identification of gram negative bacilli. For this test, peptone water was prepared.Here the colonies to be cultured were picked from the culture media using a sterilised wire loop and inoculated into the prepared peptone water and incubated for 24 hrs at 37°c after which 1-2 drops of kovacs reagent was added.

On addition of kovacs reagent, positive reaction was indicated by the appearance of a purple ring on the surface of the peptone water, while a clear solution was an indication of a negative reaction.

According to the standard chart, Escherichia *coli, citrobacter*sp, *proteus*sp *and klebsiella*sp was indole positive.Proper bacteria identification was made based on morphological characteristics of the growth on culture media.

Antimicrobial sensitivity testing

Antibacterial agents can be grouped by their mode of action i.e their ability to inhibit the synthesis of the cell wall, cell membrane, protein and nucleic acid of bacteria.

In the treatment and control of infectious disease, especially when caused by pathogens that are often drug resistant, sensitivity testing is used to select effective antimicrobial drugs. In the course of this study, the disc diffusion technique was used. Here a disc of blotting paper was impregnated with a known volume and appropriate concentration of an antimicrobial (Antibiotic) and placed on a suitable culture media usually nutrient agar on a petridish uniformly inoculated with the test organism and incubated for 16-18 hours at 37°c.

The antimicrobial (antibiotics) diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc.

RESULTS

Semen samples from 115 men were evaluated from October 2017 to February 2018. The semen culture was positive (yielded growth of bacteria after 24 hours of incubation at 37°c) in 75 samples (65%) and negative (yielded no growth) in 40 samples (35%).

Escherichia coli is the most commonly isolated pathogen as deduced from the study with a prevalence of 24% while *Enterobacter agglomerans* is the least most isolated pathogen with a prevalence of 6.7% as presented in Table 1.The composition of contaminating species according to different age group indicates that *Escherichia coli* is more predominant among older males (>36 years) while *Klebsiella*species, *Proteus mirabilis, and Streptococcus* are less predominant among younger males (< 25 years). However, no case of *Enterobacter agglomerans*and *Neisseria gonorrhoea*was recorded among younger age group (< 25 years).*Neisseria gonorrhoea, Streptococcus* spand *Escherichia coli* have been shown to have a drastic effect on sperm cell with a sperm count of $<10*10^6$ml and less than 40% active motility as indicated in Table 1.

Antimicrobial sensitivity indicated that all gram + bacterial where susceptible to Co-trimoxazole and Erythromycin. While all gram – bacterial show susceptibility to Gentamycin, Tetracycline, and Ceftriaxone. However, all strains of bacteria were susceptible to Amoxyclave, Ciproxin and Ofloxacin as indicated in Table 2. Statistical Analysis indicates that no significant difference ($p>0.05$) exists between the distribution of the pathogens and the different age group.

Table 1: Comparism of semen parameters among contaminating species to confirm semen quality

s/n	Contaminating species	Number of cases	% prevalence	White blood cell count	Sperm count	Motility
1	*Chlamydia trachomatis*	11	14.7	3hpf	$<20*10^6$ml	A=20,B=20, C=20, D=40
2	*Enterobacter agglomerans*	5	6.7	2hpf	$<20*10^6$ml	A=30, B=30 C=20, D=20
3	*Escherichia coli*	18	24.0	3hpf	$<10*10^6$ml	A=10, B=20 C=20, D=50
4	*Klebsiella* species	6	8.0	2hpf	$10*10^6$ml	A=20, B=20 C=20, D=40
5	*Neisseria gonorrhoea*	6	8.0	3hpf	$<10*10^6$ml	A=10, B=30 C=20, D=40
6	*Proteus mirabilis*	8	10.6	2hpf	$<20*10^6$ml	A=10, B=30 C=30, D=30
7	*Staphylococcus* species	11	14.7	3hpf	$10*10^6$ml	A=20, B=20 C=30, D=30
8	*Streptococcus* species	10	13.3	3hpf	$<10*10^6$ml	A=15, B=20 C=15, D=50

Source: Field survey, 2018.

A= Rapidly progressive motility,

B= Slow or sluggish motility,

C=Non progressive motility,

D= immobility.

Normal semen quality[16]

White blood cell count= nil

Sperm count=$20*10^6$ml

Motility: A=30, B=30, C=20, D=20

Table 2: Distribution of antimicrobial sensitivity among contaminating species

s/n	Contaminating species	Bacterial type	Antibiotics
1	*Chlamydia trachomatis*	Gram +	Amoxyclave, Ciproxin, Ofloxacin, Co-trimoxazole, Erythromycin
2	*Enterobacter agglomerans*	Gram -	Cretriaxone, Ciproxin, Amoxyclave, Tetracycline, Ofloxacin
3	*Escherichia coli*	Gram -	Gentamycin, Amoxyclave, Ciproxin, ofloxacin, Tetracycline, Ceftriaxone
4	*Klebsiella* species	Gram -	Amoxyclave, Ciproxin, Tetracycline, Ceftriaxone, Ofloxacin
5	*Neisseria gonorrhoea*	Gram -	Amoxyclave, Ciproxacin, Ofloxacin, Tetracycline, Ceftriaxone,
6	*Proteus mirabilis*	Gram -	Ceftriaxone, Gentamicin, Ciproxacin, Oflaxacin, Amoxyclave
7	*Staphylococcus* species	Gram +	Erythromycin, Amoxyclave, Co-trimoxazole, Ciproxin, Oflaxacin
8	*Streptococcus* species	Gram +	Erythromycin, Amoxyclave, Co-trimoxazole, Ciproxin, Oflaxacin

Source: Field survey, 2018.

Table 3: Level of significance among different contaminating species

s/n	Contaminating species	Chi-square value	Remarks
1	*Chlamydia trachomatis*	0.341	No significant difference(P>0.05)
2	*Enterobacter agglomerans*	0.287	No significant difference(P>0.05)
3	*Escherichia coli*	0.375	No significant difference(P>0.05)
4	*Klebsiella* species	0.285	No significant difference(P>0.05)
5	*Neisseria gonorrhoea*	0.306	No significant difference(P>0.05)
6	*Proteus mirabilis*	0.313	No significant difference(P>0.05)
7	*Staphylococcus* species	0.341	No significant difference(P>0.05)
8	*Streptococcus* species	0.333	No significant difference(P>0.05)

Source: Field survey, 2018.

This study revealed that the overall rate of bacterial infection in semen samples recorded among one hundred and fifteen (115) men from the different study locations was low (65%) as compared to the findings of [17] who reported from Ebonyi, South east Nigeria with an infection

rate of 83.7%,[4] from Ife in Osun state with an infection rate of 70.3% and [18] from Lagos with an infection rate of 69.1%.

However, statistics from [19] 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%), Senegal (26.8%), Ethiopia (5.6%) and Kenya (19%).

Higher infection rate is also recorded in studies carried out by [20] who reported from Mexico in south America with an infection rate of 66% and [21] from Asia with an infection rate of 72% as well as [6] from Europe with an infection rate of 68%. The difference in the rate of infection observed in this study and aforementioned studies could be due to increase in disease resistance to antibiotics, increase in harmful environmental exposure and radiations, irregular lifestyle, addiction to alcohol and smoking.

The age group 26-35 years have the highest rate of infection as deduced from the study and this agrees with the findings of [4] from Ile-ife, and [17] from south-east Nigeria that the middle age group (sexually active males) are more prone to sexually transmitted infection than other age group.

Farmers (38.9%) where discovered to be more prevalent of bacterial infection in semen than any other occupation as deduced from the studies. This agrees with the findings of [18] from Lagos (26%) who suggested that poor personal hygiene and lack of knowledge on the use of contraceptive could be the major predisposing factor affecting this occupation. However, a low prevalence was recorded among students (9.3%) which agree with the findings of [2].

Twenty seven (27) samples were found to contain large number of leukocytes which significantly affect semen parameters. This agrees with the findings of [22] that most damage to fertility is caused by the entrance of leukocytes to fight infection stimulating them to release toxic substances to kill these bacteria which results in collateral damage to sperm cell. Motility was significantly reduced in all strains of bacterial except for those infected with *Enterobacter agglomerans.* This findings is similar to those of [23-24] who suggested that the rate of infection is increased in those with decrease progressive motility. Similar research have also shown that the flagella and pili of *Enterobacter agglomerans* are less toxic i.e the mechanism of sperm damage used by these bacteria passes through the expression of the adhesive properties of the flagella and pill to mannose receptors are absent. Samples infected with *Escherichia coli* have a high record of poor morphology. This is probably because *E.coli*posses enzymes such as caspases and proteases responsible for mitochondrion changes and hence alteration in membrane symmetry and DNA fragmentation[3].

Furthermore bacteria such as *Chlamydia trachomatis, Staphylococcus* spand even*Klebsiella*sp have demonstrated a high negative influence on sperm quality of these males. These may probably suggest that aside the adhesion by their flagella and pill, these species also produces toxic such as H_2O_2 and phospholipase from their proliferation which injure sperm membrane [4].

Antimicrobial sensitivity indicates that all strains of bacterial where susceptible to Amoxyclave, Ciproxin and Ofloxacin which agree with the findings of [3]. However, all strains of gram – bacterial deduced from the study show resistance to Cretriaxone except *Enterobacter agglomerans*. This agrees with the findings of [25] which suggest that *Enterobacter agglomerans* do not possess mannose receptors.

Statistical analysis indicated that no significant difference exist between the distribution of the pathogen and the different age group. This conform with the studies carried out by [2,9,26] which suggest that increase in industrialisation and exposure to harmful chemicals could predispose all age group.

CONCLUSION

Based on the findings of this research work, the level of pathogenic microorganism in semen samples among males was high. The distribution of contaminating species among different age group indicated that *Escherichia coli* is the most prevalent and commonly occurring etiological agent of infection between older males (>35 years) while *Enterobacter agglomerans* is the least prevalent among older males and less occurring etiological agent of infection between all age group in the research area. The distribution of contaminating species across different occupation indicates that Farmers have the highest distribution of pathogens as compared students with the lowest distribution. The contamination of semen samples indicated a decrease in sperm quality mainly by the induction of apoptosis and necrosis which may in part be responsible for the observed reduction of sperm motility, sperm morphology, testicular damage, induction of decapitation in spermatozoa such as H_2O_2 produced by *Klebsiella* and *Staphylococuss* species, and overall decrease in the reproductive potential among males. Antibiotic sensitivity of the different contaminating species indicated that all bacterial where susceptible to Amoxyclave, Ciproxin and OFloxacin and hence can be used in the treatment of this infection. Statistically, there was no significant difference between all contaminating species as P value was >0.05 for all contaminating species.

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