International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (9), pp. 001-005, September, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Microbiology and semen indices of sexually-active males in Benin City, Edo State, Nigeria

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Accepted 9 April, 2018

This study was designed to examine the microbiological quality and semen indices of seminal fluids of sexually active males in Benin City, Nigeria, and to determine the relationship between the presence of pathogenic micro -organisms and semen parameters. Semen was collected from 229 volunteer sexually-active men, aged 19 - 33 years (mean 24.73 ± 3.4) in Benin City, Nigeria. Volunteers were advised to abstain from sex for 3 days before semen collection and also from alcohol (including other factors that may affect semen quality). A ten-fold serial dilution of well mixed semen in physiological saline (NaCl 0.15 M) was inoculated onto blood agar, heated blood agar, MacConkey agar, Sabouraud dextrose agar slants and mycoplasmal agar enriched with 30% serum and supplemented with 100 µg/ml ceftazidine for the isolation of *Mycoplasma* species. All inoculated culture media were incubated at 37°C for 24 - 48 h. Isolates were characterized and identified by standard microbiological methods and antimicrobial sensitivity test was carried out by the disc diffusion method. The determination of spermatozoa concentration, motility characteristics and other accompanying cells was carried out adopting standard procedures. Micro-organisms (3×10^6 cfu/ml) were isolated from 80/229 (34.9%) of participants' semen. The isolates were *Staphylococcus aureus* 47/80 *(* 58.8%). *Escherichia coli* 10/80 (12.5%) *Klebsiella* spp. 6/80 (7.5%), *Candida albicans* 5/80 (6.3%) and *Mycoplasma* species 12/80 (15.0%). Sensitivity to antimicrobial agents was highest among isolates to ciprofloxacin and pefloxacin. No activity was demonstrated with amoxicillin and tetracycline. Spermatozoa concentration was zero in 10.4% of the study population, 28.4% had concentrations below World Health Organization threshold for spontaneous male fertility. Semen with pathogenic micro-organisms had significantly lower ($p < 0.001$) spermatozoa concentrations and motility parameters. Therefore, the study suggests that the presence of pathogenic micro-organisms in semen is a marker of deterioration in semen parameters and development of male infertility.

Key words: Microbiology, pathogenic microorganisms, semen, sexually active males, antimicrobial, Nigeria.

INTRODUCTION

The association of genital tract infection to spermatozoa concentration and infertility has been documented as an important parameter that affects male reproductive capacity adversely (Comhaire et al., 1999; Witken and Toot, 1983). Previous studies have shown that there is a close correlation between prostatitis, epididymitis and sexually transmitted micro-organisms and male infertility (Hawkins et al., 1996). The risk factors in development of urinary/genital tract infection in healthy men have been identified to include intercourse with infected female partners, homosexuality and lack of circumcision (Grude et al., 2001). The existence of male genital tract and or accessory gland infections has been considered as a potential hazard to male fertility (Diemer et al., 2003). Clinical and experimental studies have linked the presence of bacteria in semen and the immunological function of spermatozoa to decreased fertility (Ireton and

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Table 1. Distribution of isolated micro-organisms.

Micro-organisms	No. of cases (%)
Staphylococcus aureus	47 (58.8)
Escherichia coli	10 (12.5)
Klebsiella spp.	6 (7.5)
Candida albicans	5 (6.3)
Mycoplasma spp.	12 (15.0)

Berger 1984). These processes ultimately lead to deterioration in spermatogenesis and spermatozoal function (Keck et al., 1998).

Majority of male infertility cases in Sub-Saharan African countries have been traced to prior genital tract infection or inflammation (Alausa and Osoba, 1978; Yeboah et al., 1992). Studies have also shown that when the characteristics of semen infected and uninfected were compared, semen with micro- organisms had poor indices of fertility (Ogunbanjo et al., 1989; Merino et al., 1995; Onemu and Ibeh, 2001; Sanocka-Meclejeska et al., 2005). Previous study in Nigeria has linked positive bacterial semen cultures with poor semen quality (Onemu and Ibeh 2001; Emokpae et al., 2005). This study was intended to determine the relationship between micro-organisms and semen parameters in sexually active young men in Benin City Nigeria.

MATERIALS AND METHODS

Unmarried sexually active male volunteers who had given oral consent after the objective of the study was explained in simple language were enlisted into the study. A sterile container was given to each participant and instructed to abstain from emitting semen for three days. He was advised to wash hands and genitals with mild toilet soap and clean water. A sterile disposable towel was provided to dry skin, and thereafter, collect semen for delivery to the laboratory within one hour of collection. For culture, 0.1 ml of a ten-fold serial dilution of well mixed semen in physiological saline (NaCl 0.15 M) was inoculated onto blood agar (Oxoid CM 55) for isolation of Gram positive bacteria, heated blood agar (Oxoid CM 55) for *Neisseria gonorrheae* and *Haemphilus* species, MacConkey agar (Oxoid CM 7) for differential of enterobacterial species and Sabouraud dextrose agar stants (Oxiod CM 41) for the isolation of yeasts and yeast-like fungi and mycoplasmal agar (Oxoid CM 401) enriched with 30% serum and supplemented with 100 µg/ml ceftazidine for the isolation of *Mycoplasma* species. All inoculated culture media were incubated at 37°C. Cultures were examined for growth after 24 h, cultures without growth or insufficient growth were re-incubated for examination at 48 h. Cultures for *Mycoplasma* species were declared negative at 96 h. Cultures with growth of $\geq 10^3$ colony forming units (cfu)/ml of a single microbial type were picked for characterization and identification according to scheme described by Cowan (1974). Antimicrobial sensitivity tests were set up using the disc diffusion method of Bauer-Kirby (Baker and Breach, 1980). The determination of spermatozoa concentration, motility characteristic and other accompanying cells was carried out adopting standard procedures (WHO, 1999).

RESULTS

Seminal fluid simples were collected from 229 sexually active volunteer men aged 19 to 33 years between July 2007 and May 2009. Cultural examination of the samples yelled 80 (34.9%) isolates belonging 5 genera Table 1. *Staphylococcus aureus* 47(58.8%) was the highest isolate. This was followed by *Mycoplasma* species 12(15.0%), *Escherichia coli* 10(12.5%) *Klebsiella* species 6(7.5%). No culture yielded a mixed growth of these micro-organisms. The relationship between spermatozoa concentration and pathogenic micro-organisms is shown in Table 2. The highest number of isolated micro-organisms occurred in semen samples with spermatozoa concentration 20 - 29.9 × 10^6/ ml. This was following by those with concentration 9.9 × 10^6/ ml. The least number of micro-organisms was recovered from samples with spermatozoa concentration 40 × 10^6/ ml. The distribution of micro-organisms according to the concentrations of spermatozoa is shown in Table 3. *S. aureus* was recovered from all dilutions of spermatozoa concentration. On the other hand, *E. coli* was isolated from samples with spermatozoa concentration 20 × 10^6/ ml while *Klebsiella* species and *Candida albicans* were recovered from semen concentrations < 30 × 10^6/ ml and < 20 × 10^6/ ml, respectively. There was no significant difference ($p > 0.05$) in the rate of *Mycoplasma* species isolation from all ranges of spermatozoa concentration.

Table 4 shows the relationship between spermatozoa motility and different types of micro-organisms. The highest total and progressive motility indices were recorded from samples without pathogenic micro-organisms. Motility ratios were reduced significantly ($p < 0.001$) from semen samples with pathogenic micro-organisms. The least motility parameters were recorded with samples with *C. albicans.* However, motility indices for samples with mycoplasmal isolates were generally higher when compared with other isolates. The antimicrobial sensitivity tests for the bacterial isolates are shown in Table 5. The isolates were most sensitive to pefloxacin and ofloxacin. Amoxycillin-clavulanate however, showed superior activity (95.8%) against *S. aureus.* The activity of gentamycin against *E. coli* and *Klebsiella* species isolates was 40 and 33%, respectively. No activity was demonstrated against ampicillin and tetracycline by all the bacterial isolates.

DISCUSSION

The study of seminal fluid samples from volunteer sexually-active unmarried males in Benin City revealed that 29.7% of the study population harboured pathogenic and potentially pathogenic micro-organisms. *S. aureus* was the most frequently isolated micro-organism. Similar findings have been documented from semen cultures (Giamarellou et al., 1984; Merino et al., 1995; Onemu and Ibeh, 2001; Emopkae et al., 2005). The predominance

Table 2. Relationship between spermatozoa concentration and pathogenic micro-organisms.

Range	Mean	No. of cases	Frequency of pathogenic micro-organisms (103 cfu/ml)
0.0	0	24	6 (25.0)
≤ 9.9	3.75	43	26 (60.1)
10-19.9	14.25	22	11 (50.0)
20-29.9	26.65	16	10 (62.5)
30-39.9	33.90	29	6 (20.7)
> 40	86.27	95	10 (10.7)

Spermatozoa concentration (× 10^6/ml).

Table 3. Distribution of micro-organisms according to spermatozoa concentration.

	No. of microorganisms (%)				
Spermatozoa concentration (×10^6/ml)	*S. aureus* (n = 47)	*E. coli* (n = 10)	*Klebsiella* species (n = 6)	*C. albicans* (n = 5)	*Mycoplasme* species (n = 12)
0.0	2(2.5)	0 (0.0)	0(0.0)	2(2.5)	1(1.3)
9.9	25(31.3)	0 (0.0)	1(1.3)	2(2.5)	2(2.5)
10-19.9	8 (10.0)	0 (0.0)	3(3.8)	1(1.3)	1(1.3)
20-29.9	3(3.8)	4(5.0)	2(2.5)	0(0.0)	0(0.0)
30 – 39.9	2(2.0)	4 (5.0)	0(0.0)	0(0.0)	3(3.8)
> 40	7(8.8)	2 (2.5)	0(0.0)	0(0.0)	5(6.3)

Table 4. Spermatozoa motility according to type of microbial isolate.

Isolate	Mean motility (%)		
	No. of cases	Total	Progressive
S. aureus	47	50.6	37.5
E. coli	10	55.0	30.5
Klebsiella species	6	30.6	15.3
C. albicans	5	30.5	10.0
Mycoplasma species	12	50.5	38.5
Sample without pathogenic micro-organisms	140	68.0	56.5

of *S. aureus* may not be a surprise because of the documented success of this micro-organism as a commensal and a major pathogen of man. This may also be partly due to the ability of this micro-organism to remain resistant to many antimicrobial agents in common use (Brooks et al., 2004). *Mycoplasma* spp. was the next commonest micro-organism isolated. The association of this micro- organism with the urogenital tract of both males and females who are sexually active has been documented (Gdoura et al., 2007) . The isolation frequency of this micro-organism from semen samples with both normal and abnormal semen parameters was similar when compared with semen samples with pathogenic micro-organisms. This may be suggestive that *Mycoplasma* spp. is not a primary uropathogen or an opportunistic pathogen. This undefined role may be the reason why this micro- organism has been described as a potential pathogen or a micro- organism whose role in seminal fluid samples is unclear (Kjaergaard et al., 1997; Keck et al., 1998). The recovery of *E. coli, Klebsiella* spp. and *C. albicans* are similar in pattern to those that have been documented in infertile males (Onemu and Ibeh, 2001; Emokpae et al., 2005). It should also be noted that *N. gonorrhea* was not isolated during this study. This may not be unrelated to the report on the ever decreasing rate of infection with this micro-organisms world-wide in the last two decades (Kamwendo et al., 1996).

Data on the concentration of spermatozoa revealed that10.4% of semen examined from the study participants was azoospermic or sterile. It was also found that another 28.4% of this population was oligospermic or sub-fertile. Each of these observations suggests that

Table 5. Sensitivity of bacterial isolates to antimicrobial agents (% isolates sensitive).

Bacterial isolate	Antimicrobial agent							
	Gentamycin	Pefloxacin	Ofloxacin	Azithromycin	Amoxycilin-clavulanate	Cloxacillin	Ampicillin	Tetracyline
S. aureus (n = 47)	70.3	91.2	91.2	90	95.8	75	0	0
E. coli (n = 10)	40.0	90.0	90.0	70	50	0	0	0
Klebsiella spp. (n = 6)	33.3	83.3	83.3	50	0	0	0	0

infertility is an important problem in young sexually active males in Benin City, Nigeria. Spermatozoa concentration was 40×10^6 ml in 41.5% of semen samples. This may indirectly indicate that a semen donor screening program would yield a poor outcome when the strict criteria are applied. This is similar to prior observation in Lagos, Nigeria (Akinrinola et al., 2003).

The highest number of pathogenic micro-organisms was isolated from semen samples with spermatozoa concentration $\geq 9.9 \times 10^6$ ml. This finding is suggestive that the presence of patho-genic micro-organisms exerts a negative effect on spermatozoa concentration. This simulates earlier observation in infertile males (Giamarellou et al., 1984; Onemu and Ibeh, 2001). *S. aureus* occurred in all ranges to spermatozoa concen-tration while *E. coli* was recovered from samples with concentrations of > 20 × 10/ ml. This tends to indicate that both micro-organisms are primary urogenital pathogens in this population. However, *S. aureus* was associated with all spermatozoa concentration ranges. This may be related to its success as a major human commensal and pathogen and partly due to some strains that have the ability to rapidly develop resistance and intrinsic high resistance to several antimicrobial agents that are commonly used (Brooks et al., 2004). *Klebsiella* spp. and *C. albicans* were associated with semen that had the least spermatozoa concentrations (Table 3). This suggests that these micro-organisms are strongly associated with deterioration of semen indices. *C. albicans* was the least micro-organisms isolated but, was recovered from samples with the lowest spermatozoa concentration and azoospermic samples. This may infer that *C. albicans* presence in semen was associated with the most severe effects on semen parameters. Similar findings have been reported in infertile males (Tuttle et al., 1977; Huwe et al., 1998; Onemu and Ibeh, 2001).

The presence of pathogenic micro-organisms in seminal fluid samples was associated with significant declines ($p < 0.001$) in total and progressive motility indices when compared with samples without pathogenic micro-organisms. This is suggestive that pathogenic micro-organisms markedly affect the kinetic characteristics of spermatozoa when present in semen. Antimicrobial sensitivity tests revealed that pefloxacin and ofloxacin were the most valuable antimicrobial agents that could be employed in the management of the varieties of bacterial isolates from this study. The successful use of these antimicrobial agents in similar situations has been reported (Andriole, 1991; Childs, 1991). The activity of amoxycillin-clavulanate against *S. aureus* was much superior to other agents tested. This may be due to the potent inhibition of the type of beta-lactamase produced by most strains of *S. aureus* (Denyer et al., 2004). This antimicrobial agent may therefore be regarded as an agent with better promise when this micro-organism is incriminated. The failure of ampicillin and tetracycline may have been partly influenced by the wide misuse of these agents in Benin City, Nigeria. The presence of pathogenic micro-organisms in seminal fluid samples was strongly linked with poor spermatozoal parameters and/or reduced male reproductive potential.

ACKNOWLEDGEMENTS

The authors sincerely thank all the male participants who volunteered to donate their semen for the study. Also, they appreciate the support of the Management and Staff of University of Benin Teaching Hospital, Benin City, Nigeria.

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