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Full length Research Paper

The prevalence of microbial isolates associated with infertility in men attending clinic at OAUTHC, Ile-Ife

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Microbial infections of the genital tracts or semen could deteriorate spermatogenesis, affect sperm motility, and alter chemical composition of the seminal fluid thereby leading to male infertility. This study was designed to investigate the new trend of prevalence of microbial isolates in the semen of infertile men and the susceptibility patterns of bacteria isolates to most of the commonly used antibiotics. Following WHO guidelines, one hundred and forty-three (143) seminal fluid samples were collected from infertile men attending infertility clinic at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife within January to December, 2011. The samples were allowed to liquefy and then cultured on MacConkey agar, Saboraud dextrose agar, and Blood agar media incubated aerobically at 37°C overnight. Staphylococcus aureus gave highest yield 51 (37.23%) followed by coagulase-negative Staphylococci 29 (21.17%) while Enterococcus faecalis 2 (1.46%) and Proteus spp 2 (1.46%) were the least isolated. Polymicrobial infection and infertility were most common among men within 30 and 49 years and 20 and 49 years of age respectively. Most of the bacteria isolated were susceptible to ciprofloxacin and ofloxacin but resistant to augmentin, amoxylin, and cotrimoxazole. The study shows that Staphylococcus aureus is the most prevalent microbe associated with infertility in infertile men while ciprofloxacin and ofloxacin are effective for the treatment of bacterial infection in infertile male.

Key words: Infertility, susceptibility patterns, seminal fluid, staphylococcus aureus, escherichia coli, proteus spp, candida albican.

INTRODUCTION

According to World Health Organization (WHO, 2010), 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. Microbial infections of the genital tracts or semen are major causes of male infertility (Onemu et al., 2010; Ekhaise et al., 2008). 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 (Mogra et al., 1981; Ibadin et al., 2008).In Nigeria, infertility is currently regarded as a major public health problem and about 20 to 30% of couples in Africa experience primary and secondary infertility (Okonofua et al., 1995; Ekhaise et al., 2011). Previous studies have identified Staphylococcus aureus, E. coli, Citrobacter spp, Klebsiella spp, Pseudomonas aeruginosa, Proteus vulgaris, and Proteus mirabilis with infertility in male partners of infertile couples (Ekhaise et al, 2011 & 2008; Momoh et al., 2011; Ibadin et al., 2008). Since fertility is important to all societies and the inability to have children has traditionally being a source of pain and anxiety; microbiological investigation of the semen of male partners in infertile couples to detect probable microbial agents causing infertility can be a useful intervention at resolving the problem of infertility (Ibadin et al., 2008; Ekhaise et al., 2008). Therefore, this study was aimed at investigating the new trend of prevalence of microbial isolates in the semen of infertile male and the susceptibility of the bacterial isolates to most of the commonly used antibiotics.

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Table 1. Frequency of Microbial Isolates from Semen

Microbial isolates	Microbial isolates in frequency	Microbial isolates in per cent
Staphylococcus aureus	51	37.23
Coagulase negative Staphylococci	29	21.17
Pseudomonas spp	10	7.29
Escherichia coli	27	19.71
Klebsiella spp	4	2.92
Enterococcus faecalis	2	1.46
Candida albican	9	6.57
Proteus spp	2	1.46
Streptococcus spp	3	2.19
	137	

Table 2. Distribution of Microbial Isolates In Relation To Patient's Age

Age range (years)	Number examined	Number infected (in %)
20-29	6	6 (100)
30-39	86	61 (70.9)
40-49	43	30 (69.8)
50-59	7	6 (85.7)
60-69	1	1 (100)
	143	104 (72.7)

Table 3. Extent of Infection in Relation to the Number of Microbes Isolated from the Semen

Age range (years)	Mixed (polymicrobial) isolates (in %)	Single microbial isolates (in %)	Total
20-29	2 (6.25)	4 (5.56)	6
30-39	16 (50.00)	45 (62.50)	61
40-49	12 (37.50)	18 (25.00)	30
50-59	2 (6.25)	4 (5.56)	6
60-69	0 (0.00)	1 (1.38)	1
	32 (30.77)	72 (69.23)	104

MATERIALS AND METHODS

One hundred and forty-three (143) patients were referred to the research and training laboratory unit of the Department of Obstetrics, Gynaecology, and Perinatology, Obafemi Awolowo University, Ile-Ife from the fertility clinic of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria for seminal fluid analysis between January and December, 2011. Semen collected from the patients in accordance with WHO (2010) guidelines was submitted to the laboratory within an hour after collection. The samples were allowed to liquefy and then inoculated on MacConkey Agar, Blood Agar, and Saboraud Dextrose agar which were incubated aerobically at 37°C for 24-48 hours. The Isolated organisms were subjected to various biochemical tests for proper identification (Monica, 2002). Also, antimicrobial susceptibility test was performed using standard disc diffusion technique. A tube of peptone water inoculated with four discrete colonies of isolated bacteria was adjusted to match the turbidity standard. A sterile cotton swab dipped into the standardized bacterial suspension was used to evenly inoculate the surface of Mueller-Hinton agar plate. The surface was allowed to dry for few minutes and antibiotic discs were placed on it using a sterile forceps. The plates were incubated at 37°C for 16-18 hours and diameters of zones of inhibition were measured in millimetre (Vandepitte et al., 2003).

RESULTS

One hundred and thirty-seven (137) microbial isolates were obtained from the seminal fluid analyzed in the laboratory as indicated in Table 1. These microorganisms were Staphylococcus aureus 51 (37.23%), coagulase negative Staphylococcus 29 (21.17%), Pseudomonas spp 10 (7.29%), Escherichia coli 27 (19.71%), Klebsiella spp 4 (2.92%), Enterococcus faecalis 2 (1.46%), Candida albican 9 (6.57%), Proteus spp 2 (1.46%), and Streptococcus spp 3 (2.19%). Out of the 143 patients examined, Table 2 shows that 104 (72.7%) were infected with at least a microbe. Also, Table 3 shows the extent of infection in relation to the number of microbes isolated

Table 4. Susceptibility Patterns of the Isolates to Antibiotics

		% of susceptible isolates								
Organisms	Total number of isolate	ERY	GEN	PEF	OFL	CPR	COT	AUG	AMX	CAZ
S. aureus	51	12 (23.5)	5 (9.8)	7 (13.7)	13 (25.4)	11 (21.5)	6 (11.7)	1 (1.9)	4 (7.8)	4 (7.8)
CoN-Staphylococcus	29	20 (68.9)	9 (31.0)	8 (27.5)	14 (48.2)	17 (58.6)	8 (27.5)	2 (6.9)	2 (6.9)	16 (55.1)
Pseudomonas spp	10	0.0	8 (80.0)	4 (40.0)	8 (80.0)	7 (70.0)	4 (40.0)	2 (20.0)	1 (10.0)	0.0
E. coli	27	1 (3.7)	13 (48.1)	15 (55.5)	17 (62.9)	19 (70.3)	10 (37.0)	7 (25.9)	8 (29.6)	7 (25.9)
Klebsiella spp	4	0.0	1 (25.0)	1 (25.0)	2 (50.0)	3 (75.0)	1 (25.0)	0.0	1 (25.0)	0.0
Streptococcus faecalis	2	1 (50.0)	0.0	0.0	2 (100.0)	1 (50.0)	0.0	0.0	0.0	1 (50.0)
Proteus spp	2	0.0	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	0.0	1 (50.0)	1 (50.0)	1 (50.0)
Streptococcus spp	3	3 (100.0)	2 (66.6)	0.0	0.0	1 (33.3)	1 (33.3)	1 (33.3)	0.0	1 (33.3)

ERY = erythromycin; GEN = gentamicin; PEF = pefloxacin; OFL = ofloxacin; CPR = ciprofloxacin; COT = cotrimoxazole; AUG = augmentin; AMX = amoxylin; CAZ = ceftazidime from the semen. Seventy-two (69.3%) infertile men were infected with single microbial isolate while thirty-two (30.77%) had more than one microbial isolate in their semen. The susceptibility patterns of the isolates to antibiotics are shown in Table 4.

DISCUSSION

According to Okonofua et al., (1995), two-thirds of the cases of infertility in Nigeria are attributable to infections. Culture-based studies are useful in determining ongoing levels of infection and can be used to answer a number of relevant questions on the relationship between infections and infertility. Usually, majority of male infertility cases in sub-Saharan African countries are not unconnected with prior urogenital tract infections (Onemu et al., 2010; Okonofua et al., 1995; Ekhaise et al., 2011).In this study, Staphylococcus aureus was the commonest bacteria isolated from the semen samples with percentage prevalence of 51 (37.23%) followed by coagulase-negative Staphylococci 29 (21.17%) and Escherichia coli 27 (19.71%). This was supported by the work of Ibadin et al., (2008) in which S. aureus (16.1%), S. saprophyticus (9.1%), and Escherichia coli (6.9%) were the main organisms isolated. According to Charanchi et al., (2012), the higher occurrence of S. aureus could be attributed to its minimal growth requirement, high resistance to environmental factors and ability to colonize and establish infection in almost every site of the body. Coagulase-negative Staphylococci are known opportunistic pathogens which are usually involved in nosocomial and human urinary tract infections (Sheik et al., 2012). Since urinary tract acts as a nidus of infection for the seminal tract, these microorganisms are capable of causing classical infections of the urogenital tract such as epididymitis and prostatitis as well as subclinical reproductive tract infections (Mogra et al., 1981; Ekhaise et al., 2011; Emokpae et al., 2009). Also, episodes of acute orchitis or epididymitis result in permanent damage to the testis or obstruction in the efferent ejaculatory ducts (Novy et al., 2008). Also, according to the studies conducted by Auroux et al., (1991) and Huwe et al., (1998), E. coli and Candida albicans, respectively, exert significant inhibitory effect on spermatozoa’s motility. Because E. coli, Proteus spp, and S. aureus may have an adverse effect on male infertility, treatment is usually recommended (Novy et al., 2008). Infertility and polymicrobial infections were common among young male partners within the age range of 20 to 49 years and 30 to 49 years respectively. This might be due to their involvement in high-risk sexual behaviour or polygamous relationships, or as a result of prior urogenital infections according to Novy et al., (2008). Polymicrobial infections are one of the reasons adduced for erectile dysfunction (Momoh et al., 2011) and many who are infected with several pathogens may lack symptoms and signs of infection. According to Brogden et al., (2005), the presence of one microorganism can predispose the host to colonization or infection by another organism. The medical community is recognizing the significance of polymicrobial diseases and the major types of microbial community interactions associated with human health and disease.Most of the isolated bacteria were susceptible to ciprofloxacin and ofloxacin. The two antibiotics are fluoroquinolones known for their high pKa and lipid solubility (Shoskes, 2001). Resistance to cotrimoxazole, augmentin, and amoxylin by the bacterial isolates could be due to genetic mutations or poor uptake of the antibiotics. Cotrimoxazole, in particular, is cheap and orally administered. This finding is in line with the report of Charanchi et al., (2012) in which the antimicrobial sensitivity patterns of urogenital bacteria among HIV-positive patients were studied. From this investigation, Staphylococcus aureus is the most prevalent microbes associated with infertility in male partners of infertile couples and the incidence of Escherichia coli, the major constituent of human intestinal flora, might be an indication of poor personal hygiene among the couples. Also, the use of fluoroquinolones (ciprofloxacin and ofloxacin) is recommended in treating bacterial infections associated with seminal fluid of infertile male. Nevertheless, proper antimicrobial susceptibility testing should be done to prevent emergence of resistant strains due to indiscriminate use of antibiotics and prescription without clear evidence of infection or adequate medical evidence. Polymicrobial infection among infertile male within the age range of 30 and 49 years could be controlled through measures such as health education on early presentation for medical treatment, drug compliance and campaign against multiple sex-partners.

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