Antibiogram and Occurence Rate of Bacteria Recovered from Pateints Attending a Fertility Clinic in Abeokuta, Nigeria

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ABSTRACT

Infertility is a growing problem in Sub-Saharan Africa with its attendant Medical and social problems. Our study is designed to examine the Microbial pattern of Urogenital specimen of patients attending a fertility clinic in Abeokuta, Ogun State, Nigeria. Three hundred and six patients attending the Federal Medical Center Abeokuta, fertility clinic were recruited for the study, comprising 108 males and 198 females. Samples collected were semen samples from male patients and Endocervical swab samples from female patients, pregnant female patients were excluded from the study. All samples were processed following standard Microbiological protocols and antibiotic susceptibility was done by disc diffusion following the Kirby-Bauer technique. Semen samples were assessed for Morphology and sperm concentration following standard protocols. An overall isolation rate of (17.8%) was obtained for all samples processed. A total of 306 subjects were recruited consisting of 108(35.3%) male subjects and 198(64.7%) female subjects, with an isolation rate of 48(56.5%) for bacteria and 37(43.5%) for fungi (Candida albicans). Male subjects gave an isolation rate of 15(17.6%) and females 33(68.8%) for bacteria and 37(100.0%) for Candida albicans. It showed that Candida albicans (43.5%) was the only fungal isolates recovered in this study. The isolation rate of various bacteria species showed that Escherichia coli 22(45.8%) was the most predominant, followed by Klebsiella pneumoniae 12(25.0%), Staphylococcus aureus 7(14.6%), and Pseudomonas aeruginosa 5(10.4%). Proteus mirabilis 1(2.1%) and Enterococcus feacalis 1(2.1%) was least prevalent. Isolation rate of isolates by pus cell size was 68(80.0%) for normal pus cell size and 17(20.0%) for large pus cell size. Enterococcus feacalis was not isolated from sperm with normal pus cell size. Proteus mirabilis and Pseudomonas aeruginosa was not isolated from sperm with large pus cell size. Azoospermia constituted 46.3% of male subjects tested, Oligozoospermia recorded 13.9% and 39.8% of subjects had normal sperm count. Bacteria isolations were highest in Azoospermic subjects with a rate of 59.3% and lowest in Oligozoospermic subjects with 6.7%. Antibiotic susceptibility showed a high activity for Ofloxacin 74.7% and Ciprofloxacin 72.7%, Tetracycline also displayed a high level of activity 72.3%, there was high level of resistance to Cefuroxime, and others showed average susceptibility. Our study shows a high bacteria isolation rate in patients attending fertility clinic at Abeokuta and a broad diversity of organisms in urogenital specimen, therefore better attention needs to be paid to detection and treatment of all forms of urogenital infections in couples attending fertility clinics in our environment.

Key Words

Urogenital, Infertility, Bacteria, Antibiotic susceptibility, Abeokuta.

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1 Introduction

Infertility can be defined as the biological inability of a man or a woman to contribute to conception. Infertility may also refer to the state of a woman who is unable to carry a pregnancy to full term (**Makar *et al.*, 2002**). Normally women experience a natural period of fertility before and during their ovulation period before returning to a natural state of infertility for the rest of their menstrual cycle (**Makar *et al.*, 2002**). Cause of infertility can be determined in about 90% of cases, but despite extensive investigations about 10% of couples never know why they cannot conceive. Between 10-30% of infertility cases have multiple causative factors, male and female infertility each account for about 30-40% of cases. In male, sperm deficit (quality and quantity) are usually responsible. Female infertility factors are more complex (**Makar *et al.*, 2002**).

Evidence has shown that urogenital infections in male and female if left untreated can lead to infertility. A good example is Clamydia trachomatis infections which are often asymptomatic in females care major causes of pelvic inflammatory disease (PID), tubal occlusion (Salpingitis), endometritis leading to infertility (**Hansfeild, 1998**). In male subjects previous studies have show a close relationship between prostititis, epidemitis, and sexually transmitted microorganisms and infertility (**Onemu *et al.*, 2010**). Also previous infection or

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existing infection of the male genital tract and assesory organs has been documented to increase the risk of infertility (***Deimer et al.*, 2003**). Clinical and experimental research has associated the recovery if bacteria isolates in semen to detioloration of spermatiogenisis and spermatozoa function which can ultimately lead to infertility (**Keck, 1998**). In sub-saharan Africa majority of cases male infertility have been traced to a previous genital tract infection or inflammation (**Onemu *et al.*, 2010**).

In Nigeria, studies have shown a relationship between positive bacteria semen cultures with poor semen quality (**Onemu and Ibeh, 2001; Emokpae *et al.*, 2005**). There is also evidence to show that increased rate of sexually transmitted infection in both males and females may contribute to the rise in infertility cases in Nigeria (**Alli *et al.*, 2011**). With the above mentioned facts we sought to investigate the occurrence rate of bacteria in urogenital samples from sexually active men and women with the objective of determining the level of isolation in relationship to infertility in Abeokuta metropolis.

2 Meterials and Methods

2.1 Study Population

A total of three hundred and six subjects, comprising 108 male and 198 female were recruited for the study all of them patients who attended the fertility clinic at Federal Medical Center Abeokuta Ogun State. Samples were collected from female patients were collected at the family planning clinic while male subjects produced semen sample from home, clinical assessment and examination were conducted at the fertility clinic by the attending physician. Pregnant women were excluded in the study.

2.2. Sample collection and Processing

Male subjects were given written or oral instruction concerning the collection and transport of semen samples. Samples were collected at the comfort of the patient's homes or a private room and transported to the laboratory within one hour. The sample was collected by masturbation and ejaculated into a clean wide mouth sterile container. High vaginal swab and Endocevical swab were collected from female patients at the family planning clinic of Fed Medical center Abeokuta and sent to the Microbiology laboratory immediately. Semen was analyzed microscopically for morphology and motility following the protocol WHO manual for the examination of semen (**WHO, 1999**). Parameters such as Appearance, Liquefaction, Viscosity and pH were recorded. Semen samples were examined under x400 magnification after the sample has been placed on a clean slide. Motility was assed by scoring either "a" Rapid progressive motility, "b" Slow progressive motility, "c" Non progressive motility and "d" Immotility. A minimum of 4 fields were viewed by a first and a second reader. Sperm concentration was determined by the WHO protocol (**WHO, 1999**) Sperm morphology was determined by making 2 smears and stained by papanicoulaou following the technique of **Meschede *et al.*** (**1993**). All samples including female endocervical swab samples were cultured on Mac Conkay agar, modified New York City agar and Chocolate agar. A wet preparation Microscopy was done for all swab samples (**Chessbrough, 1991**).

2.3. Identification of Isolates and Antibiotic susceptibility testing

Discrete colonies of isolates were picked and put into sterile peptone water, from which various biochemical tests were done to identify the organisms according to standard bacteriological practice (**Cheesbrough, 1991**). Antibiotic susceptibility testing was done using the Kirby Bauer technique for disk diffusion (**Cheesbrough, 1991**).

3 Results Analysis

Table 1 shows the frequency of occurrence of Isolates. It showed that *Candida albicans* (43.5%) was the only fungal isolates recovered in this study. The isolation rate of various bacteria species showed that *Escherichia coli* 22 (45.8%) was the most predominant, followed by *Klebsiella pneumoniae* 12 (25.0%), *Staphylococcus aureus* 7 (14.6%), and *Pseudomonas aeruginosa* 5 (10.4%). *Proteus mirabilis* 1 (2.1%) and *Enterococcus feacalis* 1 (2.1%) was least prevalent (Table 1).

Table 1. Frequency of occurrence of Isolates

Isolates	No. (%)
Candida albicans	37 (43.5)
Bacteria	48 (56.5)
Total	**85 (100.0)**

Bacteria Isolates	N=48
Escherichia coli	22 (45.8)
Enterococcus feacalis	1 (2.1)
Klebsiella pneumonia	12 (25.0)
Proteus mirabilis	1 (2.1)
Pseudomonas aeruginosa	5 (10.4)
Staphylococcus aureus	7 (14.6)
Total	**48 (56.5)**

Table 2 shows distribution of isolates in relation to sex. A total of 306 subjects were recruited consisting of 108 (35.3%) male subjects and 198 (64.7%) female subjects, with an isolation rate of 48 (56.5%) for bacteria and 37 (43.5%) for fungi (*Candida albicans*). Male subjects gave an isolation rate of 15 (17.6%) and females 33(68.8%) for bacteria and 37 (100.0%) for *Candida albicans* (Table 2).

Table 3 shows the distribution of isolates by pus cell size. Isolation rate of isolates by pus cell size was 68 (80.0%) for normal pus cell size and 17 (20.0%) for large pus cell size (Table 3). *Enterococcus feacalis* was not isolated from specimens with normal pus cell size. *Proteus mirabilis* and *Pseudomonas aeruginosa* was not isolated from specimens with large pus cell size (Table 3).

Table 4 shows the frequency of occurrence of bacteria isolates in relation to sperm cell concentration. It showed that 75.0% of the total number of bacterial isolates was recovered from those with Azoospermia. Isolation rate for Oligozoospermia (<19 x 10^6 cells/ml) was 1(2.1%) and for normal sperm count (>20 x 10^6 cell/ml) was 7 (14.6%) as shown in Table 4.

Table 5 shows the antibiotic

susceptibility pattern of the various isolates to commonly prescribed antibiotics. Values ranged from 74.7% susceptibility to Ofloxacin, 72.7% to Ciprofloxacin, 74.5% to Cotrimoxazole, 46.8% to Gentamycin and as low as 36.6% to Cefuroxime.

4 Discussion

Infertility is an increasing medical condition among married couples and also of concern among unmarried individuals in Nigeria. In Abeokuta there is also a steady increase in the attendance rate of patients with presumed infertility (Unpublished data). Lack of sufficient data in our environment concerning the relationship between cuture positive urogenital specimen and infertility viz a viz the relationship between the two prompted the conception of this study. A total of 306 subjects were studied comprising 34.5% male and 64.7% female, a total isolation rate of 85 (27.9%) with 48 (15.7%) bacteria and 37 (12.1%) fungi. This finding is similar to that of a study done in south eastern Nigeria (**Agbolahon *et al.*, 2007**).

In our study, *Candida albicans* was only isolated in female patients, this is contradictory to a study done in Benin city Nigeria which studied only male subjects and had an isolation rate of 5 (6.3%) in males (**Onemu *et al.*, 2011**). The high rate of Candia albicans isolated has been reported in our environment by several workers (**Isibor *et al.*, 2011; Nwadioha *et al.*, 2010**). The high rate of recovery of this organism has been attributed to the anatomy of the female genital tract as well as the very close proximity of the female genital tract and the anus which harbors candida.

Bacteria species distribution among subjects ranged from 22 (45.85%) for *Escherichia coli*, 12 (25%) for *Klebsiela pneumonia*, 5 (10.4%) for *Pseudomonas aureginosa* with the least prevalent organism *Proteus mirabilis* 1 (2%), *Staphylococcus aureus* gave 7 (14.5%). The bacteria distribution recorded in our study is in agreement with most studies of similar nature for instance a study by **Alli *et al.*** at Ibadan recorded a rate of

Table 2. Distribution of Isolates by sex

Isolates	No. (%)	Male (%)	Female (%)
Candida albicans	37 (43.5)	0 (0.0)	37 (100.0)
Bacteria	48 (56.5)	15 (31.3)	33 (68.7)
Total	**85 (100.0)**	**15 (17.6)**	**70 (82.4)**
Bacteria Isolates	**N=48**		
Escherichia coli	22 (45.8)	8 (36.4)	14 (63.6)
Enterococcus feacalis	1 (2.1)	0 (0.0)	1 (100.0)
Klebsiella pneumonia	12 (25.0)	3 (25.0)	9 (75.0)
Proteus mirabilis	1 (2.1)	0 (0.0)	1 (100.0)
Pseudomonas aeruginosa	5 (10.4)	0 (0.0)	5 (100.0)
Staphylococcus aureus	7 (14.6)	4 (57.1)	3 (42.9)
Total	**48 (56.5)**	**15 (31.3)**	**33 (68.7)**

Table 3. Distribution of Isolates by pus cell size

Isolates	No. (%)	Normal pus cell (%)	Large pus cell (%)
Candida albicans	37 (43.5)	31 (83.8)	6 (16.2)
Bacteria	48 (56.5)	37 (77.1)	11 (22.9)
Total	**85 (100.0)**	**68 (80.0)**	**17 (20.0)**
Bacteria Isolates	**N=48**		
Escherichia coli	22 (45.8)	17 (77.3)	5 (22.7)
Enterococcus feacalis	1 (2.1)	0 (0.0)	1 (100.0)
Klebsiella pneumonia	12 (25.0)	11 (91.7)	1 (8.3)
Proteus mirabilis	1 (2.1)	1 (100.0)	0 (0.0)
Pseudomonas aeruginosa	5 (10.4)	5 (100.0)	0 (0.0)
Staphylococcus aureus	7 (14.6)	3 (42.9)	4 (57.1)
Total	**48 (56.5)**	**15 (31.3)**	**11 (22.9)**

Table 4. Frequency of occurrence of Bacteria isolates by Sperm cell concentration

Bacteria Isolates	Sperm Concentration		
	Azoospermia N (%)	Oligozoospermia N (%)	Normalization N (%)
Escherichia coli	14 (24.1)	0 (0.0)	4 (11.6)
Enterococcus feacalis	1 (1.7)	0 (0.0)	0 (0.0)
Klebsiella pneumonia	10 (17.2)	0 (0.0)	2 (5.7)
Proteus mirabilis	1 (1.7)	0 (0.0)	0 (0.0)
Pseudomonas aeruginosa	5 (8.6)	0 (0.0)	0 (0.0)
Staphylococcus aureus	4 (6.8)	1 (6.7)	1 (2.7)
No growth	23 (40.7)	14 (93.3)	28 (80)

Key: Azoospermia(N)=58, Oligozoospermia(N)=15, Normalization(N)=35

Table 5. Antibiotic susceptibility pattern of various bacteria

Symbol code	Disc content (mg)	Resistance (%)	Intermediate (%)	Sensitive (%)
GEN	10	20 (42.5)	5 (10.6)	22 (46.8)
CXM	30	30 (63.8)	0 (0.0)	17 (36.2)
AMC	30	15 (31.9)	31 (31.9)	17 (36.2)
NIT	300	10 (21.3)	10 (21.3)	27 (57.4)
COT	25	30 (63.8)	5 (10.6)	25 (74.5)
NA	30	12 (25.5)	10 (21.3)	25 (53.2)
OFX	30	0 (0.0)	10 (21.3)	37 (74.7)
TET	30	13 (27.6)	0 (0.0)	34 (72.3)
CXC	5	15 (31.9)	10 (21.3)	22 (46.8)
AMX	25	18 (38.3)	6 (12.8)	24 (51.1)
CIP	5	0 (0.0)	10 (21.3)	37 (72.7)

25% isolation rate for Escherichia coli and *Klebseila pnuemoniae* (**Alli *et al.*, 2011**) and another study done at Lagos university teaching hospital recorded a rate of 12.1% for Esherichia coli (**Anorlu *et al.*, 2004**). The abundance of Esherichia coli and Klebseilla species can also be attributed to the close proximity of the female genital tract to the anus as these organisms are commensails of the gastrointestinal tract of humans collonising it mostly as normal flora unless in rare cases of immunosuppression where they become pathogenic.

Our study reveals that Azoospermic subjects constituted 53.7% of male population, Oligozoosspemia recorded 13.8% and normal subjects had 32.5%, this distribution is similar to a study done at Benin City which recoded a rate of 38.8% for Azoospermic and Oligozoospermic patients (Onemu et al 2010). This shows that following the strict criteria set by WHO (**WHO, 1999**) about 68% of our male study population are likely to have an unfavorable outcome with regards to achieving conception with a female partner, this follows suit to a similar study done in Lagos a low number of potential sperm donors in young adult males(**Akinrinola *et al.*, 2003**). Distribution of bacteria isolates according to sperm cell concentration revealed that, Azoospermic subjects recorded the highest isolation rate of 59.3% followed by Normal count and Oligozoospermic subjects having the lowest bacteria isolation rate. This distribution further agrees with other reports that relates recurrent bacteria infection to high risk of male infertility occurrence (**Onemu *et al.*, 2010**). The distribution of isolated organisms in relationship to pus cell size was also investigated details of result is shown in table 2, results were consistent with normal microscopic diagnostic creiteria in an infected sample (**Cheesbruogh, 1991**), with the highest isolation rate seen in large sized pus cell samples. Antibiotic susceptibility pattern is shown in table 4 reveals that the highest level of resistance was demonstrated by Cefuroxime with 63.8% while Ciprofloxacin and Ofloxacin recorded 0% resistance, sensitivity was highest in the Qunolones , with Ciprofloxacin having 72.7% and Ofloxacin 74.7%, Tetracyclin also displayed a high level of sensitivity to isolates tested with 72.3% sensitive. This shows the high activity of the Quinolones to pathogenic bacteria with a very low level of resistance development in this subpopulation of patients despite reports of abuse of this class of antibiotics. It is however worrysome to discover the high level of resistance recorded to a very potent 2nd generation Cephalosporin (Ceturoxime) in our study setting this further confirms the existence and increasing incidence of extended spectrum beta lactamase (ESBL) bacteria in Abeokuta as revealed by earlier studies (**Motayo *et al.*, 2011**).

5 Conclusion

Our study thereby reveals the presence of diverse pathogenic bacteria in samples of patients reporting for Infertility problems, thereby highlighting the importance of detection and prompt treatment of all forms of Gynaecological and Urogenital infections in couples attempting to achieving conception and looking forward to bringing fort a new life in Abeokuta metropolis.

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