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Antimicrobial Assessment of Bacterial and Fungal Isolates from Women with Infertility Challenges in Imo State, Nigeria

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Abstract	Original Research Article

A study was conducted to isolate and identify bacterial and fungal microbes associated with women with infertility in Imo State, Nigeria, as well as conduct an antimicrobial assessment of the isolates. The prevalence of these isolates were partitioned based on age, marital status, number of sexual partners and contraceptive use on one hand, as well as level of knowledge, attitude and practices of women to infertility on the other hand. Six species of bacteria and one fungal specie was isolated from the Endo Cervical Swabs (ECS) of women which includes *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species*, *Proteus mirabilis*, *Streptococcus pyogenes* and *Neisseria gonorrhoeae*, while the fungal specie is *Candida albicans*. While *Staph. aureus* remains the most significant pathogenic microbe among the women (31.4%), *Neisseria gonorrhoeae* had the least infection (5.8%). *Candida albicans* was the only fungal specie infecting 13.9% of the women. While the seven pathogens infected all the age groups tested, *Staph.aureus, Neisseria gonorrhoeae* and *Strept.pyogen* were more prevalent in age group 18-25years. In the three marital status considered i.e. married, separated and widowed, the separated women (36.36%) were predominantly infected with *Staph.aureus* and *E.coli*, followed by widowed women, while the married ones were least infected. Meanwhile the women with 3 sex partners had the highest infection of the pathogenic isolates (33.33%). The antibiotic susceptibility test revealed that Ciprofloxacin and L-flox (99% & 98%) appear to be the most effective invitro antibiotic for all the bacterial isolates. Fluzoral (84.20%) was most susceptible to Candida albicans. In terms of attitude and practices of women to infertility, the study revealed that men are mostly attributed as the main source of these pathogens and are blamed for difficulty/inability to conceive.

Keywords: Endo-cervices, Microbial, infertility, Women, Attitude.

INTRODUCTION

Infertility is defined according to the WHO as the inability to establish a pregnancy within two years for couples of reproductive age who are having intercourse without contraception [1]. It is further defined by [2] as the inability of a couple to conceive after 12 months of unprotected sexual intercourse. Primary infertility refers to those who have never been pregnant while secondary infertility refers to those who have had at least one proven pregnancy (including an abortion). Infertility is currently regarded as a major public health problem in Nigeria. In some rural parts of Nigeria, approximately 30% of couples may have difficulties in achieving a desired conception [3]. This rate is also becoming prevalent in urban communities, cities and towns. Infertility of couples affects 19% of the general population [4] while in Nigerian communities, about 9.2% and 21.1% represent primary and secondary infertility respectively [3]. In a similar study in South-Eastern Nigeria, 65% and 35% prevalent rate were reported for primary and secondary infertility respectively [5]. The causes of infertility have been divided into four major categories according to Seyed *et al*., (2007): 1) The female factor 2) The male factor 3) Combined factors and 4) Unexplained infertility. Infection remains one of the major causes of infertility

either in men or in women. Bacterial and fungal infections have been recognized as having an association with infertility [6, 7]. However, many investigations have linked the possibility that those infections of the reproductive tract that are subclinical might be etiological factors in unexplained infertility.

This study therefore aims at assessing and identifying the bacterial and fungal isolates from endo cervices of women with fertility difficulty in Imo State. Specifically, the study describes the aerobic and facultative anaerobic micro- organisms colonizing the endo cervices of women with infertility due to female factor and unexplained infertility; and the antibiotic sensitivity of some of the isolates. Also to determine the age, marital status, number of sexual partner and contraceptive use related prevalence of bacteria and fungi among women with fertility problems in Imo State Nigeria, as well as determine the level of knowledge, attitude and practices of the respondents to infertility issues.

MATERIALS AND METHODS

Study area
This study was carried out mainly at the Imo State University Teaching Hospital, Orlu, Nigeria, and partly at Federal Medical Centre Owerri and General Hospital Umuguma, Nigeria. Imo State is situated in South-eastern Nigeria and it lies between Lat. 5^0 30, 6^0 15N and Longitude $6^0$38, $7^0$18E of equator. The mean annual rainfall is 250mm, temperature range is 26.5 27.5 0C and humidity range of 70 80%. There are two tertiary health institutions in the State - Federal Medical Centre Owerri and Imo State University Teaching Hospital Orlu as well as about 13 General Hospitals.

Study samples
The samples used for this study were Endo-Cervical Smear (ECS) collected from women with fertility difficulties in Imo State.

Test organisms
The organisms used for this study are bacteria and fungi species isolated from Endo-Cervical Smear (ECS) of women with fertility difficulty in Imo State.

Test media
The media used for this study are oxoid brand of MacConkey agar (without salt), Blood agar and Nutrient agar, Biotech brand of Mueller Hington agar, Peptone water and Saboraud dextrose agar.

Instrument for data collection
The instrument used for data collection in this study is a structured questionnaire comprising 3 sections: Section A: Socio- demographic data comprising of 10 questions, Section B: Fertility study comprising of 10 questions and Section C: Knowledge, attitude and practices (KAP) comprising of 11 questions.

Selection of respondents
A total of 411 female respondents aged between 18 to 50 years were selected for this study out of 600 respondents proposed. The respondents were selected from out-patients and in- patients records and wards at Imo State University Teaching Hospital Orlu. The respondents were approached on individual basis within the working hours and the objectives of the study were explained to them. Their willingness to participate in the study was requested and their consent documented. Those who indicated willingness to participate in the study were enlisted and given questionnaires to complete as well as schedule time for sample collection.

The questionnaires were administered through person-to-person contact after explaining the objectives of the study to each respondent and obtaining her willingness to participate in the study. Two medical laboratory scientists working in the Laboratory department of the Hospital were recruited as Field assistants. The willing respondents completed the questionnaires with the required information and submitted it on the spot to the field assistants. Each respondent was directed to the Gynaecology Department of the hospital where their Endo cervical smear (ECS) shall be collected on the date scheduled for them, for microbial analysis.

Method of sample collection
The test samples (ECS) were collected using sterile swab stick and sterile disposable speculum. The Gynaecologist in charge collected the samples from each respondent and send it to the Microbiology Department of the hospital for immediate analysis. Each collected sample was labelled with the respondent's index number indicated on the research questionnaire and consent forms. The swab samples were analysed within 3 minutes of collection.

Methods of sterilization of materials and preparation of media
Asceptic standard was ensured in this study. The materials used were sterilized by standard laboratory methods as described by [8, 9]. The media used for this study were prepared according to the manufacturer s instructions on the product labels.

Identification of test organisms
The test organisms isolated from ECS were identified using their growth morphology on the different culture media, grams staining, motility test (Hanging drop method), and Biochemical identification tests: catalase, coagulase, oxidase, citrate utilization, indole production, urease production, Hydrogen sulphide production, Nitrate/Nitrite reduction, Methyl red, Voges Proskeur, and sugar fermentation tests as described by [8, 9]. The findings were compared with Bergy's manual of determinative bacteriology (Holt, 1998).

Antimicrobial effects of test samples
The antimicrobial effects of the selected antibiotics and antifungal drugs on microbial isolates from ECS of women with fertility problems were examined using commercially prepared antibiotic discs for bacterial isolates and Gel diffusion technique for fungal isolates as described by [9].

Method of analysis of data
The data obtained from the questionnaire study were analysed using descriptive statistics such as percentages, means, frequencies and charts.

RESULTS

The results of isolation and identification of bacterial and fungal microbes associated with women with infertility challenges in Imo state shows that out of 600 respondents identified for the study, 507 (84.5%) completed and submitted the questionnaires, with 411 (68.5%) responding for ECS collection and isolation of bacterial and fungal pathogens. Six species of bacteria were isolated from the respondents comprising of *Escherichia coli, Staphylococcus aureus, Klebsiella species, Neisserria gonorrhoeae, Streptococcus pyogenes, Proteus mirabilis* and one fungal pathogen - *Candida species*, with *Staphylococcus aureus* being the predominant isolate (31.4%) (Table 1).

Table 1: Microbial Infections Amongst Women with Infertility

Isolate	Number Isolated	Gram Stain	Shape	Growth on Media		Motility Test	Germ Tube Test	Possible Organism
				NA*	SDA**			
A	129 (31.4%)	+	Cocci	+	-	-	-	*Staphyloc aureus*
B	39 (9.5%)	+	Cocci	+	-	-	-	*Streptoco pyogenes*
C	57 (13.9%)	+		-	+	-	+	*Candida albicans*
D	72 (17.5%)	-	Rod	+	-	+	-	*Escherich coli*
E	39 (9.5%)	-	Rod	+	-	+	-	*Proteus mirabilis*
F	51 (12.4%)	-	Rod	+	-	-	-	*Klebsiella spp*
G	24 (5.8%)	-	Cocci	+	-	-	-	*Neisseria gonorrhoea*
Total	411							

NA* = Nutrient agar + Antifungal (Nizoral) Drug
SDA** = Saboraud Dextrose agar + Antibiotic Drug.

Age, marital status, sexual partner and contraceptive use related prevalence of bacteria and fungi among women with fertility problems.

Age prevalence of microbial isolates found in the women shows that amongst the age groups viz 18-25years (24 respondents and 5.8%), 26-35years (132 respondents and 33.1%), 36-45years (165 respondents and 40.1%) and 46-50years (90 respondents and 21.9%) studied, those within the age bracket of 18-25years are the most predominantly affected by *Staph.aureus, Neisseri gonorrhoea* and *Strept.pyogen*, while those within the age bracket of 46-50years were mostly affected by *Esch.coli* and *Candida albicans* (Figure 2).
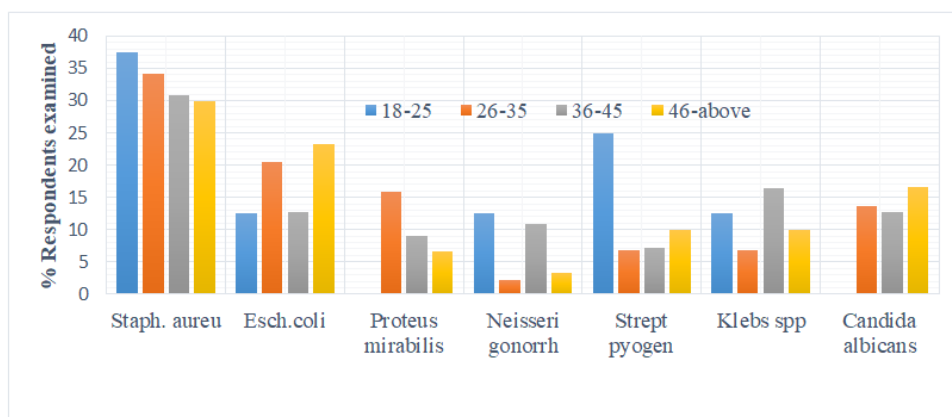

Figure 1: Endo cervical Bacterial Isolates from women with fertility challenges in Imo State Nigeria due to Age

For marital status related prevalence of microbial infection among women with infertility challenges from the 411 respondents examined, 369 (89.78%) were married, 33 (8.03%) were separated and 9 (2.19%) were widowed (Figure 3). The percentage spread of the isolates amongst the identified categories,

shows that the separated respondents were mostly affected by *Staph.aureus* and *Esch.coli,* while the widowed were mostly affected by *Candida.albicans*
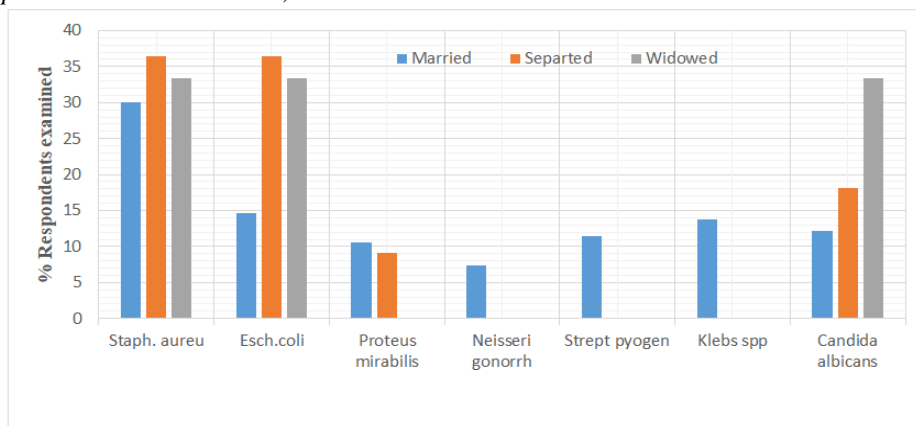

Figure 2: Endo cervical Bacterial Isolates from women with fertility challenges in Imo State Nigeria due to Marital Status

Number of sexual partner related prevalence of microbial infection among women with infertility problems shows that out of 411 respondents examined, 309 (75.18%) had one sex partner, 93 (22.63%) had 2 sex partners while 9 (2.19%) had 3 or more sex partners with the spread of the identified predominant isolates as shown (Figure 3). Those with three or more sexual partners were predominantly affected with *Staph.aureus, Neisseri.gonorrh* and *Kleps spp*. Those with two partners were mostly affected with *Strep.pyogen*, while those with single partner was affected mostly by *Esch.coli* and *Candida albicans*.
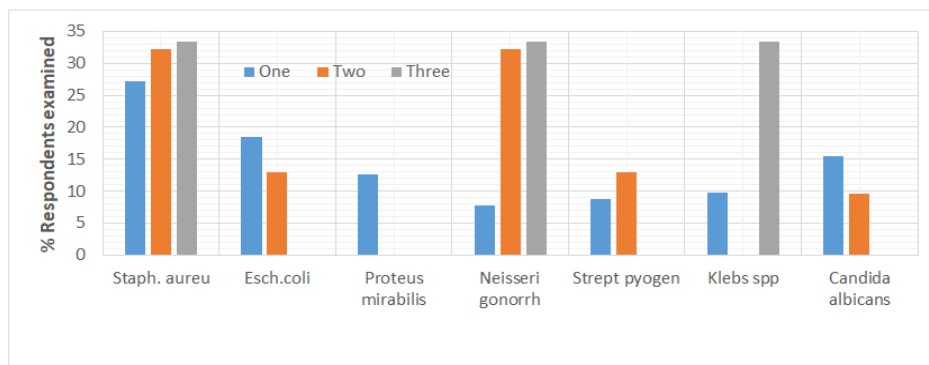

Figure 3: Endo cervical Bacterial Isolates from women with fertility challenges in Imo State Nigeria due to Number of Sexual Partners

Contraceptive use related prevalence of microbial infection among women with infertility problems of 411 respondents examined shows that 87 (20.44%) use condom occasionally while 324 (78.83%) do not use it at all. Laboratory examination of their ECS showed that the occasional users were mostly affected by *Staph.aureus, Neisseri.gonorrh, Strept pyogen* and *Candida albicans*, while non-users of contraceptive where mostly infected by *Esch.coli, Proteus.mirabilis,* and *Klebs spp* (Figure 4).
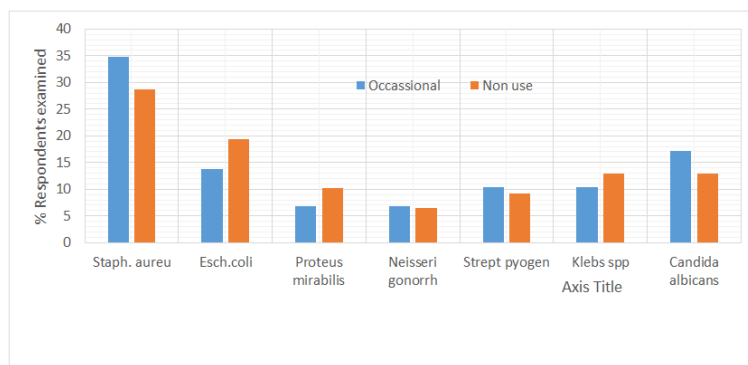

Figure 4: Endo cervical Bacterial Isolates from women with fertility challenges in Imo State Nigeria due to Contraceptive Usage

Antimicrobial susceptibility pattern on reproductive tract pathogens results shows that both bacterial and fungal pathogens were susceptible to conventional antibiotics and antifungal drugs used in Nigerian Hospitals such a Ciprofloxacin, Levofloxacin, Streptomycin, Ceftriaxone, Ofloxacin, Sparbact, Pefloxacin, Lincocin and Ceporex; with Ciprofloxacin, Levofloxacin, Ceftriaxone and Ofloxacine showing more inhibitory actions against bacterial pathogens than others (Figure 5a and 5b).
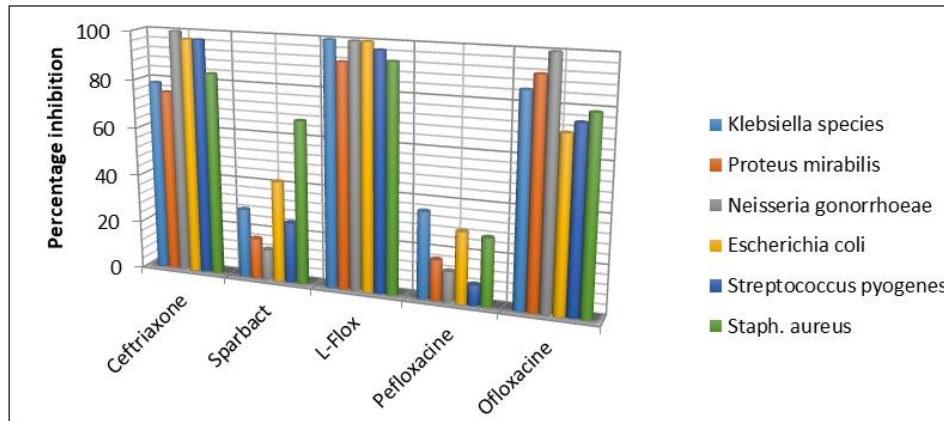

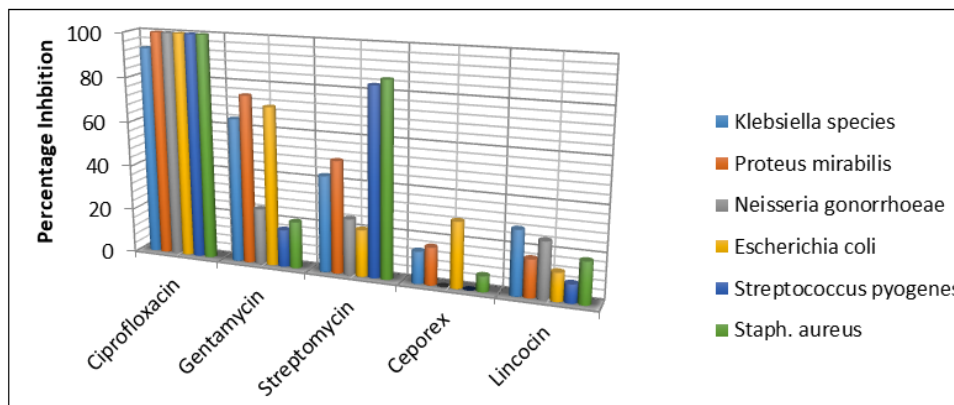**Figure 5a: Antibiotic Susceptibility pattern of Bacteria Isolates**

Figure 5b: Antibiotic Susceptibility pattern of Bacteria Isolates

Antifungal Susceptibility of Fungal Isolates

This study revealed that out of 57 *Candida albicans* isolates tested for growth inhibitory effects with three anti-fungal drugs- Fluzoral, Nystatin and Nizoral; Fluzoral exhibited growth inhibitory effect on 21 (84.2%) with mean zone of growth inhibition of 14.7mm while Nystatin exhibited growth inhibitory effects on 17 (63.2%) isolates with mean zone of growth inhibition of 10.1mm. Nirozal exhibited growth inhibitory effect on 19 (78.9%) with mean zone of growth inhibition of 11.6mm (Figure 6). This shows that Fluzoral exhibited the highest antifungal activity against the isolates while Nystatin exhibited the least.
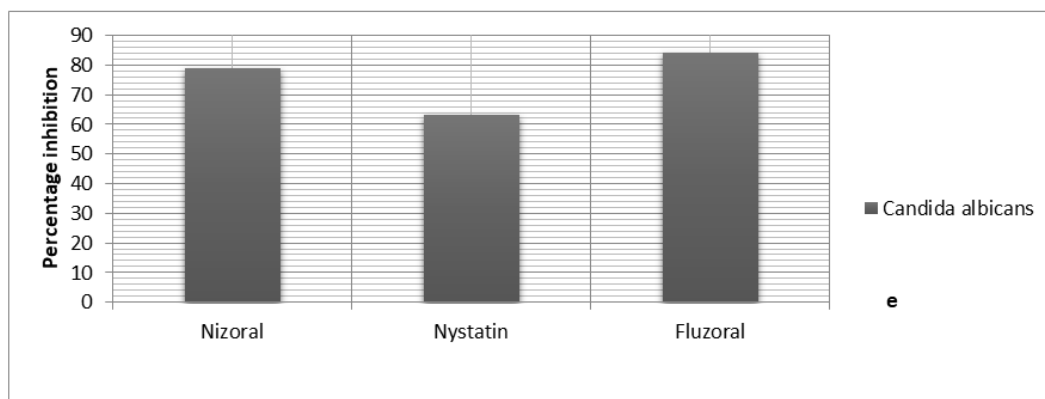

Figure 6: Antifungal Susceptibility pattern of Fungal Isolates

Knowledge, attitude and practice of respondents on infertility issues

Knowledge, attitude and practice of respondents on issues of infertility shows that majority of the respondents had not gone for Ovulation test and or Hormone assay before (Figure 7), while a slight majority has had a diagnosis of urinary Tract Infection before. Meanwhile, majority of the respondents blamed the men for their inability to conceive, while majority do not believe that traditional medicines are better than

conventional drugs in treatment of infertility. The respondents also believe that it is not the men that should determine how and when to have sex with their wife, while the majority believes that 'it depends' on the question 'should the man know and predict the wife's ovulation/ovarian cycle?'. However, almost all the respondents believed that the men are to be blamed for the transmission and spread of Urinary Tract Infection to their wives.
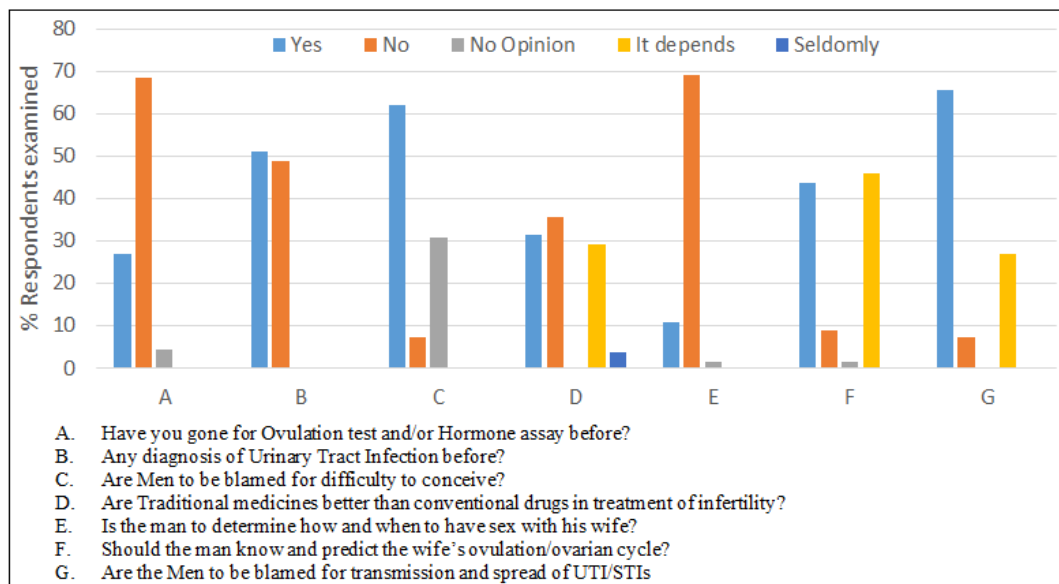

A. Have you gone for Ovulation test and/or Hormone assay before?
B. Any diagnosis of Urinary Tract Infection before?
C. Are Men to be blamed for difficulty to conceive?
D. Are Traditional medicines better than conventional drugs in treatment of infertility?
E. Is the man to determine how and when to have sex with his wife?
F. Should the man know and predict the wife's ovulation/ovarian cycle?
G. Are the Men to be blamed for transmission and spread of UTI/STIs

Figure 7: Knowledge, attitude and practice of respondents on infertility

DISCUSSION

The 31.4% rate of *Staph. aureus* infection reported in this study, shows that most of the women were infected with *Staph. aureus* while fewer were infected with *Neisseria gonorrhoeae*. This is in line with the findings of [10] who hypothesized that infertile women would be associated with high rate of cervical and vaginal colonization with microorganisms. The major pathogens he isolated included *Candida albicans*, *Staph. aureus* and *Neisseria gonorrhoeae*. Our result suggests that women with infertility issues have higher rates of infection with *Staph aureus, E. coli*, *Klebb spp*, *Proteus mirabilis*, *Streptococcus pyogenes* and *Neisseria gonorrhoeae* in this order [10] however reported that there is no statistically significant difference between infertile women and fertile women in terms of rate of cervical colonization with the major pathogens [11] also isolated *Staphylococcus* species being the predominant organism in ECS samples taken from cervices of women with unexplained infertility. This is also in accordance with earlier studies by [12] where high prevalence of *Staphylococcus* (90%) in cervical isolates had been reported.

The age related prevalence of infection among women with infertility issues reveals that the majority of the women sampled were aged between 36-45 years. In relation to isolates from infected women, those

between 18-25 years were mostly infected with *Staph.aureus, Neisseri gonorrhoea* and *Strept.pyogen*, followed by those between 26-35years. This can be ascribed to the youthful age of this group who might still be having extra marital affairs and keeping multiple partners [10] had earlier reported *Staph. aureus, Neisseria gonorrheae* and *Streptococcus spp* as being the most commonly isolated microorganisms in women with infertility who are of mean age 20.5-21.0 years. This is in contrast to an earlier report by [3] who reported that women with secondary infertility have higher rates of infection with *Neisseria gonorrhoeae, Candida albicans* and *Staph aureus* than women with primary infertility and could be ascribed to the effect of age, since women with secondary infertility were on average older than women with primary infertility.

Marital status related prevalence of infection which were grouped into married, separated and widowed showed that Married women were more in number, while the widowed are the least. In relation to the isolated microbes, separated women were more predominantly infected with *Staph. aureus and E. coli* followed by widowed women, while widowed women were more infected with *Candida albicans*. This suggests that separation or demise of their life partner may have resulted in having multiple sex partners thereby exposing them to high microbial infection.

However, *Proteus mirabilis*, *Neisseria gonorrhoeae, Strept.pyogens* and *Klebbs spp* were more prevalent in married women.

Meanwhile number of sex partner-related prevalence of infection has shown that those with upto three sex partners had the highest infection (33.33%) of *Staph aureus, Neiserria gonorrhea* and *Klebbs spp* respectively while those with 2 sex partners had more prevalence of *Strept pyogen.* This result indicates that the more the number of sexual partners, the higher the microbial infection.

Contraceptive use (Condom)-related prevalence of microbial infection among women with infertility problems reveals that those who do not use condom were infected more with *E.coli, Proteus mirabilis* and *Klebbs spp* while those who use it occasionally were more infected with *Staph aureus, Neisseria gonorrhea, Strept pyogen* and *Candida albicans*. This suggests that occasional users are those who may have other sex partners outside their husbands. In addition, it is an indication that condoms do not give absolute protection from microbial infection; hence, faithfulness to one sexual partner (particularly the spouse) is encouraged.

On the comparative analysis of antibiotic susceptibility on test bacterial isolates, it showed that of the 10 antibiotics tested on the 6 bacterial isolates, Ciprofloxacin had the highest susceptibility on the isolates, followed by L-flox. Ceporex showed the least susceptibility followed by Lincocin. The study also revealed that *Staph aureus, Strept pyogens* and *Neiserria gonorrhoeae* were resistant to Gentamycin, a common antibiotic. It supports the findings of [13] which suggests that due to intense antibiotic pressure occasioned by the widespread and inappropriate use of antibiotics by the users and the production of beta lactamases by the isolates, these results in the acute resistance. Meanwhile the antifungal susceptibility of fungal isolates reveals that Fluzoral was more susceptible to *Candida albicans*. Although the others were quite susceptible, Fluzoral remains the most active. This suggests that women with infertility issues can effectively eliminate *Candida albicans* invivo with Fluzoral.

The attitude and knowledge of respondents with infertility issues shows that men were blamed for difficulty/inability to conceive, it shows that majority of men who have infections without treatment may result in low sperm counts hence the blame for inability to conceive. This suggests that respondents strongly believed that infection is the main cause of lows sperm count and it is in accordance with the report by [4] who posited that genital infections seem to be the most appropriate explanation for high incidence of abnormal semen parameters. Also [14] reported that microbial infection of the semen is major causes of male infertility.

CONCLUSION

- This study revealed that *Staphylococcus aureus* remains the major pathogen isolated in women with infertility issues in Imo State.
- Amongst the age groups, relative to the prevalence of bacterial and fungal isolates, women in the age group of 18-25years were mostly infected with most of the isolates signifying a high prevalent group. While the separated and widowed group exhibited more prevalence of *Staph aureus, E.coli* and *Candida albicans*; the married ones showed more prevalence for the others. Meanwhile, having more than one sex partner predisposes one to more pathogenic infection.
- Although there is media hype on the use of condoms for protection against infections, this study has revealed that occasional users had a significant infection of these pathogenic microbes.
- This study has also shown that Ciprofloxacin and L-flox are the most susceptible antibiotics on all the bacterial isolates while Fluzoral proved to be the most susceptible antifungal compound on the fungal isolates.
- The knowledge, attitude and practice of respondents with infertility issues also revealed many findings. We discovered that men are blamed by women for inability to conceive as well as being the main agent for the transmission of STIs and UTIs. Many women in Imo State do not have the awareness of ovulation test and or hormonal assays as a tool to identify causes of infertility which could be due to improper ovulation and or hormonal imbalance. We also identified that whether the type of infertility is primary or secondary, women of Imo State always have one sex partner at a time.

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