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Evaluation of the bacterial agents associated with PID among women of reproductive age at Kampala International University Teaching Hospital.

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ABSTRACT

Pelvic inflammatory disease (PID) is major health problem in developed and developing country involving more young women. It is associated with high rate of female reproductive health morbidity; it can complicate with ectopic pregnancy, infertility and chronic pelvic pain. A poor response therapy increases the likelihood of these complications; this could be due to an increase in antimicrobial resistant pathogens. The aim of this research was to determine common bacterial pathogens from endocervical swab of women with PID at Kampala International University Teaching Hospital. This was a cross-sectional study conducted among women who attended gynecology clinic at Kampala International University Teaching Hospital. Consecutive enrolment of 324 participants who consented to participate was done daily until a required sample size was realized from November 2019 to January 2020. Structured questionnaires were used to collect data on associated factors; endocervical swab was taken from patient clinically diagnosed with PID. Culturing for colony characteristics followed by Gram stain was used for provisional identity of pathogenic bacteria. Further identification was done by a set of biochemical tests. Data was analyzed using STATA VERSION 14.2. Not being educated, having two or more sexual partners, previous history of PID and induced abortion, also the previous use of contraceptives specifically the use of IUD ,were all significantly associated with Pelvic inflammatory disease (P value <0.05). The isolated bacteria was *Staphylococcus aureus* and the least-prevalent pathogen was *citrobacter species*. In conclusion, *Staphylococcus aureus* is the commonest organism isolated from the endocervical swab of patients with PID.
Keywords: Bacteriology, Pelvic, Inflammatory, Disease and Women

INTRODUCTION

Pelvic inflammatory disease is a condition caused by various microorganisms and present with severe clinical presentation, poor response to therapy, increased risk of chronic pelvic pain, ectopic pregnancy and infertility [1]. Evidence emanating from various centers indicates that *Neisseria gonorrhea* and *Chlamydia trachomatis* are the commonest organisms responsible for pelvic inflammatory disease worldwide and they are predominantly sexually transmitted [2]. Pelvic inflammatory disease is predominantly sexually transmitted and caused by specific pathogens within a specific population, and other organisms less commonly seen in that population. Bacterial vaginosis (BV) may lead to vaginal inflammation, which could facilitate ascending infection with BV-associated organisms such as *Gardenella vaginalis.* [3] in Zambia did a study on female genital tuberculosis and found that *Mycobacterium tuberculosis* and *Schistosoma* species may appear the cause of chronic PID. This was seen in 5-10% of women with infertility issues in the study above. [4] in the study on identification of serious complications of pelvic inflammatory desease in the USA, stated that *Neisseria gonorrhea* was not the primary organism associated with pelvic inflammatory disease, but that it remained the second most frequently sexually transmitted disease after chlamydial infection [4].

[1] in their study on bacterial isolates associated with pelvic inflammatory disease among female patients attending some hospitals in Abuja Nigeria, found that the most common bacteria isolated using endocervical swabs of patient suspected with PID were *Staphylococcus aureus* at 34%, followed by *streptococcus pyogenes* 21%, *streptococcus faecalis* 17%, *Klebsiella pneumonia, Pseudomonas,* and *Proteus rettgers* had respectively 9%, 6% and 4%; they also reported that the Antibiogram patterns of pathogenic isolates in endocervical swabs revealed varied resistance to most of the antibiotics employed. Cefotaxim was established in this study as the best antimicrobial agent for treatment of pelvic inflammatory disease due to Gram-positive and Gram-negative bacteria isolated from the women examined. [5] in their study on *Mycoplasma genitalium*, an emerging cause of pelvic inflammatory disease done in USA reported that different researches showed *Mycoplasma genitalium* as a leading cause of nongonoccal and nonchlamidial cause of pelvic inflammatory disease and has been associated with resistance and treatment failure by cefoxitin and doxycycline.

[6] in their study on *Mycoplasma genitalium*: An organism commonly associated with cervicitis among West African sex workers, the prevalence of *Mycoplasma genitalium* infection associated with pelvic inflammatory disease among female sex workers was 26.3% , compared to 16.0% for *Neisseria gonorrhea*, and 3.4% for *Chlamydia trachomatis*. [7] in Cameroon in their study on prevalence and bacterial pathogens associated with PID showed also that the most common bacteria isolated in endocervical samples of women with pelvic inflammatory disease were genital tract Mycoplasma with a prevalence of 54.8%, *Neisseria gonorrhea* and *Chlamydia trachomatis* were not isolated in endocervical swabs of their participants [6].

There are no standardized techniques of the susceptibility testing Chlamydia *trachomatis* due to variability of types of cell culture system used, inoculums size, timing and duration of antibiotic application to the cell culture. It is unclear whether the end points measured during in vitro susceptibility testing are relevant when applied to a naturally occurring infection with dividing and non-dividing bacteria that infect multiple cell types in vivo [8]. [9] in their study on prevalence and pattern of bacterial isolates in cases of pelvic inflammatory disease at a Tertiary Hospital in Osogbo, Nigeria showed that the most common bacteria isolate in women with pelvic inflammatory disease were *Staphylococcus aureus, E. coli, Pseudomonas aeroginosa* and *Klebsiella pneumonia* these microorganism were more sensitive to gentamicin, cefuroxim, and erythromycin. In the same country [10], in the study done in Maiduguri, *Staphylococcus aureus* were also the predominant bacteria isolates in endocervical swabs followed by *E. coli* and *Neisseria gonorrhea* these bacteria were more susceptible to ofloxacin, ceftazidin, rifampicin and less susceptibility was recorded with trimethoprim-sulthamethoxazole, and ampicillin.

[11], in their study on complications of pelvic inflammatory disease in Kenyatta national referral hospital reported that patient with tubo ovarian abscess were selected represented 22% of all the gynecological admission, and when the samples from these abscesses were taken for bacterial identification, it was found that pathogens were isolated in 82.1% of participant and the most common was enterobacteriaceae in 50% of cases, closely resembled to gut flora. In this same country [12] in their study on detection of novel organism associated with salpingitis using of 16S DNA polymerase reaction, they enrolled 44 patients who had laparoscopically confirmed salpingitis, the specimen from the fallopian tubes were taken and subjected to a broad range of 16S DNA polymerase chain reaction it identified novel model, possibly uncultivable bacteria associated with salpingitis, it was identified as bacterial phylotype closely related to *Leptotrechia, Neisseria Gonorrhea* was isolated in 10%.

In Uganda, there is paucity of current published information about the causative microbial agent for PID. However, the Study done in Mbarara Regional Referral hospital by [12,13,14,15] on screening for genital Chlamydia in reproductive aged women in western Uganda found the prevalence of genital Chlamydia was 26%. The study did not consider the other microorgasms that can cause PID as this is known as a polymicrobiol condition and, the susceptibility of this microorganism was not established because the study was using the indirect exam to identify the patiens who were carriers on Chlamydia antibody. The above literature supports the fact that the causative agent for pelvic inflammatory disease can vary depending upon geographic location and also in Uganda studies are still needed to establish the causative pathogen of pelvic inflammatory disease with their antibacterial susceptibility patterns.

Aim of the study

To determine the bacterial agents associated with PID among women of reproductive age presenting at Kampala International University Teaching Hospital.

Specific objective

1. To determine the common bacterial pathogens and antibacterial susceptibility pattern among women with pelvic inflammatory disease attending gynecology clinic at Kampala University International Teaching Hospital.

Research question

1. What are the characteristics of the most bacterial pathogens implicated in pelvic inflammatory disease among women attending gynecology clinic at Kampala International University Teaching Hospital.

METHODOLOGY

Study design

This was a cross sectional study. Laboratory investigations was done to achieve the prevalence pattern in women with pelvic inflammatory disease attending gynecology clinic at Kampala international university teaching hospital. Association between PID and different factors was established.

Study area

The study was conducted at Kampala International University Teaching Hospital found in Ishaka Bushenyi Municipality at approximately 60km from Mbarara town along Mbarara Kasese highway. The study population were coming from the districts of Bushenyi, Rubirizi, Sheema, and Mitooma as well as from the nearby districts.

Study site

The study was conducted in the gynecological outpatient clinic in the department of obstetrics and gynecology. The department runs daily from Monday to Friday and receives an average of 20 patients of which 25% are diagnosed with pelvic inflammatory disease. It is run by specialists, residents, intern doctors and midwives. The main laboratory of KIU-TH has a microbiology section which is well equipped and staffed to carry out culture and sensitivity as well as other microbiological tests like growth and isolation of several microorganisms. The equipment that helps to perform different exams within the microbiology laboratory in this hospital includes incubator, microscope, hot air oven, refrigerator, autoclave, and safety cabinet and gas cylinder. It is also well facilitated with enough stains which help in identifying different microorganisms.

Study population

The study populations were all women of reproductive age in the catchment area

Target population

All women of reproductive age attending gynecology clinic at Kampala international university teaching hospital shall be considered for inclusion in this study.

Accessible population

All women of reproductive age attending gynecology clinic who meet the inclusion criteria of the study

Selection criteria

Inclusion criteria

All the women at the reproductive age attending gynecology clinic of Kampala international university teaching hospital as well as emancipated minors.

Exclusion criteria

Women on antibiotics, pregnant women, unconscious patients who cannot consent and minors were excluded from the study.

Sample size determination

$$n = \frac{z^2 pq}{d^2} \longrightarrow n = \frac{(Z_\alpha)^2 x\, p(1-p)}{(d)^2}$$

Where;
n = Desired sample size
z = Standard normal deviate at 95% level of confidence; z= 1.96
p = prevalence of Chlamydia trachomatis in reproductive aged women , using a study done in Mbarara by [12]
d=Level of precision= 0.05

$$n = \frac{(1.96)^2 - 0.2(1-0.2)}{(0.05)^2}$$

N_2= 295

Sampling technique

Consecutive sampling method was used to select participants who consented to be part of the study. All the women of reproductive age who met the inclusion criteria was invited to participate in the study, the participants was enrolled according to their order of arrival in gynecology clinic and this was carried out on a daily basis until the required sample size was achieved.

Data collection instruments

A pretested questionnaire was administered to each participant who consented to participate to the study in order to collect information on socio-demographic, gynecological and sexual behavior factors that related to the development of pelvic inflammatory disease in. A detailed history was taken in English, translated in local language where necessary for women who could not understand English physical examination was carried out and the endocervical sample was taken from patient with symptoms and of PID in order to achieve all the objectives.

Study procedure

History taking:

Women of reproductive age who attended gynecology clinic of Kampala international university teaching hospital were informed about the study, a written consent were sought then, and demographic data were inquired. Their chief complaints were taken and detailed history to look for symptoms and risk factors of developing pelvic inflammatory disease.

Physical examination and sample collection

Patient were counseled for the examination a written consent was sought and signed then a physical examination for features of pelvic inflammatory disease which are; lower abdominal tenderness, adnexal tenderness and cervical motion tenderness. The patient were put on examination bed in lithotomy position, vulva was inspected for the presence of any discharge. A sterile speculum was inserted to look for the presence of cervical discharge. During this time a sterile swab stick was used, to collect the

endocervical sample, the sterile swab was inserted in the endocervical canal 20 to 30 millimeters and rotated at 360^0 on the endocervical walls, immediately swab was put in the amies transport medium to ensure the possibility of capturing all the bacteria [1]. This sample was collected by the principle investigator in the presence of a female nurse as a research assistant, it was labeled with patient`s serial number and taken to the laboratory by the research assistant for immediate analysis of the specimen.

The patient was given treatment according to Uganda clinical guideline as the researcher continued to follow up the result in the laboratory for a period of 72 hours. Laparoscopy is not going to be considered since it is not available in the research setting.

Sample processing and analysis

Isolation

Samples collected using a sterile procedure with the endocervical swab stick was inoculated on blood agar, chocolate agar, Mac Conkey agar, Thayer Martin medium, and different biochemical tests were used. After, they were incubated both aerobically and anaerobically at 37^oC for 24-48hrs. Colony morphology were observed according to shape, size, elevation, margin and surface characteristics. Rapid diagnostic test was used in order to identify the *Chlamydia trachomatis* antibody careers within the endocervical sample of the participants, the isolation of Chlamydia which uses living cells (McCoy cell) was not done due lack of this specific media to culture *Chlamydia trachomatis,* this rapid Chlamydia test was used to determine the pourcentage of Chlamydia carriers among the patient with pelvic inflammatory disease.

Direct gram microscopy

A direct smear was made for Gram stain according to [13]; a drop of sterile normal saline was added on the center of a clean dried glass slide and the swab containing the sample rolled in the drop of normal saline spreading it on the glass slide in a circular motion to make a thin smear. The smear was then allowed to air dry and then heat fixed by passing it at least three times over a Bunsen flame. The slide was placed on the staining rack and flood with crystal violet solution for 60 seconds, wash with clean water and cover with lugol's Iodine and then it was allowed to act for a minute. The slide was washed in clean water and then decolorized with 50% acetone alcohol under slow running tap water until a faint pink color is observed or no more color tend to flow from the smear. The process of discoloration was not exceeding 30 seconds. After decolorizing, it was washed in clean water and counter stain with neutral red solution. The slide was again washed in clean water; air dried and observed under the microscope with X100 objective lens (oil immersion lens). Gram positive bacteria were observed as blue or purple color and Gram negative as red or pink color. Also, the morphology and shape of the bacteria were identified whether they are cocci, diplococcic, cocci in chains, clusters, and whether they are rods in appearance [13].

Identification of bacterial isolates

Biochemical tests

The isolates were identified using the conventional biochemical tests such as catalase, optochin, coagulase, indole, citrate utilization, urea utilization, triple sugar iron agar fermentation, MR-VP test and oxidase as described below;

Catalase test

Catalase test was carried out according to the method described by [13], to determine the ability of the isolate to produce the enzyme, catalase. A drop of 3% hydrogen peroxide was added to a loop full of the test organisms. Presence of bubbles indicated catalase activity.

Indole test

Indole test was carried out according to the method described by [13] to determine the ability of the isolate to degrade amino acid tryptophan and produce tryptophanase, enzyme were tested. 1% tryptophan broth in a test tube were inoculated with isolate and incubated at 37°C for 48 hours. After 48 hours, 1milliliter of chloroform was added to the broth. The test tube was shaken gently, and 2.1 ml of Kovac's reagent were added and again shaken

gently, this was allowed to stand for 20 minutes. The formation of red coloration at the top layer, indicated positive while yellow coloration indicated negative.

Urease test

Urease test was done according to the method described by [13] to determine the ability to hydrolyze urea to produce ammonia and carbon dioxide. Test organism was inoculated into urease broth and incubated at 30°C for 72 hours. Purplish pink coloration of the medium indicates positive reaction.

Citrate utilization

This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and incubated for 24 to 72 hours. The development of deep blue color after incubation indicated a positive result [13].

Triple sugar iron test

Triple sugar iron test was carried out according to the method described by [13]; the test determines the ability of the organism to ferment the three sugar component of the medium: glucose, lactose and sucrose. The medium contains a pH indicator (phenol red) and a detection system (thiosulphate and ferrous sulphate) for hydrogen sulphide (H2S). The medium was prepared as an agar slant. The test organism was inoculated by stabbing the medium using sterilized straight wire loop and the surface of the slope were also streaked with the test organism. The tests were incubated at 37°C for 72 hours. After incubation, gas production was determined by observing the cracking of the medium, and production of H2S was observed by the blackening of the bottom of the medium.

Coagulase test

This test was used to identify *Staphylococcus Auraus* which produces the enzyme coagulase. The rapid slide test was done by placing a drop of distilled water on each end of slide. Then a colony of the test organism (previously checked by Gram staining) was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. Formation of clumps of the organisms within 10 seconds was suggestive of a positive test while absence of these clumps indicated negative results. For suspected Staphylococcus aureus isolates which turn negative for the rapid slide test, tube test was done by emulsifying several isolated colonies of test organism in 1 ml of diluted rabbit plasma (1:5) dilution to give a milky suspension. The tubes were then incubated at 35°C in water bath for 4 hours. These were examined at intervals of 1, 2 and 4 hours for clot formation by tilting the tube through 90°**.** If the test is still negative, the tube was left at room temperature overnight and examined again for Staphylococcus aureus that produce a delayed clot [13].

Oxidase test

The test was used in identification of organisms which produce the enzyme cytochrome oxidase. A filter paper soaked with the substrate tetra methyl-p-phenylenediamine Dihydrochloride was moistened with sterile distilled water. Using a piece of stick or glass rod, a colony of the test organism was smeared on the filter paper. The development of a blue-purple color within a 10 seconds indicated positive test while absence or formation of a blue-purple color after 10 seconds was considered negative [13].

Data analysis plan

Data from questionnaires were entered in Microsoft Excel 2010, and thereafter exported to STATA 14.1. Socio-demographic, sexual behaviors and gynecologic factors were summarized as means and mediams, standard deviations and interquartile range (for continuous variables) were determined. Proportions, percentages and frequencies were used for categorical variables using STATA 14.1. Prevalence of pelvic inflammatory disease among reproductive aged women attending Kampala international university teaching hospital were summarized as frequencies and percentages and presented using pie chart. The 95% confidence interval was used for estimation purposes**.**

Ethical considerations

Informed consent:

Informed consent and respect for participant's voluntary recruitment was observed. Informed consent for participants were obtained and signed after fully explaining the details of the study to them in English and local languages where necessary (copy attached at Appendix). Participants were not forced to enroll themselves if they don't want to, they were free to withdraw from the study any time they wish without coercion or compromise of care they are entitled to.

Risks and adverse events to study participants

Patients may undergo pain during swabbing and speculum examination, however, the process of obtaining a swab was done gently and professionally to minimize risk of pain and minimize re-infection as far as possible. Additionally, culture and sensitivity are the recommended guidelines prior to antibiotic therapy to minimize the risk of antibiotic resistance.

Benefits of the research

The community benefited from dissemination of findings on the most active antibiotic that should be prescribed to these patients at KIU-TH. Such finding has significant role in contributing to reduction of mortality and morbidity due to PID.

Privacy and confidentiality

Identification of participants was by means of numerical codes. Details of respondents were kept confidential for privacy purpose throughout the course of research. Respect of the respondents' rights and fair treatment were strictly adhered to thus minimizing harm and discomfort to them. There was no disclosure of participant's information to the public without their consent; the endocervical swab was collected in presence of a female nurse as a research assistant with the agreement of the participant.

RESULTS

Table 1: Socio demographic factors

Characteristics	Frequency	%
Age (years)		
<20	31	9.6
20-29	205	63.3
30-39	71	22.0
40-49	17	5.1
Education		
None	11	3.4
Primary	99	30.6
Secondary	111	34.4
Tertiary	103	31.6
Occupation		
None	127	39.2
Farmer	85	26.2
Professionals	51	15.7
Business	31	9.6
Manual laborer	30	2.3
Monthly income (UGX)		
None	10	3.1
<300000	230	71.8
300000-600000	66	26.5
>600.000	18	5.6
Marital status		
Single	86	26.5
Married	238	73.5

The above table illustrates that 63.3% of participants are aged of 20-29 years, 34.4% have secondary education, 39.2% have no occupation, 71.8% of participants have a monthly income of less than 300.000 Uganda Shillings and 73.5% are married.

Table 2 Gynecological factors

Characteristics	Frequency	%
Parity		
Zero	98	30.3
1-3	153	47.2
>3	73	22.5
Had PID before		
No	224	69.1
Yes	100	30.9
Had miscarriage before		
No	264	81.5
Yes	60	18.5
Use Contraceptive		
No	132	40.7
Yes	192	59.3
Intra Uterine Procedure		
No	281	86.7
Yes	43	13.3
Type contraception		
Condoms	38	19.8
Pills	61	31.8
Injectables	65	33.8
IUD	28	14.6
Type of miscarriage		
Spontaneous	41	68.3
Induced	19	31.7

From the above table, 47.2% of the study participants had delivered at least one to three times, 69.1% had had miscarriage of which 68.3% were spontaneous, 59.3% of the study participants had ever used contraceptive methods of which 33.6% had used injectable contraceptive methods and 86.7% had not had intrauterine procedures.

Table 3: Sexual behavior factors

Characteristics	Frequency	Percent
Number of of sexual partners		
None	20	6.2
One	253	78.0
More than one	51	15.8
Age of initiation sexual activity(year)		
< 15	25	7.7
16-20	242	74.7
>20	57	17.6
Condom Use		
Sometimes	84	25.9
Every time	38	11.8
Never	202	62.3
Smoking		
Never smoke	316	97.5
Ever smoke	8	2.5

The above table shows that, the age of initiation of sexual activity for the majority of participants was 16-20years in 74.7%, most of the study participants denied the use of condoms with 62.3% and 97.5% were non smokers.

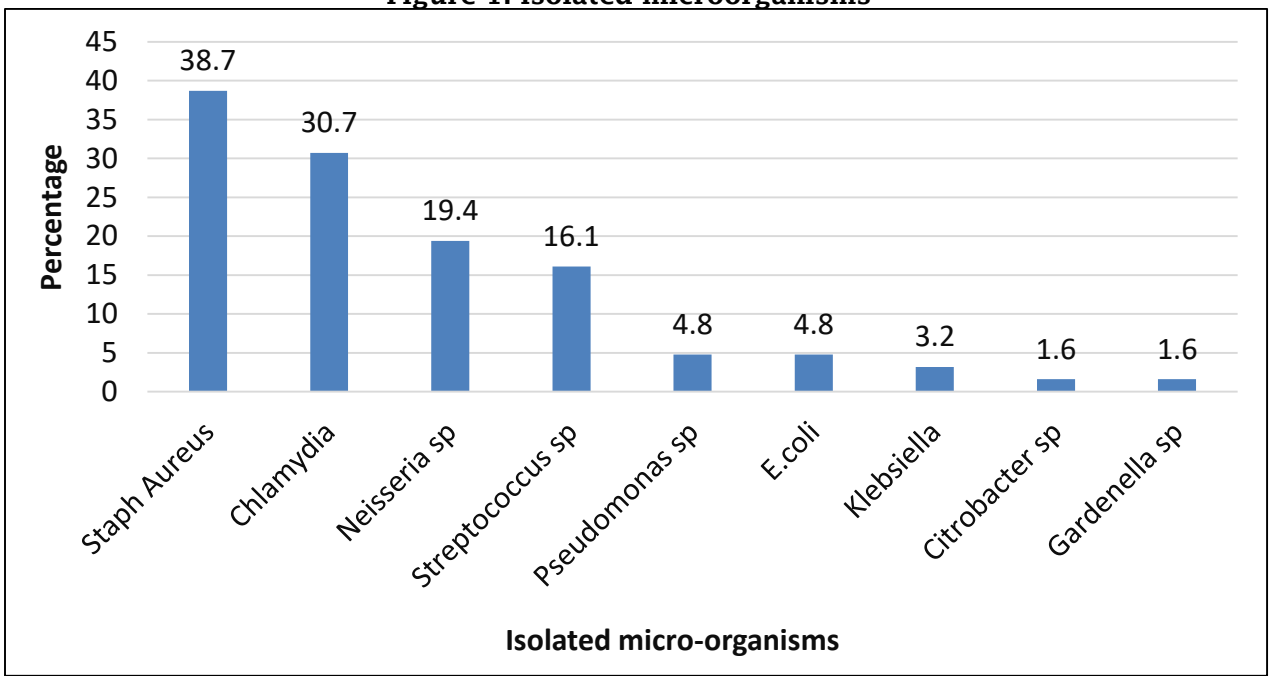
Common bacteria pathogens

Table 4: Prevalence of the isolated bacteria

Bacteria I Isolates	Fr(%)
Staphylococcus Aureus	24(38.7)
Chlamydia trachomatis	19(30.7)
Neisseria species	12(19.7)
Streptococcus species	10(16.4)
Pseudomonas species	03(4.8)
Escherichia coli	03(4.8)
Klebsiella	32(3.2)
Gardenella species	01(1.6)
Citrobacter species	01(1.6)

From the above table, *Sphilococcus aureus* was the most prevalent bacteria isolated with 38.7% followed by *Chlamydia trachomatis* which was indirectly identified using Chlamydia rapid diagnostic test with 30.7%, *Neisseria and streptococcus* were isolated in the proportion of 19.6% and 16.4% respectively.

Figure 1: Isolated microorganisms



From the above a total eight microorganism were identified, the most common being *staphylococcus Aureus* with 38.7% followed by Chlamydia identified indirectly with 30.7%, *Neisseria and Streptococcus* were in respectively 19.4% and 16.1%.

Antibacterial susceptibility for the commonest micro organisms

Table 5: Susceptibility of Common bacteria isolates

Drug	Bacteria Isolates		
	Staph Aureus	**Neisseria spp**	**Streptococcus spp**
Doxycilin	00	02(16.7)	02(20)
Cefixim	00	00	00
Ampicillin	00	00	02(20)
Ciprofloxacin	1(4.4)	00	1(10)
Azithromycin	**2(8.3)**	**05(41.7)**	**05(50)**
Levofloxacin	3(12)	2(16.7)	1(10)
Ceftriaxone	3(12)	2(16.7)	00
Nitrofurantoine	12(50)	1(8.3)	00
Vancomycin	13(54)	00	5(50)
Imipenem	19(79)	08(66.)	9(90)
Gentamycin	12(50)	06(54.6)	4(44.4)

The above table shows the antibacterial susceptibility pattern against the common bacterial isolates among women with pelvic inflammatory disease. The most common bacteria are sensitive to Imipenem at 79.1% for *staphylococcus Aureus, Neisseria species and streptococcus species* were also sensitive to imipenem with respectively 66.7 and 90%.

DISCUSSION

Common bacteria pathogens and antibacterial susceptibility

According to this study *Staphylococcus aureus* was the most common isolated bacterium accounting for 37.8% of PID cases, followed by *Chlamydia trachomatis* and *neisseria species* with respectively 30% and 16%. Though this is higher than of *28.6%* of *Staphilococcus in* the study of [14,15,16] in Nigeria it was followed by *Escerichia coli* with 22.9. The study of [15] in Cameroon found that common bacteria isolates were Genital tract *mycoplasma* with 54.3% followed by *Chlamydia trachomatis* with 37.1% this was high compare our study, he also reported a low prevalence of *Staphilococcus Aureus* with 2.9%.

This was also lower to the results of [9] in Nigeria with 41.4% for *Staphilococcus Aureus* followed by *Klebsiella species* with 24.4% and *E. coli* with 12%. This result was also higher to Spencer who found a prevalence of 16% for *Staphilococcus aureus* which was followed by *E. coli* 12% and *Streptococcus species* 8%. The prevalence of *Chlamydia trachomatis* in this was 30%, this was higher to the one found by [12] in Mbarara regional referral Hospital (2017) which was 26.4%.

CONCLUSION

The common bacteria pathogens isolated were *Staphylococcus Aureus, Chlamydia Trachomatis, Neisseria species and Streptococcus species*; Imipenem was the most sensitive antibiotic for both of the bacteria

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