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Antibiotics Susceptibility Pattern of different Bacteria Associated with female Genital tract Infection in Rural Communities in North central Nigeria

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Abstract: Genital tract infections (GTI) are infections of the reproductive system which are caused by pathogenic organisms normally present in the reproductive tract, or introduced from the outside during sexual contact or medical procedures. Infectious agents can impair various important human functions, including reproduction. Bacteria, fungi, viruses and parasites are able to interfere with the reproductive function in both sexes. Antibiotic susceptibility testing is commonly used in Medical Microbiology (1) the disc diffusion technique and (2) the tube dilution method. Both methods identify the infecting pathogen and the antibiotics that are likely to inhibit its growth. This study was conducted to determine the antimicrobial susceptibility of bacteria in the genital tract of females in rural communities in FCT Abuja, Nigeria. A total of 75 samples were collected from 32 women of the age ranging from 21 to 60 years which include; clean catch mid-stream urine, High Vaginal Swab-HVS and Endocervical Swab-ECS samples. The samples were inoculated immediately after collection in the laboratory on chocolate and MacConkey agar. Microscopy of the samples was carried out using wet preparation method they were placed on a clean grease free glass slide, covered with cover slip and viewed under the microscope using low power (X10) and high power (X40) objectives. Antimicrobial susceptibility was performed on all isolated bacteria using the Kirby Bauer's disc diffusion method on Mueller Hinton agar. Five (5) different bacteria isolates namely *Staphylococcus aureus, Escherichia coli, Coliform bacilli, Serretia marcescens, Pseudomonas* were identified. The female samples within the age group of 25–52 years, showed higher prevalence of Genital Tract Infection (GTI), 61.3% of the isolates were found to be Gram positive while 28.2% were found to be Gram negative bacteria. *Staphylococcus spp* (61.3%) was the most prevalent gram-positive isolate. Most of the Gram-negative isolates resisted Nalixidic acid, Ceporex and Septrin among all other Gram-negative disc used. 92.26% and 84.52% of the Gram-negative and Gram-

positive isolates respectively, were sensitive to Ciprofloxacin. This study reveals that about 50% of female in the rural communities in Abuja between the ages of 21 and 60 years are living with GIT. Both the Gram-negative and the Gram-positive isolates were highly sensitive to Ciprofloxacin. Hence ciprofloxacin is a broad-spectrum antibiotic. The abuse of antibiotics should be discouraged to prevent the increase of antimicrobial resistant cases so as to eradicate these bacterial infections. Finally, the Public Health and epidemiology Department in Federal Capital Territory Abuja should create awareness on personal hygiene in all the rural Communities in Abuja to avoid these infections.

Keywords: Antibiotics, bacteria, gram positive, *Escherichia coli* and resistant

1. Introduction

Genital tract infection are infections that influence the genital organs. Genital tract infections are a typical reason for diminished fruitful capacity and sterility. Microorganisms can climb the female reproductive tract after vaginal passage, and cause aggravation and resulting scarring. However tubal blockage is the most considered spin-off of pelvic infections, aggravation in the conceptive tract may likewise lessen fruitful capacity by meddling with gamete transport and implantation. They are sicknesses that influence the genital organs or regenerative organs of an individual. The sickness influences the two guys and females and can cause barrenness [1].

Genital tract infection is one of the significant reasons for dreariness and difficulties in all kinds of people [2]. Untreated genital tract infections (GTIs) increment hazard of STD procurement and transmission through a few creatures' explicit pathways. Untreated STD can likewise build GTI seriousness and length through resistant concealment pathways. For ladies, untreated GTIs are a huge reason for reproductive bleakness, including pelvic inflammation infection, tubal factor fruitlessness, unfriendly birth results, and barrenness. The finding and treatment of genital diseases (GI) present a few challenges: clinical components are not explicit; numerous contaminations are asymptomatic; lab tests accessible in the field are not generally solid; blended infections are normal; accomplices should be dealt with all the while in the event of physically communicated diseases [3]. Anti-infection weakness testing, or AST, is a broadly utilized technique for assessing anti-microbial obstruction and deciding patient treatment plans in clinical settings. There are various strategies for AST like agar weakening, stock weakening and plate dispersion examines. The circle dispersion or 'Kirby-Bauer' strategy includes spreading microbes on an agar plate and setting paper circles impregnated with anti-infection on the plate. After hatching, the development of microbes is noticed. Regions around the anti-microbial plate where no bacterial development can be seen are known as 'zones of restraint'. These zones show that an anti-microbial has been fruitful in halting bacterial development or killing the microscopic organisms. By estimating the distance across of these zones, we can analyze the adequacy of anti-infection agents and screen antimicrobial obstruction. Antitoxins address a significant class of antimicrobial specialists. By definition, anti-infection agents are biochemical's delivered by microorganisms that hinder the development of, or kill, different microorganisms. By their actual nature, anti-microbial should show specific poisonous on the grounds that they are delivered by one microorganism and apply changing levels of harmfulness against others. The revelation and utilization of anti-infection agents have upset clinical practice in the 20th century. The proper meaning of an anti-infection recognizes biochemical's that are delivered by microorganisms from natural synthetic compounds that are incorporated in the lab. This differentiation is presently not significant on the grounds that natural scientists can combine the biochemical designs of many normally happening anti-toxins. Also, numerous anti-toxins in current clinical use are artificially altered types of microbial biosynthetic items.

2. Materials and Methods

2.1. Study Area

Newly voided mid-stream urine, High Vaginal Swab and Endocervical Swab were collected between June and July 2021 out of 2 unique networks in FCT Abuja to be specific AngwanRogo and Kafe. AngwanRogo is situated at Idu modern locale. The Idu modern design inside the core of Abuja covers around 588 hectares of land that is divided into 208 business plots and is the public authority's endorsed mechanical group for the Federal Capital Territory. Furthermore, Kafe is situated at Cadastral Zone C05, it is one of three locales in Abuja city, Nigeria. It is the last piece of Gwarinpa locale under the Abuja Municipal Area Council. It is a 35-kilometer drive from the focal area and contains a quickly developing lodging domain. Although the number of inhabitants in this area is obscure as a statistics has not been explicitly completed at this point.

2.2. Materials

1. Agar (Mueller Hinton agar, Chocolate agar, MacConkey agar, Nutrient agar), Speculums, Sterile swab sticks, Antibiotics disc, Sterile Petri dishes, Glass slides, Ethical endorsement structure, Microscope

2.3. Study Size

32 women participated in this investigation with a sum of 75 examples, 26 high vaginal swabs, 14 Endocervical swabs, and 35 urine samples. These 32 ladies include pregnant and non pregnant ladies, Virgins and youths. This investigation was done simply by arbitrary example assortment. Their ages ranges between 21-60 years.

2.4. Sampling Collection

High Vagina Swab Collection

The sample was collected using sterile swab stick were labelled name, age, sex, and telephone numbers. This assortment was done while wearing sterile gloves. The cotton fleece swab was embedded 2–3 cm into the vagina and pivoted for 5–10 seconds to scratch the mucosa. Individuals who have not been on anti-microbial for the past two weeks were selected.

Endocervical collection

The sample was collected using a sterile swab stick and sterile speculum and were labelled name, age, and sex and telephone number. This assortment was done in a shut room the cervix was cleaned off of vaginal discharges and bodily fluid with a clean swab and disposed of. The sterile swab stick was embedded and pivoted 10-30 sec to get exudates from the endocervical organs. The swab containing the example were put once again into the vehicle cylinder, and moved to the research facility at room temperature.

Urine collection

Participants were given well labelled sterile bottles to collect mid-stream urine. Samples were transported to the laboratory immediately after collection.

2.5. Sterilization of Materials

All glass products to be utilized were washed and appropriately flushed with refined water and permitted to dry by cleaning it in the hot air stove at 160oC for 60 minutes. The wire loope was

sterilized by flaming prior to use and then after use, the work bench was disinfected prior and then after any activity.

2.6. Preparation Of Culture Media

The media was prepared according to the manufacturers' instruction.

Culture

All samples (HVS, ECS and Urine) were inoculated separately onto the surface of the prepared chocolate agar by streaking method and incubated at 37^{o}C for 24 hours. The same process was repeated on the surface of the prepared MacConkey agar.

Microscopy

Urine

After inoculation, the urine samples were centrifuged at 2000-3000 rpm for 5-10 minutes, after which the supernatant was tapped. A drop of the deposit was put on a clean grease free glass slide and a cover slip was applied on it.

HVS and ECS

About 0.5ml normal saline was added to the HVS and ECS samples. The swabs were placed on clean grease free glass slides a drop of ordinary saline was added to it. The slides were seen under the magnifying instrument utilizing x40 and x10 target focal point.

Bacteria Isolate

Each of the bacteria isolates from the culture were isolated and inoculated separately on a freshly prepared chocolate and McConkey agar plate and incubated at 37oC for 24 hours to get a pure culture

Gram Stain

This test separates the microscopic organisms into Gram-Positive and Gram-Negative Bacteria, which helps in the characterization and separation of microorganisms. The Gram stain differentiates microbes into two groups: (1) Gram-positive microorganisms that hold the essential color (Crystal violet) and (2) Gram-negative microorganisms that take the shade of the counter stain (normally Safranin).

Results:

Gram positive – purple

Gram negative – pink

Biochemical Tests

This test is utilized to distinguish the specific organisms present in the urine, HVS (high vagina trade) tests and Endocervical tests. The following biochemical test were conducted;

Catalase test

Coagulase test

Methyl red test

Motility test

Indole test

Urease test and

Citrate test

Catalase Test

This test shows the presence of catalase, a catalyst that catalyzes the arrival of oxygen from hydrogen peroxide (H_2O_2). It is utilized to separate those microscopic organisms that produce a catalyst catalase, like *staphylococci,* from non-catalase creating microorganisms like *streptococci.* Ordinarily 3% H_2O_2 is utilized for the standard culture while 15% H_2O_2 is utilized for recognition of catalase in anaerobes.

Presence of air pockets – catalase positive

No air pockets – catalase negative

2.7. Coagulase Test

The coagulase test is a significant test that separates the types of the family *Staphylococcus aureus* from different Staphylococci species like *S. epidermidis and S. saprophyticus* based on their capacity to deliver the coagulase protein [4].

Noticeable agglutination – positive

No noticeable agglutination – negative

Methyl Red Test

MR test is utilized to decide the capacity of a creature to deliver and keep up with stable corrosive finished results from glucose maturation [5].

Dazzling red tone – positive

Yellow tone – negative

Motility Test

This test is utilized to separate motile secludes. It is utilized to separate among motile and non motile microscopic organisms [5].

Apparent development away from point of vaccination – positive

Noticeable development along the line of immunization – negative

Indole Test

This test exhibits the capacity of specific microbes to disintegrate the amino corrosive tryptophane to indole, which gathers in the medium.

Red or pink – positive

No shading change – negative

Citrate Test

Citrate agar is utilized to test a living being's capacity to use citrate as a wellspring of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts ($NH_4H_2PO_4$) as the sole wellspring of nitrogen.[5]

Development with shading change from green to blue along the inclination. - Positive

No development and No shading change; Slant stays green. – Negative

Urease Test

Urea Agar was created by Christensen in 1946 for the separation of intestinal bacilli. The urease test is utilized to decide the capacity of a living being to parted urea, through the creation of the protein urease.[5]

Radiant pink tone – positive

No shading change – negative

Antibiotic Susceptibility/Sensitivity Testing.

The paper disc diffusion technique on Mueller Hinton agar plate was used to carry out the antibiotic susceptibility testing. Commercially available gram positive and gram-negative paper disc were used. of the plant extracts in comparison with standard antibiotic gentamicin (20 mg/ml) *in vitro* on the isolates according to the methods of NCCLS (2007). Each pure culture of the bacteria isolates were grown on nutrient agar. The colonies of each of the organisms were inoculated into the Mueller Hinton broth (Oxoid, England), incubated for 4 hours at 37°C and diluted with sterile saline to a density visually equivalent to McFarland Standard. The prepared 0.5 McFarland standard organisms were inoculated in duplicate onto the surface of the Mueller Hinton agar by spreading method. The commercially prepared gra- positive and gram-negative antibiotic disc were aseptically placed on the inoculated plates well as the standard drug, gentamicin (GEN, 20 mg/ml) and sterile water separately, which served as the positive and negative controls respectively. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured with the use of a metric rule.

2.8. Statistical Analysis

Information was investigated utilizing the Statistical Package for Social Sciences (SPSS) programming adaptation.

3. Results

A total number of 32 women with ages ranging from (21-60 years) participated in this study. With a total of 75 samples, 26 High Vaginal Swab samples, 14 Endocervical Swab samples, and 35 urine samples. Out of 75 samples 62 were positive, HVS 16, ECS 12 and urine 32. The remaining samples that showed no bacteria growth were discarded. Overall, 20 women had genital tract infection. The most commonly identified bacteria in the genitals were *Staphylococcus aureus* followed by *coliform*. The reviewed cases were grouped into ages ranging from (21-30 years), (31-40years), (41-50), (51-60) (Table.1). Ages ranging from (41-50) had the least pathogens isolated from them. Table 2 shows the morphological features of each bacterium isolates on MacConkey agar. Table .3: shows the Bacteria distribution of positive cases and negatives cases with *Staphylococcus aureus* having the highest positive case and the least negative cases, *Pseudomonas* and *Serratia marcescens* having the least positive cases and the highest negative cases. Table 4-9: Shows the Susceptibility and resistance, zone of inhibition of each bacterium isolates to different antibiotics. Fig 1 shows the percentage of prevalence of each organism. The result shows that all samples contained different types of bacteria. The results obtained are shown in the tables below.

Table 1. Age distribution of sample population

Age	Total Numbers	Positive cases
21-30	13	8
31-40	13	8
41-50	1	1
51-60	2	2
Total	29	19

Table 2. Morphological features of bacteria isolate on MacConkey agar.

Forms	*Staphylococcus aureus*	*Coliform*	*Escherichia coli*	*Serratia marcescens*	Pseudomonas
Elevation	Covex	Covex	Raised	Opaque	Flat
Shape	Circular	Circular	Circular	Circular	Irregular
Edge	Smooth	Smooth	Smooth	Smooth	Rough
Colour	White	White	Yellow	Pink	Green
Consistency	Moist	Moist	Moist	Moist	Dry
Margin	Entire	Entire	Entire	Umbonate	Undulate
Size	1mm	2mm	2mm	1mm	2mm

Table 3. Bacteria distribution in the samples

Bacterial isolates	Positive cases	Negative cases
Staphylococci aureus	38	37
Coliform bacilli	15	60
Escherichia coli	7	68
Serratia marcescens	1	74
Pseudomonas	1	74

Table 4. Susceptibility and resistance of the bacteria isolates to antibiotics

Bacterial	Antibiotics/ zone of inhibition(mm)									
	CH	LEV	RD	OFX	CPX	PEF	CN	S	E	AU
Staphylococcus aureus	20	20	20	-	-	17	20	15	19	-
Coliform bacilli	-	-	-	23	-	25	20	19	-	-
E. coli	-	-	-	18	-	20	-	-	-	-
Serratia marcescens	-	-	-	17	-	-	15	19	-	20
Pseudomonas	-	-	-	17	-	-	-	11	-	-

0 < 9mm Resistance

10> and above Sensitive

KEY

AU (Augmentin)

LEV (Levofloxacin)

CPX (Ciproflox

Table 5. Susceptibility and resistance of *Staphylococcus aureus* isolates to antibiotics

Antibiotics	Zone of inhibition(mm)	Interpretation
Ciprofloxacin	25	S
Norfloxacin	15	I
Gentamycin	20	S
Amoxil	14	I
Streptomycin	15	I
Rifampicin	20	S
Erythromycin	19	I
Chloramphenicol	20	S
Ampiclox	14	I
Levofloxacin	20	S

S = Sensitive

I = Intermediate

Based on the clinical laboratory standard institute 2012

Table 6. Susceptibility and Resistance of *Coliform bacilli* isolate to antibiotics

Antibiotics	Zone of inhibition(mm)	Interpretation
Tarivid	23	S
Reflacine	25	S
Ciprofloxacin	25	S
Augmentin	5	R
Gentamycin	20	S
Streptomycin	19	I
Ceporex	5	R
Nalidixic acid	5	R
Septrin	5	R
Amplicin	19	I

S = Sensitive

I = Intermediate

R = Resistance

Based on the clinical laboratory standard institute 2012

Table 7. Susceptibility and Resistance of *Escherichia coli* isolate to antibiotics

Antibiotics	Zone of inhibition(mm)	Interpretation
Tarivid	17	I
Reflacine	18	I
Ciprofloxacin	20	S
Augmentin	20	S
Gentamycin	15	I
Streptomycin	19	I
Ceporex	10	I
Nalidixic acid	5	R
Septrin	19	I
Amplicin	20	S

Table 8. Susceptibility and Resistance of *Serratia marcescens* isolate to antibiotics

Antibiotics	Zone of inhibition(mm)	Interpretation
Tarivid	19	I
Reflacine	5	R
Ciprofloxacin	23	S
Augmentin	8	R
Gentamycin	19	I
Streptomycin	22	S
Ceporex	9	R
Nalidixic acid	10	I
Septrin	5	R
Amplicin	5	R

Table 9. Susceptibility and Resistance of *Pseudomas* isolate

Antibiotics	Zone of inhibition(mm)	Interpretation
Tarivid	10	I
Reflacine	5	R
Ciprofloxacin	25	S
Augmentin	8	R
Gentamycin	20	S
Streptomycin	10	R
Ceporex	5	R
Nalidixic acid	5	R
Septrin	3	R
Amplicin	20	S

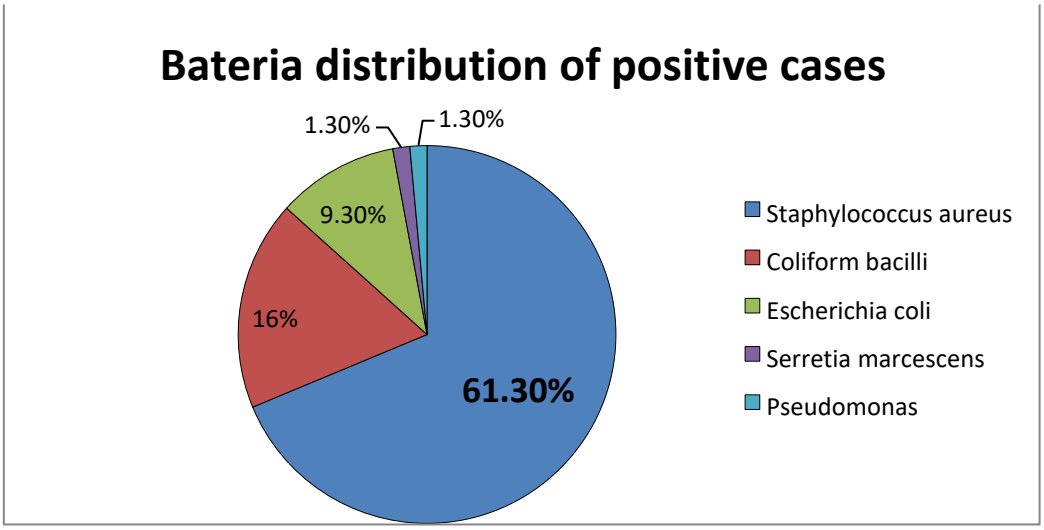

Figure 1. Pie chart showing the test results for HVS, ECS and urine in rural communities in FCT.

A total of 75 samples in which 38 samples yielded growth of *Staphylococcus aureus*, 15 samples yielded growth of *Coliform*, 7 sample yielded growth of *Escherichia coli*, 1 sample yielded growth of *Serretia marcescens*, and 1 sample yielded growth of *Pseudomonas.*

4. Discussion

The general investigation gives important information to look at and screen the anti-biotic susceptibility among various microorganisms in the female genital tract to work on productive treatment. Expanding antimicrobial resistance has been accounted around the world.

In this examination, a collection of microscopic organisms (bacteria) was isolated from the endocervix, vagina and urine of the female with indications of GTI. Furthermore, *Staphylococcus aureus* had the most elevated predominance rate, trailed by *Coliform bacilli, E..coli, Serratia marcescens,* and *Pseudomonas spp.* Most of these isolates are in the WHO's rundown of need bacterial microorganisms for examination, disclosure and improvement of new anti-microbials [6]. Most of the members were ladies of reproductive age, which shows that if they are not well treated it can lead to infertility cases. The presence of countless bacteria in High Vaginal Swab and urine sample highlights genital disease which was seen in ladies 21-60 years particularly in Angwan Rogo and their transmission is connected to poor, unhygienic and ecological conditions. Pseudomonas which was found in the urine and furthermore known to cause UTI was less oftentimes recuperated in the urine. The high perception of GTI causing bacteria in this investigation can be portrayed because of helpless sterilization and cleanliness rehearses.

Not all the test results uncovered pathogenic life forms, as about 20% of the outcomes over the period yielded no form of bacteria. *Staphylococcus aureus* which is the most prevalent aerobe in this research lines up for certain investigations which demonstrates *Staphylococcus aureus* as the prevailing species [7]. Rather than discoveries of most investigations *proteus spp, Enterobacter spp, Streptococcus, Citrobacter spp, Klebsiella spp* [8] were not detached in this examination. Vaginal microbes were observed to be more in grown-up ladies between the ages of 20-50. This is an all-around announced finding in a few different examinations [9].

Ciprofloxacin or Ciproflox were observed to be the best antibiotics against the microorganisms detaches in this investigation. In past examinations as indicated by [10] detached 42 Gram positive and Gram-negative microscopic organisms of the female bacterial vaginosis co-amoxiclav had the most elevated medication opposition score of 40% for Gram-negative bacteria. E. coli, the Gram-negative bacterium most commonly neglected, showed a serious level of protection from co-amoxiclav. Also, Gram-positive microscopic organisms' disengages' general medication opposition rates went from 31.1% for cefotaxime to 62.5% for penicillin. [11] demonstrated E. coli was exceptionally delicate to Aminoglycoside gathering of anti-infection agents. Which are expansive range anti-infection agents of high intensity that have been customarily utilized for the therapy of genuine Gram-negative contaminations.

This study shows that both Gram negative and gram-positive bacteria are more sensitive to Ciproflox which has a place with a gathering of wide range anti-microbials called fluoroquinolones. Aminoglycosides class of antibiotics showed 66% affectability against E. coli followed by the other gathering of anti-toxins. Present investigation shows that *Staphylococcus aureus* is profoundly sensitive to Cipoflox (25), gentamycin (20), Rifampicin (20), Chloramphenicol (20), levofloxacin (20). *Staphylococcus aureus* was sensitive to imipenem (96.7%), levofloxacin (86.7%), chloramphenicol (83.3%), cefoxitin (76.7%), ciprofloxacin (66.7%), gentamycin (63.3%), antibiotic medication and sulfamethoxazole-trimethoprim (56.7%), and vancomycin and doxycycline (half). *Coliorm* was sensitive to Reflacin (25), Ciproflox (25), Tarivid (23). *Escherichia coli* was sensitive to Ciproflox (20), Augmentin (20), Ampicillin (20). While *Escherichia coli* was sensitive to Nitrofurantoin (96.4%), Norflaxocin (90.6%), Gentamicin (79.6%) And Ciprofloxacin). *Serratia marcescens* was exceptionally sensitive to Ciproflox (23), Streptomycin (22). Most strains are vulnerable to amikacin, yet reports show expanding protection from gentamicin and tobramycin. Quinolones additionally are profoundly dynamic against most strains. Pseudomonas spp was profoundly helpless to ciproflox (25), Gentamycin (20), Ampicillin (20). Anti-microbial susceptibility test results uncovered normal weakness paces of 73.8%, 70.1%, 66.2%, 59.5%, and 34.3% to ciprofloxacin, gentamicin, levofloxacin ceftazidime, and carbenicillin separately [12].

Ciprofloxacin, a second era expansive range fluoroquinolone, is dynamic against both Gram-positive and Gram-negative microorganisms. Ciprofloxacin has a high oral bioavailability and an enormous volume of circulation. It is utilized for the treatment of a wide scope of diseases including urinary parcel contaminations brought about by powerless microbes. Following oral therapy, ciprofloxacin is quickly ingested in the GIT by uninvolved dispersion and arrives at the pinnacle serum fixation under 2 hours. Ciprofloxacin exhibits a fixation subordinate penetrability and it is generally dispersed in body tissues and liquids including bile, prostatic tissues, gingival liquid, and lungs. The pathway of ciprofloxacin is by both renal and non-renal pathways.

5. Conclusion

Nalixidic acid, Ceporex and Septrin were found the most resistant gram-negative antibiotics among all gram-negative bacteria. Ciproflox was found to be the most susceptible antibiotic against isolated gram negative and gram-positive bacteria which showed 92.26% and 84.52% susceptibility, respectively.

Ethical Clearance

Endorsement was allowed for this work from the FCT Health Research Ethics Committee (FCT HREC). Garki, Abuja-Abuja

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