Isolation of Multidrug - Resistant *Escherichia coli* from Urogenital Samples of Patients with Pelvic Inflammatory Disease in Niger State

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ABSTRACT

This study was conducted to determine the prevalence of multidrug resistant (MDR) *Escherichia coli* isolated from patients with PID attending nine hospitals in Niger State. A total of 1170 endocervical swabs (ECS) and urine samples were collected using sterile swap stick and sample containers. The samples were transported on ice pack to the Microbiology laboratory, Federal University of Technology, Minna for analysis.Screening for the presence of *Escherichia coli* was done using streak method of inoculation on Nutrient agar and Macconkey agar. Isolates of *Escherichia coli* were identified through Gram staining and other biochemical tests. Seventy two (22.5%) and 98 (24.5%) *Escherichia coli* isolates were obtained from both ECS and urine respectively. The antibiotic susceptibility profile of the isolates to ten (10) commonly prescribed antibiotics was determined using Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The antibiogram showed that a total of 79 (34.6%) *Escherichia coli* isolates out of 228 multidrug resistant bacteria expressed multidrug resistant characteristics, and were resistant to more than three (3) classes of antibiotics. The multidrug resistant *Escherichia coli* exhibited 100% resistance to: Gentamicin in General Hospital Suleja; Cephalexin and Nalidixic acid in General Hospital Minna; Gentamicin and Streptomycin in General Hospital Kuta; Augmentin and Sulfamethoxazole in General Hospital Agaie; Cephalexin and Nalidixic acid in General Hospital Lapai; Augmentin in General Hospital Wushishi; Perfloxacin and Augmentin in General Hospital Nasko; followed by 92.9% resistance to Ofloxacin in General Hospital Bida and 80% resistance to Gentamicin and Cephalexin in General Hospital Kontagora. The results of this study confirmed the presence of multidrug resistant *Escherichia coli* in Niger State, hence there is a need for public health workers, to create awareness on the misuse of antibiotics, to prevent and curtail treatment failure due to antibiotic resistance.

Keywords: Pelvic inflammatory disease; Urogenital; Multidrug resistant *Escherichia coli*

INTRODUCTION

Pelvic inflammatory disease (PID) is basically a disease of various organs (such as the ovaries, fallopian tubes, uterus and endometrium) located in the upper genital tracts of a female. However, this disease is one of the top three prevalent gynaecological problems, basically associated with female reproductive damages such as; fallopian tubal blockage, endometriosis

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and oophoritis, which inturn leads to 10% infertility and 0.5% mortality among women in the reproductive ages of 25-34 years (Usman, 2016). The emergence of this life threatening disease is basically associated with organisms referred to as urogenital pathogens.

Overtime urogenital pathogens especially members of enterobacteriaceae such as: *Escherichia coli, Salmonella* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Proteus* sp (Meštrović, 2017) have been regarded as causative agents of PID in most developing countries such as Nigeria and as such, PID is basically regarded as a polymicrobial infection (Centre for Disease Control, 2015). However, based on quest to attain a stable healthy condition, most female patients misuse and abuse existing antibiotics, which inturn lead to the emergence of resistant urogenital pathogens, especially those resistant to multidrug classes of antibiotics.

Occurrence of multi drug resistant urogenital pathogens among females in hospitals and communities, have increased hospital delay, treatment failure, morbidity and mortality (Spencer *et al.*, 2014). It is therefore imperative to determine multi drug resistant enterobacteriaceae such as *E.coli* which is fast becoming a urogenital pathogen associated with most PID patients in most developing countries.

METHODOLOGY

Description of the Study Area

The study was conducted in Niger State, Nigeria. Niger State is located in the middle belt zone of the country. It lies between latitude 8˚20'N and 11˚30'N and longitudes 3˚30'E and 7˚20'E. It shares common boundaries with other states namely: Zamfara States to the north-west; Kaduna State to the north-west; Kebbi State to the north-west, Kogi State to the north-cental; Federal Capital Territory (FCT) and Kwara State to the north-central and north-central respectively. The state covers a land area of about (76,363km^2) square km (29,484 square miles). About 85% of the populace in the state is involved in agriculture, particularly farming and they are majorly rural dwellers, while about 15% are involved in are urban dwellers, involved in white collar jobs, businesses, crafts and arts.

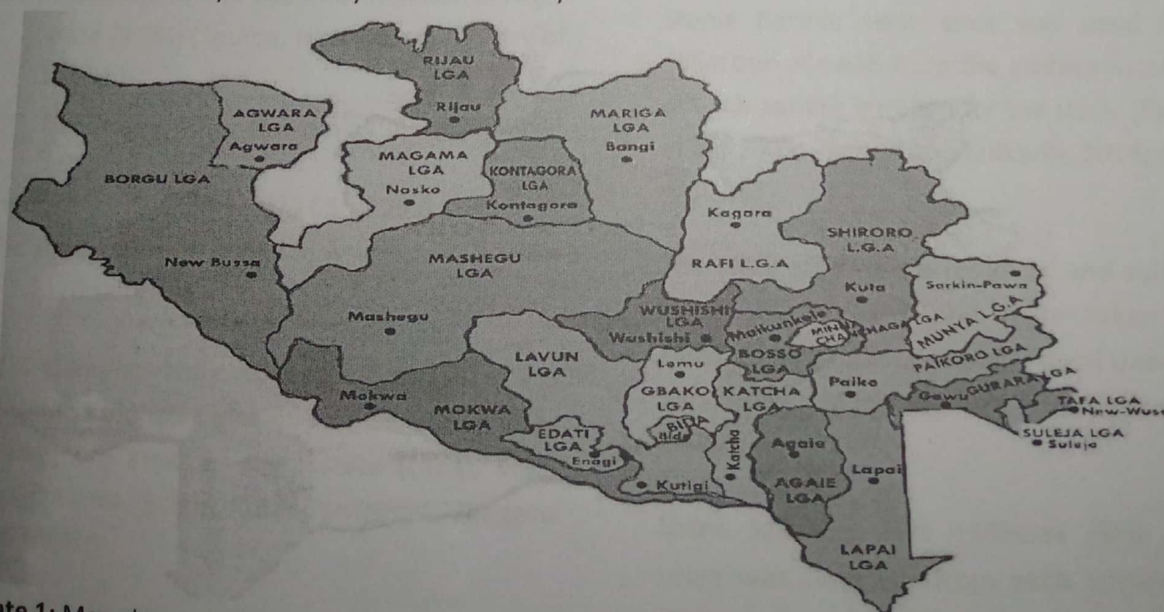

Plate 1: Map showing the various Local Government Areas in Niger State.
Source: Niger State Bureau of Statistics (2010)

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The state has three zones, each zone with a distinct climate pattern and a defined agricultural system. Zone A is found in the southern part of the state and it comprises of Agaie, Bida, Edati, Katcha, Gbako, Lapai, Lavun and Mokwa Local Government Areas; while zone B comprises of Bosso, Chanchaga, Gurara, Kuta, Paikoro Rafi, Shiroro, Suleja and Tafa Local Government Areas and zone C comprises of Agwara, Borgu, Kontogora, Magama, Mariga, Mashegu, Rijau and Wushishi Local Government Areas. Nine (9) Local Government Areas (comprising of 3 Local Government Areas from each zone) were randomly selected for this study.

Sample Size

The number of samples collected, were calculated using the equation below (Idakwo, 2015).

$$n = \frac{T^2 \text{ x } P(1-P)}{m^2}$$

where

n= required sample size

T= Confidence level at 95% (standard value of 1.96)

P= Prevalence rate of bacterial infection in Niger state (9.3%) (Source, Niger State Ministry of Health)

m= Margin of error at 5% (Standard value of 0.05)

n= (1.96²x 0.093 x 0.907)/0.05²=129.6 ≅ 130

The sample size for each L.G.A was (n) =130 x 9= 1170 samples.

1170 samples were collected from nine (9) general hospitals located in selected local government areas of Niger state (Lapai, Bida, Agaie, Minna, Suleja, Kuta, Kontogora, Zungeru and Nasko).

Inclusion and Exclusion Criteria

Female patients within the age of 15-54years diagnosed of PID and are attendees of the selected hospitals were recruited for this study. Female patients above 54years and less than 15 years not diagnosed of PID and who are not attendees of the selected hospitals were excluded from this study.

Ethical Clearance

Ethical clearance for this study was sought from the Niger State Hospital Management board, Research and Ethics Committee.

Collection of Demographic and Clinical Data

A structured questionnaire was administered to obtain patient's demographic data (such as patient's location, age and awareness of the disease); patient's previous medical history; patient's socio-economic factors (such as patient's family status, occupation and education) and risk factors (such as douching frequency, source of water and type of toilet facility) as described by Kolo (2016) and Oseni *et al.* (2017).

Collection and Transportation of Samples

Sterile flexible swab stick was used for the collection of swab from the endocervical region of each patient enrolled for the study (Einwalter *et al.*, 2005; Pachori and Kulkarni, 2016; Oseni *et al.*, 2017).

The swab sticks were removed and submerged into normal saline and were taken to the Microbiology Laboratory of Federal University of Technology, Minna for further analysis (Enwa *et al.*, 2015).

Urine Samples: Five millilitres (5ml) of fresh urine was collected from each female patient diagnosed of PID into a universal bottle. The urine samples were transported to the

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Microbiology Laboratory of Federal University of Technology, Minna under cold condition (Hunter *et al.*, 2013). The urine samples were stored at 4°C for 24 hours for further analysis (Hunter *et al.*, 2013).

Direct Examination

Saline wet preparation was carried out in order to rule out the presence of *Trichomonas vaginalis* which is characteristically associated with a yellow-green discharge, itching, redness and swelling (Spencer *et al.*, 2014).

Culture of Bacteria

The endocervical swabs and urine samples were cultured and incubated on the following media such as Nutrient agar, MacConkey agar and Salmonella- Shigella agar at 37°C for 24hours for the isolation of Gram negative and Gram positive bacteria. Pure culture of each isolate was obtained by continuous sub-culturing using the streak method. The pure isolates were stored on a nutrient agar slant for further identification and characterization (Kolo, 2016).

Gram Staining Technique

Smear of the isolate were prepared with a wire loop by emulsifying a colony of the isolate with a drop of distilled water on a clean glass slide free of grease and was used to make a thin smear. The smear was air – dried and was heat fixed. The smear was flooded with crystal violet stain for sixty seconds and was washed with clean running tap water. The slide was tipped off and flooded with lugol's iodine for sixty seconds and washed with clean water. The smear was decolourized rapidly using alcohol and washed immediately with clean water. The smear was flooded with neutral red stain (safranin) for two minutes, washed with clean water and the back was wiped with clean cotton wool and the smear was allowed to air dry. The dried slides were examined microscopically under oil immersion, using x40 objective lens and the results recorded (Cheesbrough, 2010; Kolo, 2016).

Biochemical Tests

The bacterial isolates was identified based on the following conventional biochemical tests such as; Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar iron test (Cheesbrough, 2010).

Coagulase test

A drop of distilled water was placed on two separate slides and a colony of the test isolates was emulsified on the two slides to make a thick suspension. A loopful of plasma was added to one of the suspension. Observation for agglutination reaction was done within 10seconds of adding plasma cells (Cheesbrough, 2010).

Catalase test

Three milliliters of 3% hydrogen peroxide solution was added into a sterile test tube. A sterile wire loop was used to pick colonies of the test isolates and was immersed in the hydrogen peroxide solution. Observation for bubbles was done immediately and results recorded (Cheesbrough, 2010).

Triple sugar iron agar test

The test was performed to determine the ability of bacteria to ferment various carbohydrates such as glucose, lactose and sucrose. Inoculation with the test organism was done by stabbing through the centre of the medium to the bottom of the tube and the test organism was streaked on the surface of the agar slants. The agar slants were incubated at 37°C for 24hours. Observations for colour change of the phenol red indicator to yellow both at the butt and slants (due to the fermentation of either glucose,

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lactose or sucrose), gas production indicated by bubbles or cracks on the medium and hydrogen sulphide (H_2S) production indicated by black pigment coloration was done and recorded (Cheesbrough, 2010).

Citrate test

Simmon citrate agar slants were prepared and the surfaces streaked with isolates and incubated at 37°C for 24hours. Observation for colour change was done and the results recorded (Cheesbrough, 2010).

Indole test

A wire loop of the test organisms were inoculated in the test tubes containing peptone broth at 37°C for 24hours. After 24 hours, 0.5ml of Kovac reagents was added into the test tubes and the solution was thoroughly mixed. Observations for colour change was made and results recorded (Cheesbrough, 2010)

Oxidase test

A piece of filter paper was placed in a sterile Petri dish and two drops of freshly prepared oxidase reagent (referred to as tetra-p-diaminechloride) was applied onto the piece of filter paper. A colony of the test organisms was introduced onto the soaked filtered paper. Observation for blue- purple colour within few seconds was done and result recorded (Cheesbrough, 2010).

Antimicrobial Susceptibility Testing of Isolates

Preparation of turbidity standard for the inoculums

The McFarland standard was employed in the standardization of the test organisms. Morphologically similar colonies of each test organism were transferred aseptically from an agar plate culture into a tube containing 4 to 5 ml of nutrient broth. The broth was subjected to agitation and was incubated at 37°C until it achieved or exceeded the turbidity of the 0.5 McFarland standard. The turbidity of the actively growing culture in the broth was adjusted with sterile saline to obtain turbidity that was optically comparable to that of the 0.5 McFarland standard (Lalitha, 2004).

Inoculation of Plates

Susceptibility test of the isolates was carried out using Kirby- Bauer disc diffusion method on Mueller-Hinton agar (Clinical and Laboratory Standards Institute, 2014). A sterile cotton swab stick was dipped into the adjusted suspension. The swab stick was rotated several times by pressing it firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab stick (Kolo, 2016). The surface of the sterile agar was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two or more times, rotating the plates approximately 60° each time, to ensure uniform distribution of bacteria on the plates. The inoculated plates was left for 10 minutes to ensure prediffussion of the organisms and to allow excess surface moisture to be absorbed, before the agar plates were impregnated with discs. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C for 24h (Clinical and Laboratory Standards Institute, 2014).

Reading Plates and Interpreting Results

After 24h hours of incubation, each plate was examined and the diameters of the zones of inhibition were measured, including the diameter of the disc. Zones of inhibition were measured to the nearest whole millimeter using a meter rule (Kolo, 2016).

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Screening for Antibiotics resistant bacterial isolates

Antibiotics available in the study area were used for the study. The antibiotic include penicillin G, Augmentin, cefotaxime, ceftriaxone, tetracycline, trimethoprim/sulfamethoxazole, gentamycin, ofloxacin and chloramphenicol test discs (AB BIODISK, Sweden) were employed (Spencer *et al.*, 2014).

Screening for Multi drug resistance bacteria

Bacteria isolates resistant to three or more classes of antibiotics according to the clinical laboratory standard institute (CLSI, 2014) guidelines were termed multi-drug resistant (MDR) bacteria (Magiorakos *et al.*, 2012; Iliyasu *et al.*, 2015).

RESULTS AND DISCUSSION

Results

Out of 1170 endocervical swaps and urine samples screened, only 720(62%) samples revealed the presence of bacteria (Table 1).

Table 1: Prevalence of PID in nine general hospitals

Samples	NSS	NPS	Prevalence (%)
Endocervical swap	1170	320	27.3
Urine	1170	400	34.2
Total		720	62

Key: NNS= Number of Samples Screened; NPS= Number of Positive Samples

The *Escherichia coli* isolated and identified are indicated in Table 2. It was observed that *E.coli* occurred in both endocervical swaps (ECS) and urine samples (Table 2).

Table 2: Occurrence of *E.coli* in nine general hospitals

	ECS		URINE		TOTAL	
	Number of *E.coli* isolates	% Frequency	Number of *E.coli* isolates	% Frequency	Number of *E.coli* isolates	% Frequency
Escherichia coli	72	22.5	98	24.5	170	23.6
Total	320		400		720	

A total of 228 multidrug resistant bacteria were isolated from this study. *Escherichia coli* isolates from endocervical 31 (34.5%) and urine 48 (34. 8%) samples were isolated respectively. Table 3 shows the percentage frequency of multidrug resistant *Escherichia coli* from urine and endocervical samples respectively.

Table 3: Multi drug resistant *E.coli* in patients with PID from nine general hospital

	ECS		URINE		TOTAL	
Organisms	Number of MDR- *E.coli*	% Frequency	Number of MDR- *E.coli*	% Frequency	Number of MDR- *E.coli*	% Frequency
Escherichia coli	31	34.4	48	34.8	79	34.6
Total	90		138		228	

Key: ECS= Endocervical swap

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Table 4: Susceptibility Pattern of multidrug resistant *Escherichia coli* in patients with PID from nine general hospitals

Hospitals	No of Isolates	Susceptibility pattern	OFX(%)	PEF(%)	CPX(%)	AU(%)	CN(%)	S(%)	CEP(%)	NA(%)	SXT(%)	PN(%)
G.H.S	8	S	1(12.5)	2(25)	0(0)	2(25)	0(0)	0(0)	1(12.5)	0(0)	2(25)	0(0)
		I	0(0)	1(12.5)	4(50)	1(12.5)	0(0)	2(12.5)	0(0)	1(12.5)	3(37.5)	0(0)
		R	7(87.5)	5(62.5)	4(50)	5(62.5)	8(100)	6(75)	7(87.5)	7(87.5)	3(37.5)	8(100)
G.H.M	5	S	1(20)	1(20)	2(40)	3(60)	2(40)	0(0)	0(0)	0(0)	2(40)	2(40)
		I	1(20)	1(20)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	2(40)	0(0)
		R	3(60)	3(60)	3(60)	2(40)	3(60)	4(80)	5(100)	5(100)	1(20)	3(60)
G.H.K	6	S	1(16.7)	0(0)	2(33.3)	0(0)	0(0)	0(0)	2(33.3)	0(0)	2(33.3)	0(0)
		I	0(0)	2(33.3)	0(0)	2(33.3)	0(0)	0(0)	0(0)	1(16.7)	0(0)	1(16.7)
		R	5(83.3)	4(66.7)	4(66.7)	4(66.7)	6(100)	6(100)	4(66.7)	5(83.3)	4(66.7)	5(83.3)
G.H.B	14	S	0(0)	9(64.3)	9(64.3)	2(14.3)	5(35.7)	2(14.3)	1(7.1)	0(0)	6(42.9)	3(21.4)
		I	1(7.1)	2(14.3)	3(21.4)	3(21.4)	0(0)	4(28.6)	4(28.6)	2(14.3)	3(21.4)	2(14.3)
		R	13(92.9)	3(21.4)	2(14.3)	9(64.3)	9(64.3)	8(57.1)	9(64.3)	12(85.7)	5(35.7)	9(64.3)
G.H.A	11	S	3(27.3)	1(9.1)	0(0)	0(0)	0(0)	0(0)	1(9.1)	1(9.1)	0(0)	1(9.1)
		I	3(27.3)	2(18.2)	3(27.3)	0(0)	2(18.2)	1(9.1)	1(9.1)	1(9.1)	0(0)	1(9.1)
		R	5(45.4)	8(72.7)	8(72.7)	11(100)	9(81.8)	10(90.9)	9(81.8)	9(81.8)	11(100)	9(81.8)
G.H.L	12	S	2(16.7)	7(58.3)	1(8.3)	1(8.3)	3(25)	1(8.3)	0(0)	0(0)	2(16.7)	0(0)
		I	0(0)	2(16.7)	5(41.7)	1(8.3)	0(0)	2(16.7)	0(0)	0(0)	1(8.3)	2(16.7)
		R	10(83.3)	3(25)	6(50)	10(83.3)	9(75)	9(75)	12(100)	12(100)	9(75)	10(83.3)
G.H.KN	10	S	5(50)	0(0)	4(40)	2(20)	1(10)	0(0)	0(0)	2(20)	2(20)	4(40)
		I	1(10)	5(50)	1(10)	1(10)	1(10)	3(30)	2(20)	1(10)	1(10)	1(10)
		R	4(40)	5(50)	5(50)	7(70)	8(80)	7(70)	8(80)	7(70)	7(70)	5(50)
G.H.W	8	S	0(0)	0(0)	5(62.5)	0(0)	1(12.5)	0(0)	1(12.5)	0(0)	3(37.5)	2(25)
		I	1(12.5)	3(37.5)	2(25)	0(0)	1(12.5)	4(50)	3(37.5)	1(12.5)	1(12.5)	2(25)
		R	7(87.5)	5(62.5)	1(12.5)	8(100)	6(75)	4(50)	4(50)	7(87.5)	4(50)	4(50)
G.H.N	5	S	1(20)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	1(20)	2(40)
		I	1(20)	0(0)	1(20)	0(0)	0(0)	1(20)	3(60)	1(20)	0(0)	0(0)
		R	3(60)	5(100)	4(80)	5(100)	4(80)	4(80)	2(40)	4(80)	4(80)	3(60)

Key:OFX:Ofloxacin;PEF:Perfloxacin;CPX:Ciprofloxacin;NA:Nalidixicacid;CN:Gentamicin;ST:Streptomycin;PN:Ampicillin;Cep:Cephalexin;AU:Augmentin;SXT:Sulfamethoxazole; S: Susceptible; I:Intermidiate; R:Resistance; G.H.S: General Hospital Suleja; G.H.M: General Hospital Minna; G.H.K: General Hospital Kuta; G.H.B: General Hospital Bida ; G.H.A: General Hospital Agaie; G.H.L: General Hospital Lapai; G.H.KN: General Hospital Kontagora; G.H.W: General Hospital Wushishi; G.H.N: General Hospital Nasko

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DISCUSSION

Pelvic inflammatory disease is basically the disease of female genital organs. It is usually said to occur when bacterial ascends from the vagina to the upper genital tract. This disease occurs as cervicitis, endometritis, oophoritis and salpingitis and in severe cases it can result to infertility (Oseni *et al.,* 2017; Ahmed 2017).

This study indicates that 720(62%) o f the samples collected from PID patients were positive for bacterial growth. This is based on the silent spread of bacterial organisms to the upper genital tract which results to high degree of damages such as miscarriage, preterm labor and ectopic pregnancy in the infected females (Naaz *et al.,* 2016). These therefore lead to infertility among the female population. This is in agreement with the findings of Pachori and Kulkarni (2016) and Naaz *et al.* (2016) who reported that higher rates of bacterial infections such as 60%, 57% and 30% in Africa, Asia and Indian respectively.

The high occurrence of *E.coli* 170(23.6%) revealed in this study could be based on the fact that *E.coli* predominantly colonize the gastrointestinal tract and as such is the main causative agent of urinary tract infections, and this frequently exposes this organism to the vagina due to its proximity to the periurethral openings and the perianal areas. This results is in agreement with the findings of Erdem *et al.* (2018), who revealed that majority of the organisms isolated from patients with urogenital infections are *E.coli*.

Furthermore, the high occurrence of multidrug resistant *E.coli* in this study could be attributed to the fact that *E.coli* is highly associated with the transfer of genetic materials such as plasmids, referred to as the resistance (R) - plasmids through various gene transfer processes (such as conjugation) among non-resistant *E.coli*. This result agrees with the findings of Isighoni (2016) and Nikado (2009).

The hundred percent (100%) resistance exhibited by multidrug resistant *E.coli* to eight (8) antibiotics namely; Gentamicin, Ampicllin, Cephalexin, Nalidixic acid, Streptomycin, Augmentin Sulfamethoxazole and Perfloxacin could be attributed to the fact that these multidrug resistant *E.coli* have been exposed to various antibiotics due to the misuse of these antibiotics by the populace in these study area. This finding agrees with the results of Anuli *et al.* (2016) and Anejo-Okopi *et al.* (2015).

Conclusion and Recommendations

This study basically revealed high resistance profile of multidrug resistant *E.coli* associated with pelvic inflammatory disease (PID) and as such have resulted to the rapid dissemination of resistant genes in the study area, which led to prolonged hospital stay, high mobidity and high mortality among reproductive age women with pelvic inflammatory disease (PID). Therefore, it is necessary that the government enforce certain measures such as; constant awareness on the misuse of antibiotics and laws against self medication and illegal purchase of drugs across the counter, to help curb the menace associated with pelvic inflammatory disease resulted from resistant organisms.

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