Prevalence and Associated Risk Factors of Uropathogenic *Escherichia coli* Isolates from Catheterized Persons at Ilorin Tertiary Hospital, Nigeria

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Background and study aim: Urinary tract infection (UTI) is among the most common complications of catheterized persons in hospital settings especially, those caused by extended spectrum beta lactamase (ESBL) and biofilm producing *Escherichia coli*.This hospital based, cross sectional study was carried out to determine the prevalence and associated factors associated with Uropathogenic *Escherichia coli* (UPEC) isolates from catheterized persons (inpatients and outpatients) attending Ilorin Tertiary hospital, Nigeria

Materials and Methods: Between 2nd April and 30th June 2016, urine samples from 113 catheterized inpatient and outpatients were evaluated. Female subjects accounted for 47(41.6%) of the study population. Standard microbiological methods and Analytical Profile Index (API) 20E system were used for the isolation and identification of UPEC, respectively. Tissue culture plate (TCP) technique was used to demonstrate biofilm production potentials.

Results: The prevalence of catheter associated urinary tract infection (CA-UTI) in this study was 70.8% most of which are Gram negative bacilli belonging to the Enterobacteriaceae family with *Escherichia coli* 44(55.0%) being the most predominant pathogen. Extended spectrum beta lactamase (ESBL) *E coli* in this study was 27.2% of which all (100%) were strong biofilm producers. Female subjects had relatively higher prevalence of UPEC isolates, 29 (65.9%) than the male counterparts. Whereas, the highest cases of UPEC was in 61-70 years age group, 29.5%. There was significant association between UPEC and age and gender of subjects ($p<0.05$).

Conclusion: Findings from this study shows that *E. coli* is still the most common uropathogenic bacteria isolate in catheterized persons. Biofilm production confers some degree of ESBL production and antibiotic resistance.

INTRODUCTION

Some strains of *E. coli* are broadly categorized as extra intestinal pathogenic *E. coli* (EPEC) **[1]**. Uropathogenic *E. coli* (UPEC) has several virulence factors that allow it to colonize host mucosal surfaces, injure and invade host tissues, overcome hosts defense mechanisms and incite a host inflammatory response **[1]**. UPEC can colonize the bladder through the urinary tract and cause cystitis; this organism is also able to move through the ureters to the kidneys and cause pyelonephritis **[2]**.

E coli isolated from the urinary tract often express specific virulence properties that are not prevalent among isolates from normal fecal flora. The *E.coli* virulence factors (VFs) that cause urinary tract infection include adhesions, α-hemolysin (Hly), cytotoxic necrotizing

factor, fimbriae, aerobactin-mediated iron uptake, K1 capsular polysaccharide, and biofilm formation. These factors ultimately lead to tissue damage **[3]**. The ability of bacteria to attach to uroepithelial cells through specific fimbriae and adhesions is critical for the initiation of infection **[4]**.

Biofilm formation is a dynamic process that can bring about wide variety of physiological events such as antibiotic tolerance, expression of virulence factors and increased resistance to host defense mechanisms **[5]**. Its emergence as an important ground of morbidity and mortality among hospitalized patients, visitors and staff is responsible for billions of dollars drained in treatment and work lost as such call for concern **[5]**.

CA-UTIs are the most common nosocomial infections and a vast majority of them are caused by biofilm formed on urinary catheters. Catheterization and implanted medical devices will continue to increase especially with the aging population; hence the incidence of biofilm implicated infections will continue to rise **[6]**. Not many studies have documented biofilm formation especially amongst uropathogens in sub-Saharan Africa.

Catheterization and implanted medical devices will continue to increase especially with the aging population; hence the incidence of ESBL implicated *E coli* infections will continue to rise **[6]**. Upsurge in antimicrobial resistance and the consequential complications of CA-UTI mediated by biofilms warrants a comprehensive understanding of the imperative role of specific urovirulence determinants especially the role of ESBL production in affected individuals. In view of these, the present study sought to determine the prevalence and associated risk factors of ESBL *E coli* isolated from catheter urine of in- and out-patients attending University of Ilorin Teaching Hospital.

MATERIALS AND METHODS

Study Area:

This study was conducted at the Department of Medical Microbiology and Parasitology of the University of Ilorin Teaching hospital (UITH), Ilorin. UITH belongs to the second generation of Teaching Hospitals in Nigeria. It is a tertiary health care centre and the only referral centre in Kwara State with a capacity of over 450beds and an average of 10,000 to 12,000 annual admissions in-patient and out-patient's visits respectively in the last five years as captured by the Department of Health Information Management, UITH, in Ilorin, 2015. It is located in the North central region of Nigeria. The Hospital provides quality health care services to the neighboring states like Oyo, Kogi, Niger, Osun, and Ekiti states. Ilorin is the capital of Kwara state in Nigeria, West Africa. Ilorin coordinates on the globe at 8°30′N 4°33′E. Ilorin's central location makes it easily accessible to all parts of the country. The infection control unit of UITH comprised of infectious diseases physicians, nurses, pharmacists and microbiologists. Infection prevention and control work on daily basis to prevent or control the spread of infections in the hospital wards, departments and units and the community. Clinicians suspecting occurrence of healthcare-associated infection (HCAI) report this to the Chief Medical Director or the Chairman, HCAICC (healthcare-associated infection control committee) or Infection control officer (ICO). All details regarding the patient, procedures, medication etc. are made available. Surveillance for HCAIs are prospectively monitored in high risk units of the hospital. High risk areas of the hospital include Operation Theatres (OT), Intensive care units (ICU), Transfusion services unit, Wards, Glutaraldehyde storage and monitoring unit, Kitchen/ Food handlers, Drinkable water source, Central Sterile Supply unit among others. Swabs of surfaces and air environment of OT are sampled and bacteriologically cultured once in six months. Sterilization are done in accordance to culture results. Samples (1ml) of in-use disinfectants, hand wash agents are taken and sent to the microbiology laboratory also once in 6 months for sterility analysis. Records of all microbiological analysis are kept with nurse in charge of OT and infection control nurse (ICN). In case of unacceptable results decision on corrective measures are taken by HCAICC.

Sample Size Determination:

Sample size was determined from a previous cross-sectional study by Fattahi **[7]**, who reported prevalence of Biofilm forming UPEC as 92.0%. With this, a sample size of 113 was calculated. Hence 113 study participants were enrolled.

Study Design:

This was a hospital-based cross sectional study.

Study Population:
The study population comprises of catheterized in-patients and out-patients of all age groups and gender at clinics /units of the hospital such as General Outpatient Department (GOPD), Urology clinic, Male and Female Surgery wards, Accident and Emergency unit.

Sampling Technique:
Random sampling technique was employed for the selection of patients that met the inclusion criteria.

Inclusion Criteria:
1. Patients (both inpatient and outpatient) with indwelling catheter ≥ 48 hours calendar days.
2. Those not on any antibiotics within the last 72 hours before recruitments.

Exclusion Criteria:
1. Patients (both inpatient and outpatient) with indwelling catheter <24hrs.
2. Those on antibiotics within 72 hours of enrollment in the study.

Analytical Laboratory Procedures:
Catheter Urine Sample Collection and Transportation
Urine sampling from patient with an indwelling urinary catheter was obtained from a sampling port using aseptic technique. Where there is no sampling port, the drain tubing was detached from the catheter bag and about 10-15ml urine is allowed to drain aseptically into a sterile receptacle. Collected urine in the catheter bag was not considered. All sample collection was carried with the assistance of an assigned Doctor. Samples collected were promptly transported to the Medical Microbiology and Parasitology department for analysis. However the samples that were unavoidably delayed were refrigerated at 2-8^{0}C for not more than 4hours.

Culture and Isolation:
A modified semi-quantitative culture technique was used. Standard calibrated bacteriological loop (to determine the colony forming unit, CFU) was used to aseptically transfer 0.001 ml of a well mixed urine sample on appropriately well labeled culture of Cysteine Lactose Electrolyte Deficient (CLED) agar (Biomarker) and 5.0% Sheep Blood Agar (Biomarker) media. All culture media were prepared according to the manufacturer's specifications. Incubation was at 37°C for 18-24 hours aerobically.

Characteristic of Isolates:
E. coli appears on CLED as Large, elevated opaque yellow lactose fermenting colonies with a slightly deeper yellow center. E.coli appears on Blood Agar as large, opaque, sticky and colorless, ± narrow clear hemolysis zone.

Bacterial Biochemical Testing
A Commercially available phenotypic qualitativ API 20E system (Bio Merieux, France) for Enterobacteriaceae identification was used for the biochemical confirmatory identification of UPEC isolates **[8]**.

Extended Spectrum beta Lactamase (ESBL) Detection:
The double disk synergy test (DDST) using third generation cephalosporins (3GS) which includes ceftrazidime (30ug) and ceftriaxone (30ug) antibiotic discs alongside amoxicillin–clavulanate (10ug) disc were used for the detection of ESBL production amongst UPEC isolates. This was done by placing the antibiotic disk at distance of 20mm from each other (center to center). Following incubation at 37^{0}C aerobically for 18-24hrs, a positive synergistic effect showed inhibition zone between disks. A >5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone is taken as a positive ESBL production **[8]**.

Biofilm Assay Using Tissue Culture Plate (TCP) Method:
A phenotypic quantitative Tissue Culture Plate (TCP) Method first developed and described by Christensen et al **[10]** and considered as the gold standard for biofilm detection was used for the detection of biofilm production in all UPEC isolates.

Antibiotic Susceptibility Testing:
This was performed according to the Clinical and Laboratory Standards Institute (CLSI), 2017 guide-lines. In-vitro antibiotic susceptibility testing of established biofilm producing UPEC isolated was carried out by modified Kirby-Bauer disc diffusion method on Muller-Hinton (MH) agar plates. The following Oxoid™ antibiotic susceptibility disks (ThermoFisher™, UK) were used; Amoxicillin-Clavulanate (10ug), Ceftriaxone (30ug), Ceftazidime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Nitrofurantoin (300 µg), and Imipenem (10ug).

Quality Control:
International reference strains of Escherichia coli ATCC 25922 served as positive control.

Questionnaire:
Structured open ended questionnaires were prepared and administered on the spot to each participant. The participants were asked about sociodemographic data and clinical presentations/ diagnosis at enrollment for this study. Data accessed from questionnaires included their age, gender, marital status, education level, occupation, number of wives, sexual activity and places of resident.

Statistical Analysis:
The values obtained from this study were entered into a computer; data were analyzed using Statistical Package for Social Science (SPSS) version 21. Data were presented as bar-chart, pie chart and frequency tables. Chi-square test was used determine association between age, gender and UPEC isolates from subjects. P value < 0.05 was considered as statistical significant.

RESULTS

Catheter urine samples from One hundred and thirteen (113) patients comprising of both inpatients and outpatients who met the inclusion criteria set out for this study were collected within the month of April –June, 2016.

Table 1: Sociodemographic characteristics of sampled population

Variables		Frequency	Percentage
Residence	a. Rural	51	45.1
	b. Urban	62	54.9
	Total	113	100
Sexual activity (last 10 days)	a. No	93	82.3
	b. Yes	20	17.7
	Total	113	100
Age	≤40	13	11.5
	41 – 50	5	4.4
	51 – 60	13	11.5
	61- 70	46	40.7
	>70	36	31.8
	Total	113	100
Gender	a. Female	47	41.6
	b. Male	66	58.4
	Total	113	100
Marital Status	a. Single	4	3.5
	b. Married	45	39.8
	c. Widow/ Widower	64	56.6
	d. Total	113	100
Educational level	a. No formal education.	51	51.3
	b. Primary	36	31.8
	c. Secondary	24	21.2
	d. Tertiary	2	1.7
	Total	113	100
Occupation status	a. Unemployed	46	40.7
	b. Employed	26	23.0
	c. Self-employed	41	36.3
	Total	113	100
Family structure	a. Monogamous	54	47.8
	b. Polygamous	59	52.2
	Total	113	100

Male patients formed the majority of the sampled patients with 58.4% while age group range of 61-70 (40.7%) recorded the highest participants in this study. More of the subjects reside in urnan areas, 62 (54.9%) resident than those from rural areas, 51 (45.1%). Majority of the subjects had no sexual activity for the last 10 days before enrollment in the study, 93 (82.3%). Widows/ widowers made the highest proportion of subjects, 64 (56.6%). Majority of them were unemployed, 46 (40.7%) (Table 1).

Table 2: Clinical presentation of subjects enrolled for this study

Clinical presentations	Frequency	Percent (%)
Spinal Cord Injury	1	9.0
Trauma Injury	5	4.4
Unknown	7	6.2
Neurogenic Bladder/retention (BPH)	8	7.1
Bladder Outlet Obstruction (BOO)	11	9.7
Incontinence	20	17.7
Post-Operative Surgical Care	23	20.4
Palliative Care for terminally ill/Old age	38	33.6
Total	113	100.0

As shown in Table 2, palliative care for terminally ill/old age patients had the highest frequency of 33.6% while patients with spinal cord injury had the least of 9.0%.

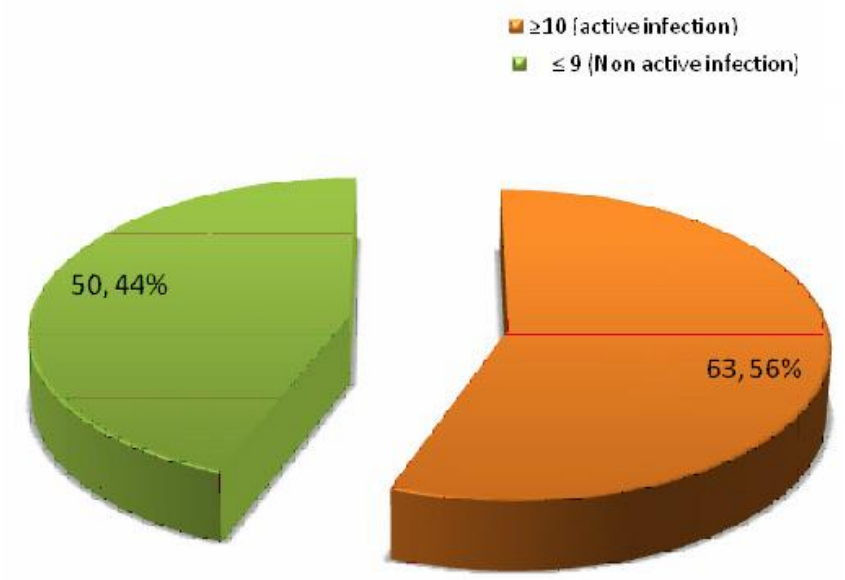

Figure 1: Pyuria level of the total sampled population

Microscopic examination of the catheter urine shows that 63 (55.8%) recorded Pyuria level ≥10 indicative of active infection as presented in Figure 1.

Table 3: Cultural outcome of catheter urine after 24hrs of incubation at 37^0C

Report	Frequency	Percent
Significant Pure culture isolates	80	70.8
No growth (No visible colony)	14	12.4
Mixed Growth (More than two isolates)	19	16.8
Total	113	100.0

As shown in Table 3, prevalence of CA-UTI was 80 (70.8%) while those with mixed growth accounted for 19 (16.8%).

Table 4: Relationship between gender and age amongst UPEC isolates

VARIABLES	UPEC			
	POSITIVE (%)	NEGATIVE (%)	X^2	ρ
GENDER			17.647	0.000028
Female	29 (65.9)	18 (26.1)		
Male	15 (34.1)	51 (73.9)		
AGE GROUPS				
≤ 40	11 (25.0)	2 (2.9)	17.647	0.0014
41 – 50	3 (6.8)	2 (2.9)		
51 – 60	7 (15.9)	6 (8.7)		
61 – 70	13 (29.5)	33 (47.8)		
≥ 71	10 (22.7)	26 (37.7)		

Distribution of UPEC isolates across gender shows 29 (65.9%) were females. While the highest occurrence across age groups were observed amongst 61-70 years with 29.5%. There was significant association between UPEC and age and gender of subjects ($p<0.05$).

Table 5: Distribution of UPEC isolates amongst patient's category

VARIABLE	UPEC			
	Positive (%)	Negative (%)	X^2	*P*
Patients category Inpatients	32(72.7)	22(31.9)	17.963	0.000023
Outpatients	12(27.3)	47(68.1)		

The occurrence of UPEC isolates was more frequent among Inpatients 32(72.7%) as shown in table 5. This was statistically significant association between frequency of UPEC isolated with patients' categories ($p<0.05$).

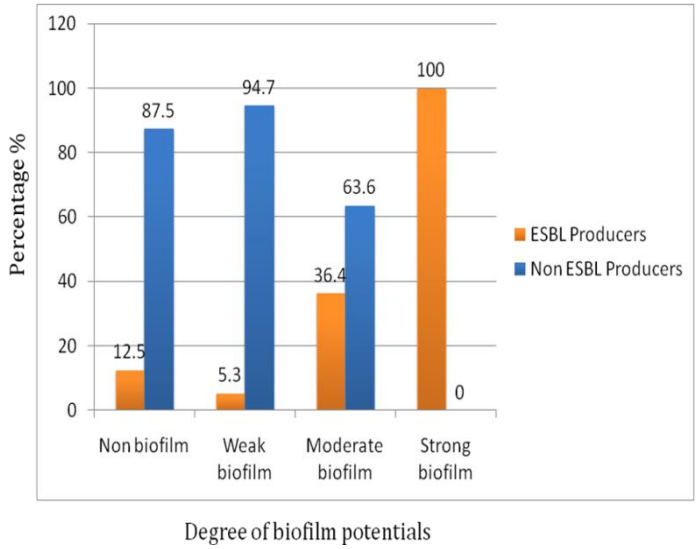

Figure 2: Relationship between biofilm and ESBL Production
Figure 2 shows a statistical significant difference ($p<0.05$) in the incidence of ESBL across varied degree levels of biofilm formation. Highest incidence was observed in strong biofilm producers (100.0%).

Table 6: Antibiotic Susceptibility Pattern of UPEC Isolates (n=44)

Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
Amoxicillin-Clavulanate	13(29.54)	0(0.0)	31(70.45)
Ceftriaxone	29(65.9)	0(0.0)	15(30.09)
Ceftazidime	25(56.81)	0(0.0)	19(43.18)
Ciprofloxacin	22(50.0)	1(2.3)	21(47.72)
Gentamicin	6(13.64)	0(0.0)	38(86.36)
Nitrofurantoin	31(70.45)	0(0.0)	13(29.54)
Imipenem	41(93.2)	3(6.8)	0(0.0)

As shown in table 6, UPEC isolates exhibited high resistance to Gentamicin and Amoxicillin-Clavulanate of 86.36% and 70.45% respectively. Imipenem showed 100% effectiveness with no resistant UPEC isolate.

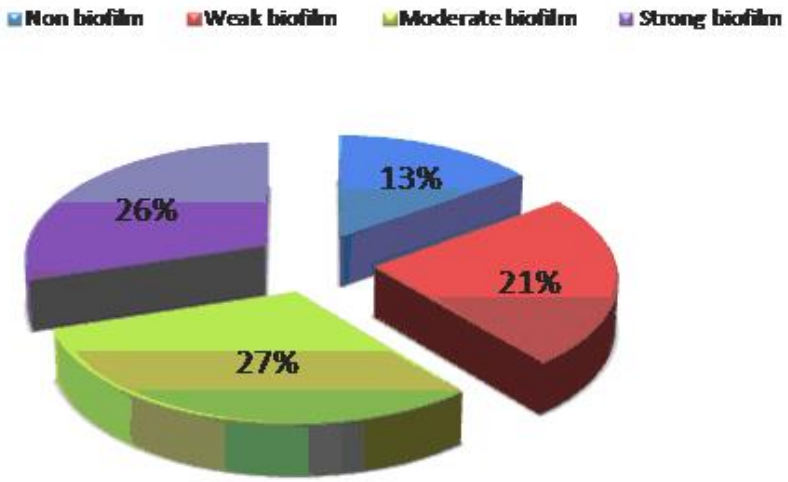

Figure 3: Resistance patterns and degree of biofilm formation
Average antibiotic resistance pattern amongst UPEC across the four grade levels of biofilm production showed Strong 26.0%, Moderate 27.0%, Weak 21.0% and Non biofilm producers 13.0% respectively. Non biofilm producing UPEC showed the least resistance pattern.

DISCUSSION

This present study involved the recruitment and evaluation of 113 patients with indwelling urinary catheter, within the period of 2nd April and 30th June, 2016. These patients comprises of both outpatients and inpatients. The prevalence of CA-UTI in the study area is (70.8%), 55.0% of which is caused by UPEC isolates. Prevalence of biofilm production potential by UPEC isolates was 31.8% respectively. Isolation and phenotypic identification of UPEC by standard microbiological methods and miniaturized API 20E system showed that 85.5% of the significant isolates observed in this study were gram negative bacilli belonging to the Enterobacteriacea. This observation is in agreement with Nicolle **[10]**, who reported that most uropathogens are bacteria derived from the bowels that find their ways to the urinary tract through the urethra.

UPEC accounted for 44(55.0%) of the total uropathogens isolated, hence the most predominant pathogen. Comparatively the trio of Michael **[11]** and Nermeen et al **[12]** reported similar findings where UPEC was observed as the most prevalent uropathogen despite variation in the prevalence rates of 24.14% and 31.7% respectively. These variations may be partly explained by the differences in study populations and also in the inclusion criteria used by centers in selecting urine samples for culture. Antibiotic intake prior to presentation at the hospital may also be a key factor in bacterial yield. This study finding was however in contrast with Gruneberg **[13]** who posited that UPEC as the leading cause of urinary tract infections was being replaced by other members of the Enterobacteriaceae.

Distribution of UPEC across age groups confirmed that UPEC is common in all age groups. The highest frequency of 29.5% was observed amongst 61-70 years while the least occurrence of 6.8% was observed amongst 41-50 years. The high incidence as observed in advanced aged groups can be attributed to the presence of a number of risk factors some of which includes prostatic enlargements in males, diabetes mellitus, reduced ambulation, osteoporosis, interventional instrumentations like catheterization and weak bladder sphincter **[14]**.

Further analysis shows that out of the total of 54(47.8%) inpatients evaluated in this study, 32(72.7%) yielded significant growth of UPEC as summarized in Table 4.5, which was statistically significant ($p<0.05$). Similar observation was also reported by Ponnusamy and Nagappan **[15]** where 62.96% inpatients yielded UPEC isolates as compared to 37.03% from outpatients. Other previous studies which reported similar findings includes Hassan et al **[16]**; Jigna and Pratibha **[17]** who reported incidence rate of 83.3% and 60.0% respectively. There was significant statistical difference ($p<0.05$) observed in the distribution of UPEC isolates across gender of which female subjects accounted for a notable frequency of 29(65.9%) despite higher male recruitment of 66(58.4%). This finding was in line with other research such as that of Ponnusamy and Nagappan **[17]**; Kashef et al. **[18]** who reported prevalence in females as 56.0% and 60.0% respectively. This disparity between gender could be traceable to several factors which include anatomic differences such as the proximity of the female urethra to the anal region, hormonal effects and behavioral patterns.

Persistence of UPEC as a major ethological agent in UTI and as observed in this study and other similar research work can be attributed to the expression of a variety of virulence factors, which include adhesins (e.g., type 1 and P fimbriae) and toxins (e.g., haemolysin), cytotoxic necrotizing factor, fimbriae, aerobactin-mediated iron uptake, K1 capsular polysaccharide and biofilm formation **[19]**.

Biofilm assay using the TCP method shows that the frequency of biofilm formation potentials amongst UPEC isolates in this study was 81.8%. This finding was consistent with other studies such Niveditha et al **[19]** who reported 60.0% biofilm production rate respectively. Another study carried out by Ponnusamy et al **[20]** showed that among 100 *E. coli* strains, 72 (72.0%) strains displayed a biofilm positive phenotype.

The occurrence rate of extended spectrum beta lactamase (ESBL) production of 27.2% amongst biofilm forming UPEC isolates in this study is a double barrel tragedy of public health concern which requires the prompt attention of caregivers. The highest occurrence of ESBL was 100.0% amongst the strong biofilm producers. This high incidence of ESBL reported in this study could be an indication of creation of selective drug pressure because of commonly use of cephalosporin and other antibiotics in our region **[21]**.

UPEC isolates showed the highest susceptibility to antibiotics such as Imipenem 93.2% followed

closely by nitrofuratoin with 70.45%. The resistance pattern of UPEC isolates to the different anti-microbials agents employed in this study shows that among the antibiotic tested, resistance to gentamicin was the highest (86.36%) while resistance to Imipenem was zero (0.0%). Resistance to antimicrobial agents is well documented and has been noted since the first use of antibiotics which currently has become a worldwide problem **[22]**. This study finding reveals a higher antibiotic resistance development to the commonly used antibiotics for prophylaxis and for empirical therapy for UTI such as gentamicin and nitrofuratoin respectively. This may be due to increased consumption of these drugs, self-medication, and transfer of resistance isolates. These resistance pattern as observed is supported by the work of Hryniewicz et al **[2]** who stated that, the worldwide data is showing an increasing resistance among urinary tract pathogens urinary tract pathogens towards the conventional drugs.

LIMITATION:

This study is not without limitation, the major limitation of this study is enrolment of outpatients whom might have had the bacteria incubating in them before sampling. In addition, the inadequacy of sociodemographic variables studied. This might led more credence to risk factors data that favors contracting *E coli* infection.

CONCLUSION

Findings from this study shows that *E. coli* is still the most common uropathogenic bacteria isolate in catheterized persons. Biofilm production confers some degree of ESBL production and antibiotic resistance.

Conflict of interest:

None

Funding:

Self funded

Ethical Considerations:

Ethical approval was sought from the University of Ilorin Teaching Hospital Ethical Review Committee (approval number: ERC/PAN/2016/1528). Written and/or verbal informed consent was also obtained from all participating subjects in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. This was done via an informed consent forms duly completed by all the subjects.

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