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Plasmid Profiles of Resistant Strains of *Escherichia coli* in Men Attending Prostate Clinics in a Tertiary Hospital in Enugu State, Nigeria

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Abstract

Multi-drug resistant *Escherichia coli* has become a major threat and cause of many urinary tract infections (UTI) in Enugu, Nigeria. The study was carried out to determine the resistant plasmids of multidrug resistant *Escherichia coli* isolated from men attending prostate clinics at 82 division Hospital, Enugu. A total of 50 urine samples were collected from patients attending prostate clinic at 82 division Hospital, Enugu, presenting UTI: with their biodata. The samples were inoculated on Eosin methylene blue agar and characterized by general microbiological standard. Antimicrobial susceptibility testing was performed using Kirby-bauer disk diffusion method on

Mueller-hinton agar. Multi drug resistant isolates were selected for plasmid profiling. Plasmids were extracted by the alkaline lysis method, electrophoresed on 0.8% agarose gel and profiled using a gel-photo documentation system gel. *Escherichia coli* were isolated at 72%. *E. coli* isolate showed high resistance to most of the test agents. *E.coli* strains were observed to resistant to more than nine antibiotics. The resistant plasmid DNA were detectable in all of the 11 multi drug resistant *Escherichiacoli* having single sized plasmids of the same weight 18kbp. 6 strains (lanes 6,7,8,9,10 and 11) had multiple sized plasmids at 9,8,8,8 and 9kbp respectively. They were all resistant to amoxycillin, ciproflox, nalidixic acid, reflaxine, pafloxacin and chloramphenicol. The study has highlighted the emergence of multidrug resistant plasmid among *E.coli* causing urinary tract infections in Enugu, Nigeria. There is a high level of resistance to many antimicrobials that are frequently used in Enugu, Nigeria.

Key words: Plasmids, Antibacterial resistance, antimicrobials, *Escherichia coli,* Urinary tract infections

1. Introduction

Urinary tract Infection (UTI)is a significant disease and a major cause of morbidity and mortality and are commonly spread [32,33]. Occurrence of urinary pathogens occurs among different age groups, sex, catherization, hospitalization and previous exposure to antimicrobial[39]. Urinary tract infections present with burning sensations during urination, dysuria, pain in the back or lower abdominal pain, fever or chills and frequent or intense urine urge. In Nigeria, *E. coli*, Proteus spp and klebsiella spp have been isolated in 90.0% of UTI reported cases[15,19]. *E.coli* is mostly implicated in UTI and is associated with asymptomatic bacteriuria and symptomatic UTI caused by uropathogenic *E. coli* [12,17].

E.coli is ubiquitous in humans and can act as an extraintestinal pathogen (EXPEC) causing both community as well as hospital acquired urinary tract infections and sepsis often leading to serious secondary health issues[37]. The widespread and injudicious use of antibiotics at the community level cause a high level of resistance patterns of microorganisms to common antibiotics. UTI caused by multidrug resistant (MDR)*E.coli* increases cost of treatment, morbidity and mortality[25,26]. These MDR isolates has serious implications for the empiric therapy against pathogenic isolates and for the possible co-selection of antimicrobial resistant, mediated by multi drug resistant plasmid[35,36].Antibiotic resistance in *E. coli* can arise by mutations in diverse targets or by acquisition of preexisting genes whose products target antibiotics for alteration or efflux[10,30].Mobile resistance genes have the greatest potential for spread of antimicrobial resistance in the microbiome. Bacterial plasmids are self-replicating, extrachromosomal elements that are key agent of change in microbial population. They promote the dissemination of a variety of traits, including virulence, enhanced fitness, resistance to antimicrobial agents, and metabolism of rare substances. *E. coli* from clinical isolates are known to harbor plasmids of different molecular sizes[9]. *E.coli* shows high resistance to ampicillin, amoxicillin, tetracycline and trimethoprim – sulfamethoxazole[30,32]. Resistance patterns of uropathogenic *E. coli* to various antibiotics has also been reported[1]. Multiple drug resistance isolates causing UTI has serious implications for the empiric therapy against pathogenic isolates and for the possible co-selection of antimicrobial resistant mediated by multi-drug resistant plasmids. Antibiotic sensitivity patterns vary in different locations, the widespread occurrence of drug resistant *E.coli* and other pathogens in developing countries and Nigeria in particular has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide basis for developing rational prescription programs, making policy decisions and assessing the

effectiveness of both. In this study, we aimed to determine the prevalence and the resistant plasmids of multi-drug resistant *Escherichia coli* isolated from men attending prostrate clinics in parts of Enugu State, Nigeria.

II. Materials and Methods

Sampling

A total of fifty (50) clean-voided, mid-stream urine samples were collected from men attending '82 Division hospital, Enugu. Samples were collected from Jan to Feb 2023 and sent immediately to microbiology lab, Enugu State University of Science and Technology.

Isolation of Organisms

All the media used were prepared according to manufacturer's instructions and then autoclaved at 121^0C for 15 min. 0.1ml of urine sample was transferred on Eosin methylene blue agar and spread with a sterile inoculating loop. The plates were incubated at 37^0C for 24hr. Resultant colonies were purified by transferring to nutrient agar and incubated at 37^0C for 24hr. The characteristic isolates were aseptically isolated and characterized using established microbiological methods including colonial morphology, Gram's staining reaction, haemolytic reaction, catalase and coagulase tests. Identified *E.coli* colonies were stored on agar slants at 4^0C for further use.

Inoculum Preparation

MacFaland's turbidity standard was prepared by dissolving 1m1 of barium chloride ($BaC1_2$) into 9m1 of sulphuric acid (H_2S0_4). One loopful of each of the pure isolates (*E.coli*) were transferred /into sterile 5m1 nutrient broth in a test tube and incubated at 28°C for 24hr. Each of the cultures was then adjusted to 0.5 MacFaland's turbidity standard.

Antibiotic Susceptibility Testing

The antibiotic susceptibility test was carried out as described by Kirby-Bauer disc diffusion method and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS). 0.1ml of each isolate already matched to 0.5 Macfarland's standard turbidity were transferred on Mueller hinton agar plates and incubated at 37^0C for 24 h. Ten common antimicrobial drugs were use: ciprofloxacin (CPX) (10ug), Nalidixic acid (NA)(10ug), Gentamycin(CN)(10ug),Amoxycillin(AMX)(30ug),septrin(S)(30ug), reflacine(RD)(10ug),perfloxacin(PEF)(30ug),chloramphenicol (CH)(30ug),ofloxacin(OFX)(10ug), levofloxacin (LEV)(30ug)(Maxicare medical laboratory).The size of the area of suppressed growth(zone of inhibition) was determined by the concentration of the antibiotics present in the area, therefore, the diameter of the inhibition zones denotes, the relative susceptibility to a particular antibiotic. Inhibition zones of diameters were measured after 24h of incubation at 37^0C.

Plasmid DNA Extraction

The DNA extraction was done using Zyppy™ Plasmid Miniprep. 1.5 ml of bacterial culture was centrifuged for 5 min at maximum speed (1200 rpm), the supernatant was discarded and the process repeated twice. A total of 600 µl of TE or water was added to the bacterial cell pellet and resuspended completely. A total of 100 µl of 7X Lysis Buffer (Blue) was added and mixed by

inverting the tube 6 times. The sample was allowed to stand for 2 mins. A total of 7X Lysis Buffer was added, color change was observed in the solution from opaque to clear blue, indicating complete lysis. A total of 350µl of cold Neutralization Buffer (Yellow) was added and mixed thoroughly. The sample turns to yellow when the neutralization is complete and a yellowish precipitate was formed. The sample was inverted for 3 times to ensure complete neutralization and centrifuged at maximum speed for 4 minutes. The supernatant (~900µl) was transferred into the provided Zymo-Spin™ IIN column. Care is taken to avoid disturbing the cell debris pellet. The column was placed into a collection tube and centrifuged for 30 secs. The flow-through was discarded and the column placed back into the same Collection Tube. 200µl of Endo-Wash Buffer was added to the column and centrifuged for 30 secs. A total of 400µl of Zyppy™ Wash Buffer was added to the column and centrifuged for 1 min. The column was transferred into a clean 1.5 ml microcentrifuge tube and 30 µl of Zyppy™ Elution Buffer was added directly to the column matrix and allowed to stand for one min at room temperature centrifuged for 30 seconds to elute the plasmid DNA.

Agarose Gel Electrophoresis of Extracted Plasmid DNA

An aliquot (3 µl) of the extracted plasmid DNA was mixed with 7 µl of DNA gel loading dye (Thermo Scientific™). The mixture was analyzed on 1% Agarose gel stained with 1µg/mL of ethidium bromide following protocol described by Lee *et al.* (2012). Electrophoresis was carried out at 90 volts for 45 min and visualized/ illuminated under ultraviolet transilluminator. A 1 kbp DNA ladder (New England Biolabs, USA) was used as DNA molecular weight marker.

Statistical Analysis

Frequencies were obtained and percentages were calculated for study variable. Chi-square was used to calculate and determine significance. A p-value of less than or equal to 0.05 was considered to be statistically significant($p<0.05$).

III. Results

Growth and Morphology of Isolates

Out of 50 urine sample examined 36(72%) were blue black colonies with metallic sheen characteristic of *E. coli* (Table1).

Prevalence of Isolates in The Study Area

E.coli was highly prevalent in the study area at 36(72%) (Fig 1) .

Microscopic appearance and Biochemical Characteristics of *E. coli*

E. coli appeared as Gram- negative rod shaped cells under the microscope and differed in their biochemical characteristics (Table 2).

Prevalence of Symptomatic and Asymptomatic Individuals in the test Population

Pre-experimental activity involved sharing of structured questionnaire comprising of signs and symptoms, information obtained from the respondents helped in grouping them into those that

observed symptoms and those who observed no signs and symptom. 36(72%) respondents were symptomatic while 14 (28%)were asymptomatic (Fig 2).

Macroscopic Analysis of Urine Samples from the Study Population

Physical analysis of urine from respondents recorded altered colour and smell(odour) at higher occurrence of 44 (88%) followed by turbidity at 36 (72%) and bloody urine at 8 (16%) (Table 3).

Prevalence of Genital Symptoms and Clinical Signs in Men with Urinary Tract Infection

From the questionnaire, a total of 36 men indicated having symptoms of urinary tract infections. Significant relationship at $p<0.05$ (0.0012) was observed between presence of symptoms and infection with *E. coli.*(Table 5)

Pattern of Antimicrobial Susceptibility Result of Isolates

The results of antibiotic susceptibilities of *E. coli* from positive urine samples were shown in Fig 3. It was observed that *E. coli* isolates were resistant to most of the test agents at 100%.

Prevalence of Multi-drug Resistance Among the Isolates

Multi drug resistance was defined in the study as resistance to four or more of the antibiotics tested. 100% of *E. coli* (Table 6).

Plasmid DNA profiling

Eleven (11) *E. coli* isolates were characterized by plasmid DNA profiling. Single plasmid bands were observed in all the isolates with molecular weights of approximately 18kbp (lanes

1,2,3,4,5,6,7,8,9,10 and 11). Multiple plasmid bands were observed with molecular weight of 4kbp (lanes 7,8,9and 10). Lane 7 had an additional 7kbp (Table6).

Table 1: Growth and Morphology of Isolates

Media	**Growth morphology**	**Organism isolated**
Eosin methylene blue agar	Blueblack colonies with greenish metallic sheen	*Escherichia coli*

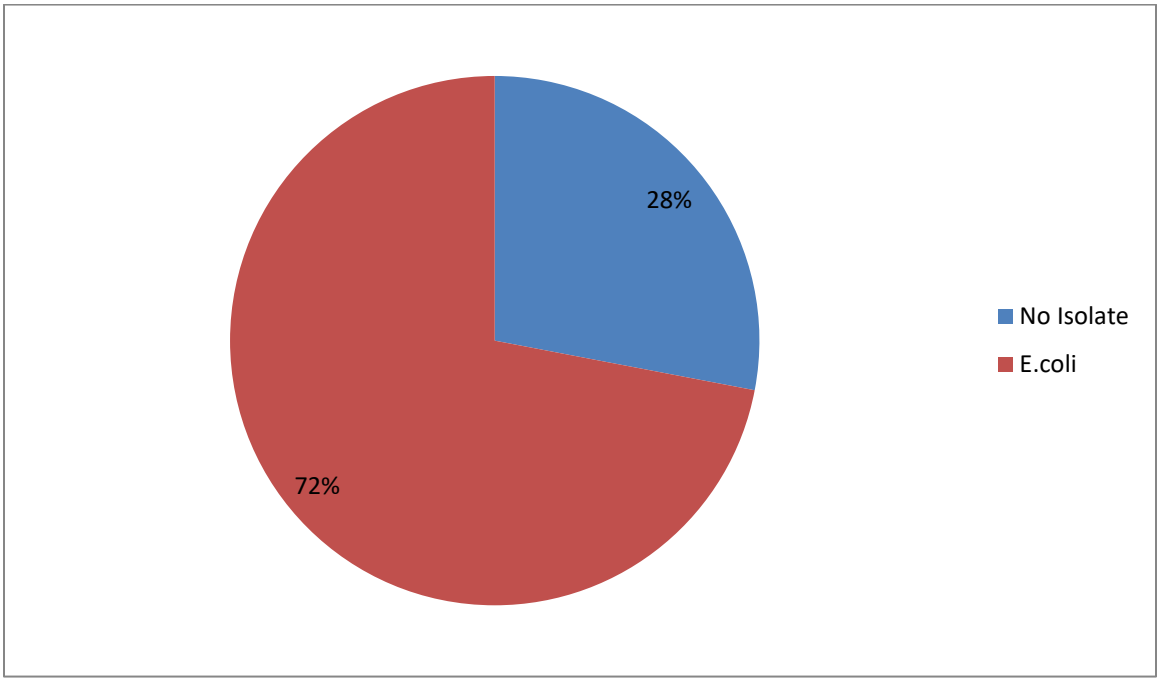

Fig 1: Distribution of *E. coli* isolates from the urine samples

Table 2: Identification and Characterization of Isolates

Biochemical characterization ***E .coli***	**Inference**
Catalase Test	+ve
Coagulase Test	-ve
Indole Test	+ve

Oxidae Test	+ve	
Citrate Test	-ve	
MethylRed Test	+ve	-
Sugar Fermentation Test		
Lactose	Acid/Gas	
Fructose	Acid	
Maltose	Acid	
Glucose	Acid	
Microscopy	Rod shape	
Gram staining	Gram –ve	

Key

+ Positive

- Negative

A Acid

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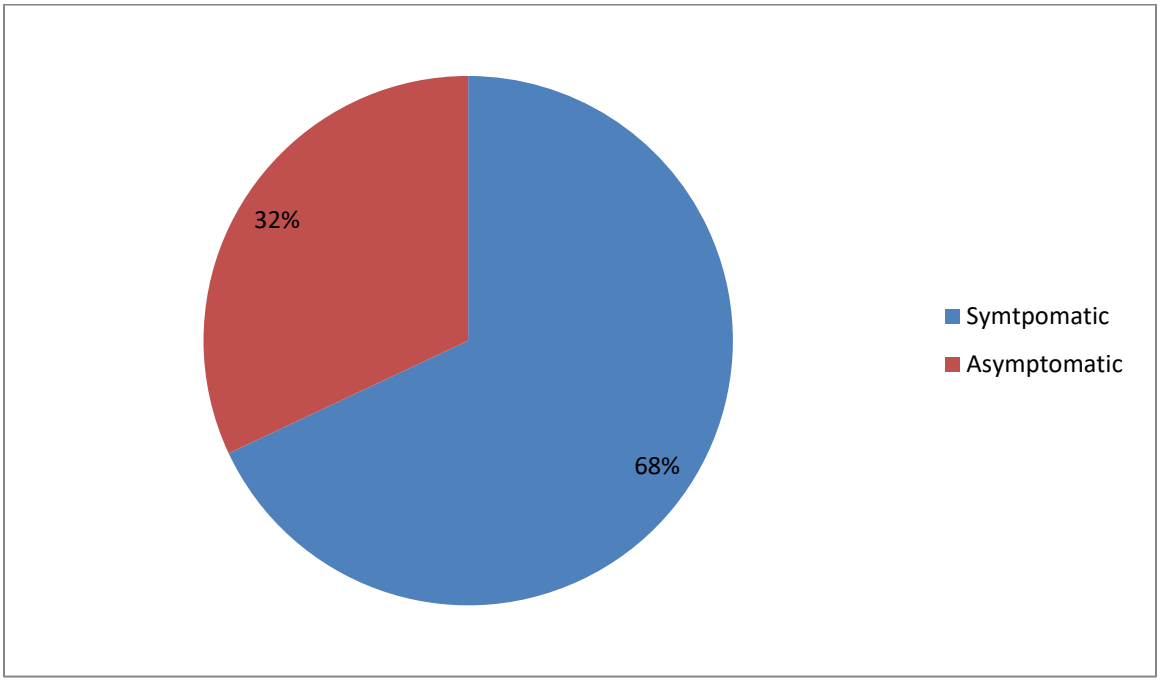

Fig 2: Prevalence of Symptomatic and Asymptomatic respondent in the Test population

Table 3: Macroscopic Analysis of Urine

Test (n=50)	**Normal Parameter**	**% of Normal Parameter**	**Abnormal Parameter**	**%of Abnormal Parameter**
Colour	Pale to dark yellow	6 (12%)	Altered colour	44 (88%)

Clarity	Clear	3 (6%)	Turbid	36 (72%)
			Bloody	8 (16%)
Odour	Slightly nutty	3 (6%)	Altered smell	44 (88%)

Table 4: Distribution of *E.coli* Amongst Men with Abnormal Urine Parameter

Abnormal Parameter	**% of Abnormal Parameter**	**% of those positive with *E.coli*(n=36)**
Altered colour	44 (88%)	36(81%)*
Turbid	36 (72%)	36 (100%)*
Bloody	8 (16%)	8(22%)*
Altered smell	44 (88%)	36 (81%)*

***significance p<0.05(0.0012)**

Table 5: Prevalence of Genital Symptoms and Clinical Signs in Men with Urinary Tract Infection

Criteria(Symptoms) n=50	**% Positive**	**% of men positive with *E.coli*(n=36)**
Pain in the scrotum	42 (84%)	36 (85%)*
Swelling and redness in the	40 (80%)	36 (90%)*

testicle		
Blood in the semen	15(10%)	5 (33%)
Fever and chills	28 (53.6%)	28 (36%)*
Dysuria	44 (88%)	36 (85%)*

*****There was significant relationship between*E.coli* and symptoms at p<0.05(0.0013).

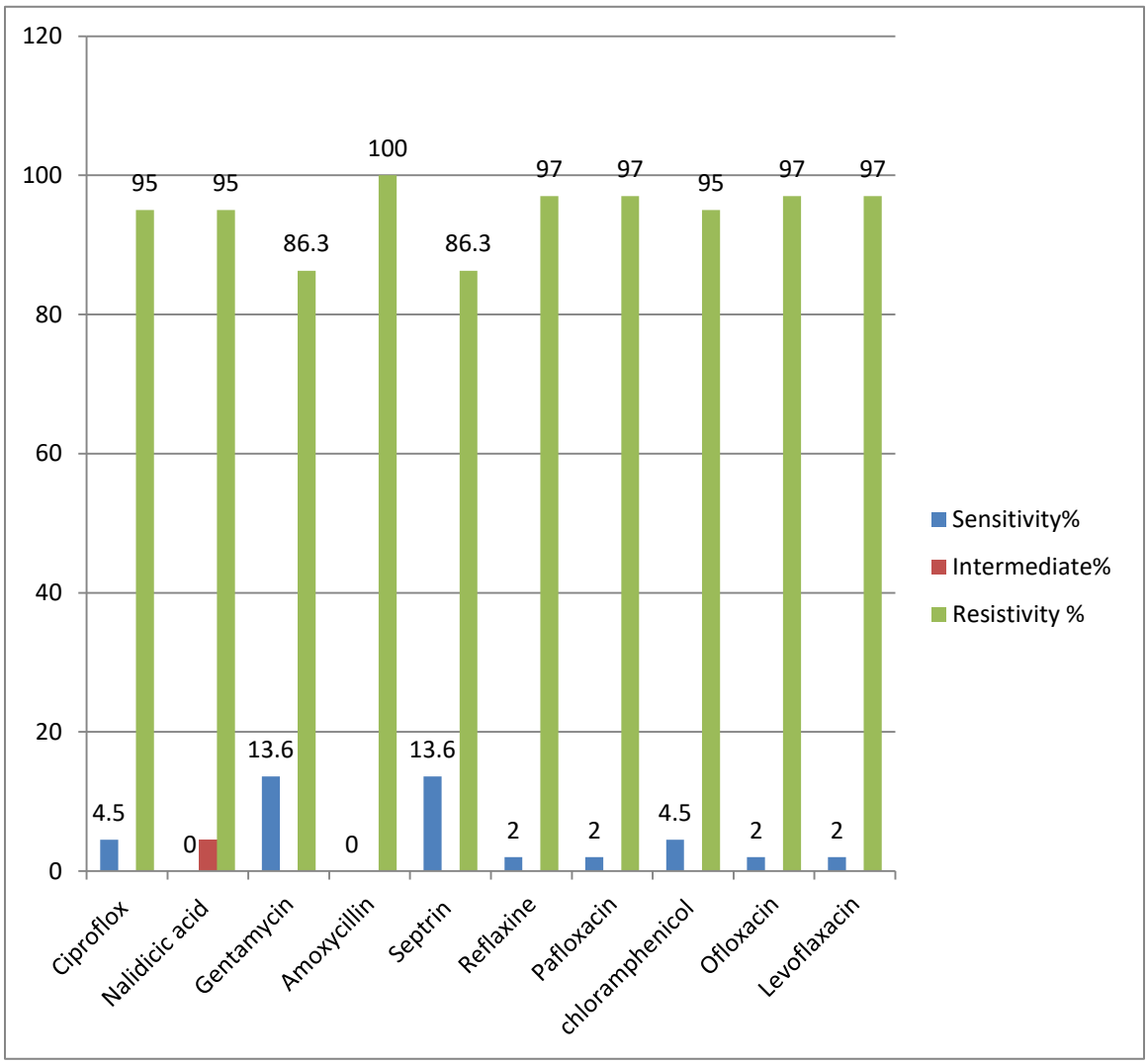

Fig 3: Pattern of Antimicrobial Susceptibility Results of *E. coli*

Table 6: Prevalence of Multidrug Resistance Pattern among the Isolates

Types of isolates	Resistance to 4 agents	Resistance to 5 agents	Resistance to 6 agents	Resistance to 7agents
E. coli(n=34)	36 (100%)	34 (100%)	34 (100%)	29 (85%)

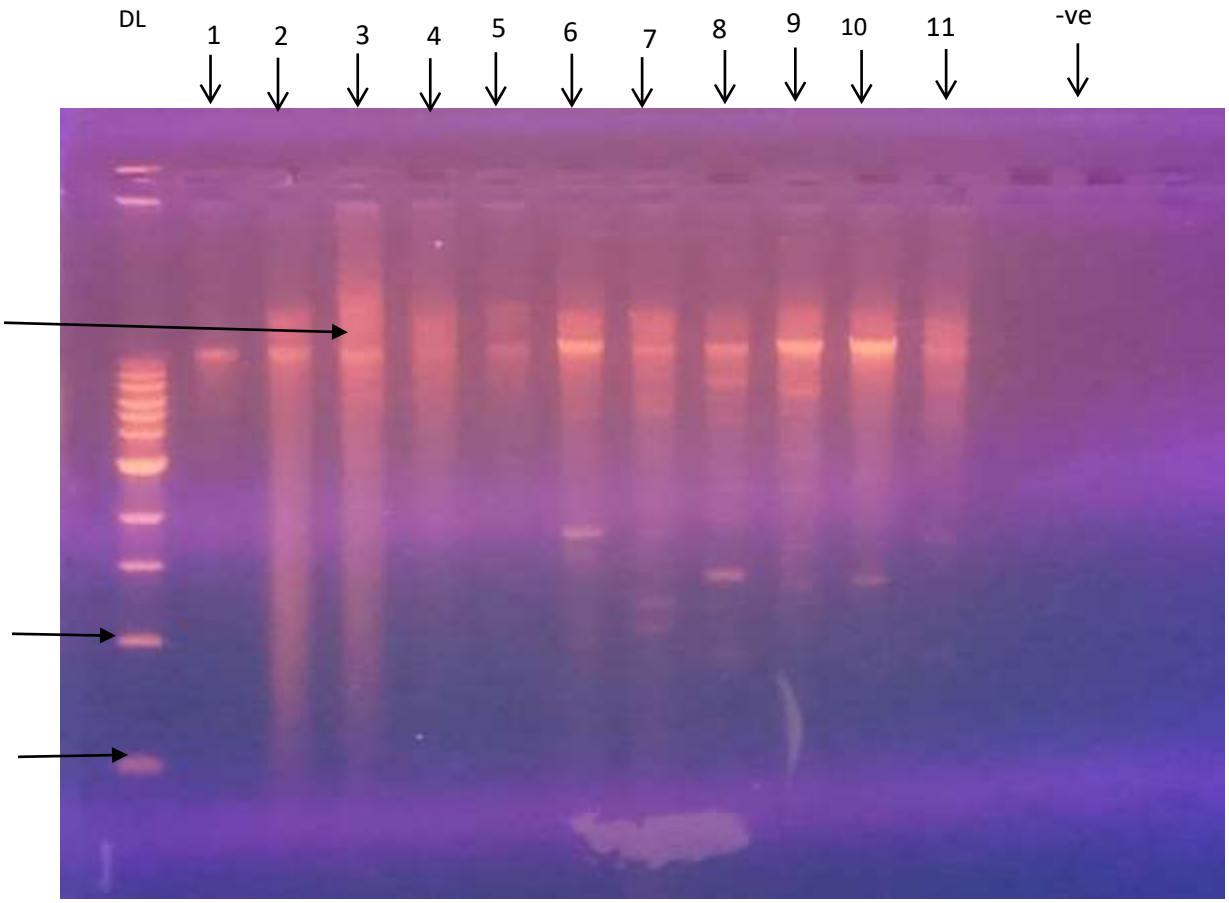

Fig 4. Plasmid DNA profiling. Lanes 1,2,3,4,5,6,7,8,9,10,11 have single bands.

Lanes 6,7,8,9,10&11 have multiple bands

Table 7: Plasmid Characterization isolate from *E.coli* strains, showing numbers and sizes and pattern of resistant antibiotics

Isolate	Number of plasmid isolated	Size of plasmid(kb)	Resistant antibiotics CPX	NA	MX	S	RD	PEF	CH	FX	LEV	
E1	1	18kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	-
E2	1	18kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	-
E3	1	18kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	CN
E4	1	18kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	CN
E5	1	18kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	CN
E6	2	18kb, 9kb	CPX	-	MX	-	RD	PEF	CH	FX	LEV	CN
E7	3	18kb,8kb, 7kb	CPX	-	MX	-	RD	PEF	CH	FX	LEV	CN
E8	2	18kb,8kb	-	NA	-	S	RD	PEF	CH	FX	LEV	CN
E9	2	18kb,8kb	CPX	-	-	S	RD	PEF	CH	FX	LEV	CN
E10	2	18kb, 8kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	CN
E11	2	18kb, 9kb	CPX	NA	MX	S	RD	PEF	CH	FX	LEV	CN

IV. DISCUSSION

UTI caused by the multi drug *E. coli* has increased in the current years probably due to the increasing and irrational use of antibiotics[3]. In this study, *E. coli* occurred at a high prevalence of 34(75%) (Fig 1). This is in line with the works of [3] who found *E.coli* the most predominant species in their study population. *E coli* has been reported as the leading causes of acute and uncomplicated UTI in ambulatory patients [4,5]. Other reporters populated that in Nigeria, *E. coli*, Proteus spp and Klebsiella spp have been isolated in 90.0% of UTI reported cases [22,27,28, 29,26]. This present study revealed *E. coli* isolates as Gram –ve rod shaped strains. (Table 2) The isolates differed in their biochemical reactions. In this study, information obtained was based on respondents. Those classified as asymptomatic men were those who did not report observing any signs and symptoms while symptomatic men observed signs and symptoms (Fig 2).

In this study only 2 (4%) recorded as asymptomatic respondents while 42 (84%) were symptomatic. According to [33] unpathogenic *E. coli* (UPEC) causes symptomatic urinary tract infection (UTI) and little is known about the mechanisms by which the strains colonize the human urinary tract. *E. coli* has also been implicated as the most common organism associated with asymptomatic bacteriuria (ABU). *E.coli* is responsible for more than 80.0% of all UTI causing both ABU and symptomatic UTI's [33,2]. This present study tries to make association of *E. coli* with signs and symptoms from respondents. The pattern of physical urine analysis showed altered colour at 44 (88%); turbid at 36 (72%);

bloody at 8 (16%) and altered at smell 44 (88%). There was association between these criteria with isolated (Table 3). This work observes a strong association between genital symptoms and clinical signs in men positive with *E. coli*. Clinical signs for the isolates were 42 (84%) for pain in the scrotum at 8% for swelling and redness in the testicle (10%) for blood in the semen at 5 (10%), 56% for fever and chills and 88% for dysuria. In the study, there was association between clinical signs in men with urinary tract infection and presence of *E.coli* as causative agents (Table 4). In this study, a high prevalence of resistance to antimicrobial agents was recorded, *E. coli* was resistant at 100% to amoxicillin resistance to fluoroquinolones drugs were recorded thus: Pafloxacin(97%); Reflacine (97%); Ofloxacin (97%); and Ciprofloxacin(95%) followed by Gentamycin and septrin respectively(Fig 4.3). Adnan 2015 and Akingbade *et al*.,2014 showed resistance of *E. coli* from urinary tract infection to cotrimazole at 66.7% and 70% respectively. Varied results have been recorded in other studies. Susceptibility to gentamycin (85.8%), levofloxacin (80%), ciprofloxacin (72.5%) and Ofloxacin (60.8%) was recorded in the works of [2], this is in contrast with the present study. Susceptibility or resistance pattern have been demonstrated in different geographic location [40,7,14,3,2], all recorded varied reaction in their susceptibility patterns. *E.coli* from clinical isolates are known to harbor plasmids of different molecular sizes[9]. According to [31] bacteria harbor antibiotic resistant genes which can be horizontally transferred to other bacteria. Multiple drug resistance in isolates capable of causing urinary tract infection is a serious public health problem as empirical therapy becomes compromised and triggers possible co-

selection of antimicrobial resistant pathogens [35,36]. In the present study, 100% of *E. coli* isolates showed multi drug resistance to test agents. 12% were completely resistant to 10 agents (Fig 3). The increasing resistance of *E. coli* strains to antibiotics makes guidance of therapy by antibiotic susceptibility test of paramount importance.

Resistance to numerous antimicrobial agents in this study may be due to indiscriminate and widespread use of these antibiotics in Enugu, Nigeria which may lead to the spread of mutant strains, insusceptible to medical treatment. All multi-drug *Escherichia coli* isolates in the study possessed plasmids with similar molecular weights of 18kbp. Sample 8 and 10 had additional bands of 9kbp while sample had an additional band of 10 kbp (Fig4). These observations suggest that a number of different plasmids and plasmid combination occur in *E. coli* strains. This agrees with previous studies of Smith *et al*., 2003 who reported that 47 of the *E.coli* isolated form animals in Lagos harbor detectable plasmids which ranged in sizes from 0.564kb to > 23kb. Dambara *et al*.,1987 reported that *E.coli* isolates possessed small plasmids of molecular sizes which ranged between 3.9kb 50kb. Umolu *et al*.,2008 also reported that *E. coli* isolates with high multi-drug resistance profiles were found to possess plasmids, though with large sizes in the range of 6.557- 23. 130kb.

V. Conclusion

This study showed that there was a high prevalence of *E. coli*. There was also emergence of multidrug resistant plasmids among *Escherichia coli* causing urinary tract infections in men

attending prostate clinic in Enugu, Nigeria. It is of public health concern as most of the susceptible drug of choice in other studies were found completely resistant in the study.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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