Original article

Molecular analysis of the resistant factor of virulent uropathogenic *Escherichia coli* in volunteered females of Elizade University

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A R T I C L E   I N F O

A B S T R A C T

Background: The antibiotic resistance, plasmid profile and virulence of *Escherichia coli* isolated from volunteered females of Elizade University, Ilara-Mokin, Ondo State was studied within a period of twenty months along with a questioner. **Methods:** A total of 300 samples of urine were collected and processed using standard microbiological culturing techniques. Identification, antibiotic susceptibility (disc diffusion), plasmid analysis, detection of virulence gene and curing of virulence were carried out. **Results:** The result showed a prevalence rate of 10.5% significant bacteriuria in sexually transmitted disease case. Majority (72.4%) were between 16-22 years (30.25±4.58) and 81% of the students had well educated parents. On a general note, *Eshericia coli* accounted for 42.5% of the bacteria isolated followed by *Staphylococcus aureus* (32.5%). All the uropathogens isolated were not susceptible to the commonly prescribed antibiotics; cefuroxime (100%) but mostly susceptible to quinolone (84%) and cephalosporin (81%). Of the twenty-five (25) *E. coli* isolated from these urine samples, fifteen (15) possess the ability to produce beta-lactamase enzyme. Eleven of these fifteen *E. coli* isolates (11/15%) were positive for type 1 pili fimbriae adhesin virulence factor and one showed the presence of shiga toxin 1. Ten of these *E. coli* had plasmid resistance gene. The curing of these resistant plasmid using high temperature incubation (at 42^0C) showed that six were cured of their resistance. **Conclusion:** These results showed that there has been no significant change in the prevalence of bacteriuria in the recent years. Guideline to prevent indiscriminate use of antibiotics is needed to prevent the resistance observed in this study.

Introduction

One of the most prevalent facultative Gram-negative bacilli in the environment and human fecal flora today is *Escherichia coli (E. coli)*. It usually inhabits the colon as an innocuous commensal. According to the special pathogenicity theory [1], special properties enabling *E. coli* to overcome host defenses in a new environment, are necessary in order for it to escape the limitations of the colonic mileu and move into new niches devoid of competition from other bacterial species [2]. Uropathogenic *E. coli* (UPEC) strains are responsible for approximately 80 % of community acquired and 30 % of nosocomial-acquired urinary tract infections (UTIs). The UPEC belong to the group of the enteropathogenic *E. coli* (EPEC). Females under 10 years of age, or between 18 and 40, are at the highest risk for community-acquired infections. Infections in children are often due to blockages in the urinary tract, resulting in pools of

stagnant urine. Uropathogenic *E. coli* can reside in the colon and then be introduced into the urethra. Urinary tract infections result from ascending colonization of the urinary tract by these strains. Infections can occur in the urethra (urethritis); bladder (cystitis), moderately severe; associated with burning and pain on voiding plus, possibly, suprapubic pain or tenderness from bladder inflammation and kidneys; (pyelonephritis) - most severe; associated with fever, chills, and flank pain from renal inflammation [3]. In order to colonize and establish a UTI, UPEC strains take advantage of assortment of virulence properties. Bacterial adherence to and colonization of the urinary tract by UPEC strains are mediated by the expression of several types of fimbriae and non-fimbriae adhesins, like most adhesins, it has been difficult to precisely define the role of any particular adhesin due to overlapping function [4]. Type I and P fimbriae, the most common fimbriae found in UPEC strains enhance virulence and are involved in initial urethral colonization. Many UPEC produce hemolysin, which may be involved in kidney disease. Certain UPEC strains possess iron sequestration systems to assist in growth; others produce a capsule that may help avoid clearance from the urinary tract.

Adherence to solid substrates is a property common to many pathogenic microorganisms, including viruses, Gram-positive and Gram-negative bacteria [5]. By attaching to host structures, microbial pathogens avoid being swept along by the normal flow of body fluids (blood, urine, intestinal contents) and eliminated, although host cells with adherent bacteria can be shed, thereby eliminating the organisms despite attachment. Adherence of *E. coli* isolates to uroepithelial cells is used to differentiate between uropathogenic and fecal strains. Different authors [6-8] suggested that uropathogenic strains may present a mean of 20 bacteria/ cell or more, while fecal strains may present a mean of about 7 bacteria/cell, and that UPEC strains causing UTI typically agglutinate human erythrocytes despite the presence of mannose (mannose-resistant hemagglutination [MRHA]) and adhere to human uroepithelial cells. Also, adherence to uroepithelial cells is usually unaffected by mannose (mannose- resistant adherence) and is more common among strains exhibiting MRHA than among those exhibiting only mannose-sensitive hemagglutination [9].

Uropathogenic *Escherichia coli* strains are responsible for the majority of uncomplicated urinary tract infections. The reasons for the geographic variations in prevalence of antibiotic resistance among *E. coli* causing urinary tract infections are poorly understood. Therapy for these infections is usually begun before results of microbiological tests are known. Furthermore, in women with acute uncomplicated cystitis, empirical therapy without a pre-therapy urine culture is often used. Consequent to this, antimicrobial resistance among uropathogens causing community-acquired UTIs, both cystitis and pyelonephritis, is increasing [9].

Multiple antibiotic resistances (MARs) in bacteria may be commonly associated with the presence of plasmids. Plasmids are circular double stranded extra-chromosomal DNA molecules, and conjugal transfers of plasmids play an important role in the spread of antibiotics resistant genes among *E. coli* and other bacterial strains. The resistance to commonly using antibiotics in bacteria creates a threat to public health in the world. In addition, the size, number and attributes of the plasmid in a bacterium remain the same for a long time and they are transferred to equally daughter cells [1]. Therefore, the aims of this research are to determine the contribution of *Escherichia coli* to asymptomatic bacteriuria in females attending the University clinic; determine some of their pathogenic traits (virulence factors), and also to molecularly characterize the resistant isolates (plasmid extraction and antibiotic resistance genes amplification through polymerase chain reaction (PCR)).

Materials and Methods

The study population

The study population included participants recruited from volunteered females Elizade University undergraduate students.

Patients' selection

Apparently healthy female Elizade university students attending classes after an informed consent of the subject and ethical clearance of reference number (UTHA/05/15/004) approved by the ethical and research committee of the institution was obtained.

Questionnaire and statistical analysis

Pregnant women were taken through an interviewer administered questionnaire consisting items such as socio-demographic data, medical history and occupational status. The data were analyzed using

statistical package for social sciences (SPSS) version 21 (Chicago, SPSS Inc, 2012).

    (a) Inclusion criteria for the choice of patients include;

        I. Age (15 years and above)

        II. Apparently healthy female students only

    (b) Exclusion criteria include:

        I. Non cooperative or unwilling students

        II. Sick and pregnant women on admission.

Sample size

Sample size was determined using the formula $n = p(1-p) Z^2/d^2$ [10], where n=is sample size, *p* is the estimated prevalence value put at 5%, Z is the confidence limit of results (1.96) and d is the level of significance or precision (0.05).

Culture media used

Three culture media were majorly used in this work; these are: nutrient agar, nutrient broth, Eosine-Methylene Blue agar. The media were prepared according to the manufacturers' instructions (at the appendix).

Sample collection and isolation of bacteria

Consent of patients were sought and given by each patient. Instructions were given on the mode of collection and the collection was made on-site. A total of 500 samples of clean catch mid-stream urine samples were collected from the pregnant women attending antenatal clinic using sterile sample bottles. The time lag between collection and processing was therefore within 5 minutes. Isolation of the bacteria was done using both streaking method and pour plate method respectively. The urine sample was shaken and a loop full of it was streaked on the already prepared agar and incubated at 37^0C for 24 hours for isolation of the bacteria. At the same time, one ml (1 ml) of the urine was aseptically transferred into a sterile empty Petri dish and a molten agar at about 45^0C was added to it and swirled gently for homogenous growth. It was then incubated at 37^0C for 24 hours and the number of bacteria that grown was counted and recorded respectively.

Sub culturing

Distinct colonies of bacteria were picked using sterile inoculating loop. These were streaked onto the surface of the prepared nutrient agar plate to obtain pure isolates for confirmation of their identities.

Identification of isolates

Identification of the bacterial isolates was done using the morphological and biochemical characteristics as outline by [11].

Antibiotic susceptibility testing

Antibiotic susceptibility of the clinical isolates was determined by disc diffusion assay following Clinical Laboratory Standard Institute (CLSI) guidelines 2013 [12]. The strains were tested for susceptibility against amoxycillin (10μg), cephazolin (10μg), gentamicin (10μg), amikacin (10μg), cefotaxime (30μg), ceftazidime (30μg), ciprofloxacin (5μg), netilmicin (30μg) and piperacillin (100μg). They are commercial antibiotic prepared and marketed by Optu disc, United States of America. All the strains were tested twice without any discrepancy between results. The strains were scored as sensitive, intermediate (moderately sensitive) or resistant according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Determination of beta-lactamase production

The simple acidimetric test described by [11] was used to detect beta-lactamase producing strains of the Isolated *E. coli.* The test is based on detecting a change in colour of the indicator of bromocresol purple from purple to yellow. A strip of Whatman No. 1 filter paper was placed at the bottom of Petri dish. Few drops (3-4 drops) of buffered crystalline penicillin bromocresol purple solution were added until the paper was almost saturated. A sterile wire loop was used to place some colonies of the test organism on the filter paper spreading it carefully to cover an area of 5 mm in diameter. The Petri dish was covered gently and incubated at room temperature for about thirty minutes (30 min) before examining the filter paper for colour change from purple-blue to yellow.

Determination of virulence factors

The *E. coli* isolates found to produce beta-lactamase enzyme were tested for virulence factors such as type1 / type 2 pili fimbriae adhesion, haemolysin and aerobactin.

Molecular analysis

DNA extractions

The bacterial cells were first cultured in nutrient broth (50 ml) and harvested by centrifuging it at 3,000 rpm for 5 minutes and subsequently washing the cells twice using sterile distilled water according to the method of [13] before extracting the DNA. DNA extraction was carried out on the samples

using the Jena Bioscience Bacteria DNA Preparation Kit. The DNA of the isolates was extracted by suspending the bacterial isolates in 200 µl of sterile distilled water in Eppendorf tubes that had been previously labelled accordingly. The tubes were covered and sealed with paraffin tape to prevent accidental opening and consequently loss of the suspension. The suspension was then heated to boiling point and allowed to boil for 7 minutes in water bath. It was then cold shocked in ice for 2 minutes and then centrifuged at 10,000 rpm for 45 seconds using a micro-centrifuge (Eppendorf centrifuge 5418 series-2015 model). The supernatant, which contain the DNA was then taken for amplification. The purity and concentration of the extracted DNA was evaluated using a NANODROP (ND 1000) Spectrophotometer (Thermo Scientific, USA). Since the amount of DNA required for PCR should be between 10-200ng, some of the extracted DNA were diluted before PCR was carried out.

Multiplex PCR amplification of EPEC virulent genes

A multiplex PCR was carried out to amplify the virulent genes homologous to EPEC. These are the eae, bfp and EAF genes. The multiplex PCR reaction was carried out using the primer pairs eae-F (5'-TCA ATG CAG TTC CGT TAT CAG TT-3') and eae-R (5'-GTA AAG TCC GTT ACC CCA ACC TG-3') with an expected amplicon size of 482bp, bfp-F (5'-GGA AGT CAA ATT CAT GGG GGT AT-3') and bfp-R (5'-GGA ATC AGA CGC AGA CTG GTA GT-3')with an expected amplicon size of 397bp, and EAF-F (5'-CAG GGT AAA AGA AAG ATG ATA A-3') and EAF-R (5'-TAT GGG GAC CAT GTA TTA TCA-3')with an expected amplicon size of 300bp. The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in a 20 µl reaction mixture containing 1X Blend Master mix buffer. Buffer (Solis Biodyne), 2.0mM MgCl₂, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 20pMol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol*DNA* polymerase (Solis Biodyne), Proofreading Enzyme, 2µl of the DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series) for an initial denaturation at 95°C for 15 minutes followed by annealing at 35 amplification cycles of 15 seconds at 95°C; 15 seconds at 55°C

and 15 seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C.

Gel electrophoresis

The amplified PCR products were separated on a 1.5% agarose gel prepared by dissolving 1 g of agarose powder (Promega, Madison, USA) in 100 ml of IX Tris-borate-EDTA (TBE) buffer inside a clean conical flask. It was then heated in a microwave oven for 3 minutes while constantly stirring with a magnetic stirrer until there was clarity. The product was allowed to cool to about 50⁰C and 0.5 µl of ethidium bromide was added before pouring into the electrophoretic gel tray sealed at both end with support to form a mould with special comb placed in it to form wells. The comb was carefully removed after the agar was set and the samples carefully (5 µl of the amplicons) placed in the wells and the plate was placed inside the electrophoretic tank containing IX TBE solution. The electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

Multiplex PCR for the identification of multidrug resistance isolates

All the available partial and full-length gene sequences of resistance gene were determined according to **Anusha et al.** [14] protocol with some modification. The standard primers for *shv, ctxm* and *tem* were obtained from Zymo Research Europe Gmbh, Germany through Inqaba Biotec, South Africa for PCR amplification.

Amplification of virulence factors from E. coli by multiplex PCR

Virulence genes like fimH and hlyA were detected by gene amplification method using multiplex PCR. Primer sequence used was designed by **Anusha et al.** [14].

Bisulfite analysis

Bisulfite profiles of the amplified DNA of the uropathogenic *E. coli* isolates were studied using four bisulfite-converted DNA primers described below were used for the PCR.

1. 5 -TCC CAG CAGT- 3; 2. 5 -GTC GTC GTCT- 3; 3. 5 -ACG GGA CCTG-3; 4 .5 -GTT AGT GCGG- 3.Each polymerase chain reaction mixture consists of 2 µl of template DNA, 1 µl of 1.6 micromolar solution of primer , 10 µl 2 X PCR master mixes (Promega, USA) and made up to 20 µl with

molecular grade water. Amplification was performed in a Bangalore Gene thermocycler.

Plasmid profile of E. coli

Plasmid DNA was extracted by alkaline lysis method of plasmid preparation. Extracted plasmids were separated with gel electrophoresis using agarose gel of 1.5% to identify the number of plasmid copies present in different isolates and the fragments were stained with ethidium bromide and they were visualized by UV-Trans illumination called Photo-documentation system (EBOX VF5; 2015 model, made in France). Standard DNA molecular weight markers were used to estimate the Plasmid size.

Plasmid curing

The tested multi-resistant E. coli isolates were cured from their own plasmids by growing them in elevated temperature at 42°C [15]. Thereafter, an appropriate dilution of cured bacterial cultures were spread on Muller Hinton agar plates and incubated at 37°C. Five random single colonies were picked up and tested for their sensitivity against tested antibiotics and PCR for determination to confirm if their resistance was plasmid related or not.

Statistical analysis

Statistical data are presented as mean ± SE (standard error). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using BMI SPSS version 21 software. For all the tests, the significance was determined at the level of $p < 0.05$.

Results

Forty-Seven (47) out of the 300 women whose urine were tested yielded significant bacteriuria giving a prevalence rate of 10.5% asymptomatic bacteriuria. Two hundred and twenty-two (72.2%) Showed no or insignificant growth while 77 (17.3%) yielded mixed growth **(Table 1)**. The summary of prevalence of asymptomatic bacteriuria relating to age group, religion, educational status and occupation is presented in table (2). Out of the 47 asymptomatic bacteriuria, 34 (72.4%) were between the age range of 25-34 years. The majority of the patients (19 out of 47 representing 40.4%) with asymptomatic bacteriuria were students. Majority (97.9%) of significant growth were Christians while only 2.1% were Muslims. Most of the asymptomatic bacteriuria were found among the well-educated population as 83.0% of the significant culture are students of the tertiary institution and almost half 48.9% were university staff. *Escherichia coli* was the most frequent bacteria (44.7%), followed by *Staphylococcus aureus* (25.5%). Other isolates found in this study included *Klebsiella aerogenes* (12.8%), *Proteus mirabilis* (8.5%) *Enterococcus faecalis* (6.4%) and *Enterobacter sp* (2.1%) **(Table 3)**. The antimicrobial susceptibility pattern of the bacteria isolated in this study revealed that Ofloxacin was the most effective antibiotic followed by ceftriazone, ceftaxidime, cotrimoxazole and ciprofloxacin. The least effective antimicrobial agents were cefuroxime sodium, amoxycillin, amoxicillin-clavulanic acid and nitrofurantoin **(Table 4)**.

The profile of bacteria in cases of asymptomatic bacteriuria in in students showed that a total of 47 bacterial growth was observed. *E. coli* growing from 21 urine samples recorded the highest percentage (44.7%) followed by *S. aureus* (25.5%). The bacteria with smallest growth occurrence was *Enterobacter* species growing from only one urine sample and giving rise to 2.1% of the total bacterial growth. The result of the profile of bacteria in cases of asymptomatic bacteriuria in students is shown in **table (3)**.

Table 4 shows the result obtained from the antibacterial evaluation of the isolates. Ten commercial antibiotics were used of which the one that exhibited the maximum inhibitory effect on the isolates. Of the 21 *E. coli* for instance that was isolated, 19 of them was inhibited by cefotaxime and only two resisted the antibiotic.

Table 1. Growth patterns of urine culture among the university populace.

Growth	Number of samples	Average colony forming unit/ml	Percent (%)
Significant growth	47	10^4	10.5
Insignificant growth/No growth	222	0	72.2
Mixed growth	31	10^2	17.3
Total	300		100

Table 2. Socio-demographic characteristics of the population.

Parameters	Frequency (N=300)	Percent (%)	Number with significant growth (N=47)	Percent (%)
Age				
≤19	1	0.2	0	0
20-24	37	8.3	3	6.4
25-29	164	36.8	17	36.2
30-34	169	37.9	17	36.2
35-39	61	13.7	10	21.3
≥40	14	3.1	0	0
Religion				
Christianity	217	93.5	46	97.9
Islam	29	6.5	1	2.1
Educational status				
None	3	0.7	0	0
Primary	3	0.7	0	0
Secondary	67	15.0	8	17.0
Tertiary	227	83.6	39	83.0
Total	300			

Table 3. The profile of bacteria in cases of asymptomatic bacteriuria in students.

Organism	Number	Percent
Escherichia coli	21	44.7
Staphylococcus aureus	12	25.5
Klebsiella species	6	12.8
Proteus species	4	8.5
Enterococcus faecalis	3	6.4
Enterobacter species	1	2.1
Total	47	100

Table 4. Antimicrobial susceptibility pattern of bacteria from asymptomatic bacteriuria in pregnancy.

Uropathogen	No Tested	CFT	CFZ	AST	AUG	CFX	CRO	NIT	GEN	COT	OFL	AMX	CPX
						% Susceptibility							
Escherichia coli	21	19 (91)	19 (91)	16 (76)	1 (5)	0	0	0	11 (52)	15 (71)	21 (100)	0	18 (86)
Staphylococcus aureus	12	9 (75)	10 (83)	0	0	0	0	0	0	10 (83)	12 (100)	0	12 (100)
Klebsiella species	6	5 (83)	5 (83)	5 (83)	1 (17)	0	0	0	4 (67)	4 (67)	6 (100)	0	5 (83)
Proteus species	4	1 (25)	3 (75)	2 (50)	1 (25)	0	0	0	0	4 (100)	4 (100)	0	4 (100)
Enterococcus faecalis	3	3 (100)	3 (100)	3 (100)	1 (33)	0	2 (67)	0	2 (67)	0	3 (100)	0	3 (100)
Enterobacter species	1	1 (100)	1 (100)	1 (100)	0	0	1 (100)	0	0	0	0	0	0
Total	47 (100%)	38 (81)	41 (87)	27 (57)	4 (0.08)	0 (0)	3 (0.06)	0 (0)	17 (36)	33 (70)	47 (100)	0 (0)	42 (89)

CFT- Cefotaxime **CFZ**- Ceftazidime **AST**-Astreonam **AUG-** Amoxyclav **CFX-** Cefuroxime **CRO-**Ceftriaxone **NIT-** Nitrofurantoin **GEN-**Gentamicin **COT**Cotrimoxazole **OFL-** Ofloxacin **AMX-** Amoxicillin **CPX-** Ciprofloxacin.

Figures in parenthesis are % values of the ones inhibited by the antibiotics.

A total of twenty-six (26) UPEC were isolated from the urine samples and identified using their morphological and biochemical characteristics as well as their sugar fermentation tests. The twenty-six isolates were subjected to beta-lactamase enzyme production test of which fifteen of them were positive (i.e. showed the presence of the enzymes). Of these number, none of them tested positive for shiga toxin virulence factor while eleven (11) others tested positive for type 1fimbriae adhesin virulence factor. The eleven *E. coli* isolates that were positive for this virulence factor were tested for gene plasmid resistance code. Their resistance was detected to be plasmid coded. The curing of the plasmid resistance gene using incubation at high temperature showed that seven of the *E. coli* isolates were cured at a temperature of 42˚C of incubation of the *E. coli*.

The plates of some of the analysis carried out in this research are shown in plates 1-6.

Plate 1. Further identification of isolates using sugar fermentation test.
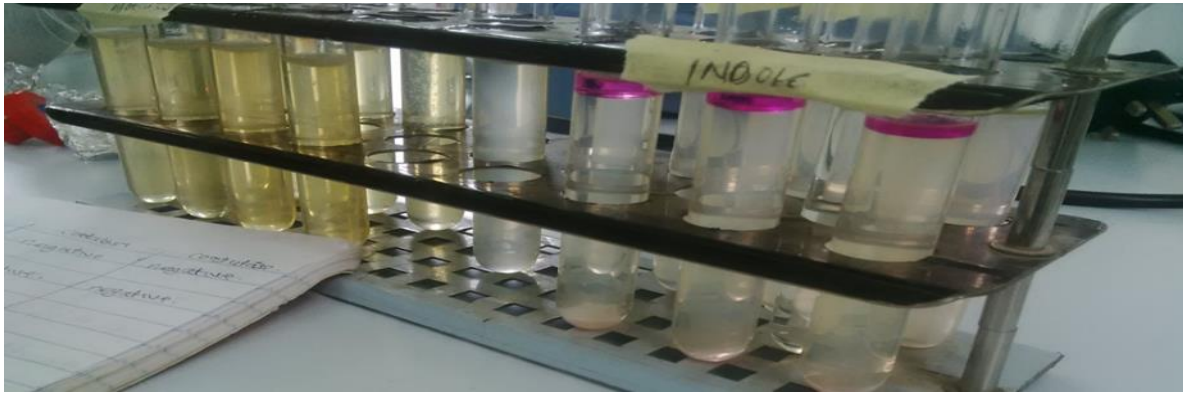
Plate 2. DNA extraction kit (Jena Bioscience Bacteria DNA Preparation Kit, by Thermo Scientific Production, USA).

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
1	Default	7/27/2015	3:38 PM	2.25	0.045	0.031	1.43	0.81	50.00	230	0.055	-0.029
2	Default	7/27/2015	3:39 PM	95.57	1.911	0.933	2.05	1.48	50.00	230	1.294	0.121
3	Default	7/27/2015	3:40 PM	203.45	4.069	2.235	1.82	1.03	50.00	230	3.932	0.243
4	Default	7/27/2015	3:41 PM	256.78	5.136	2.806	1.83	1.03	50.00	230	4.990	1.988
5	Default	7/27/2015	3:42 PM	80.95	1.619	0.784	2.07	1.58	50.00	230	1.023	-0.030

Yield of the extracted DNA

All the samples showed a DNA yield between 2.25 - 280ng, and the extracted DNA were optimally pure showing A260/A280 between 1.4 –2.0.

Virulence factor result

Plate 3. Electrophoretic gel plate of amplified gene for type 1 pili fimbriae and haemolysin virulence factors in *E. coli* isolates.
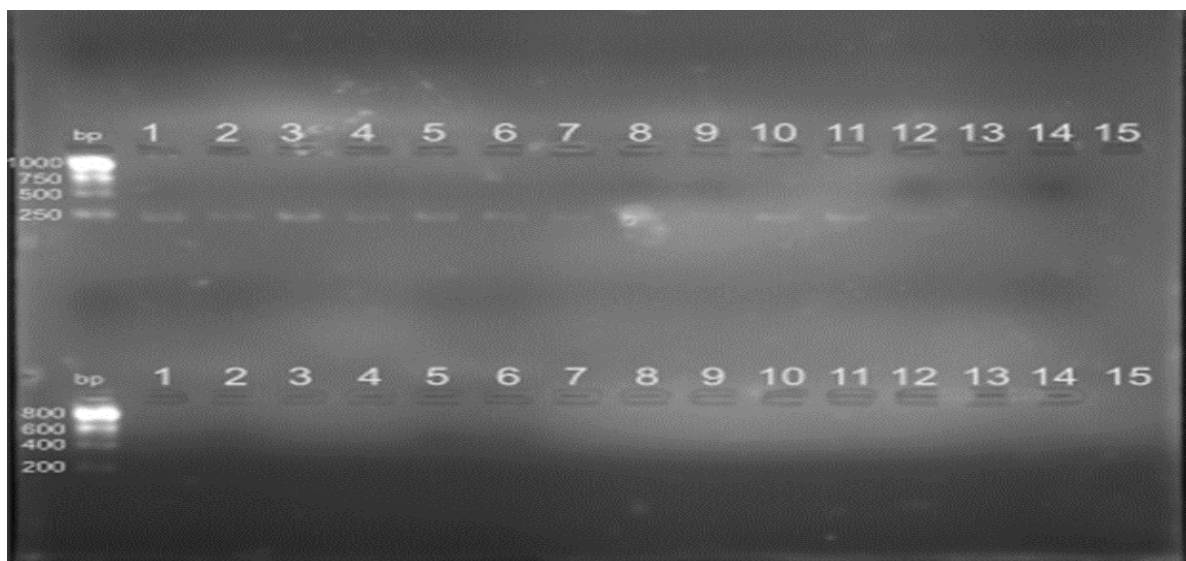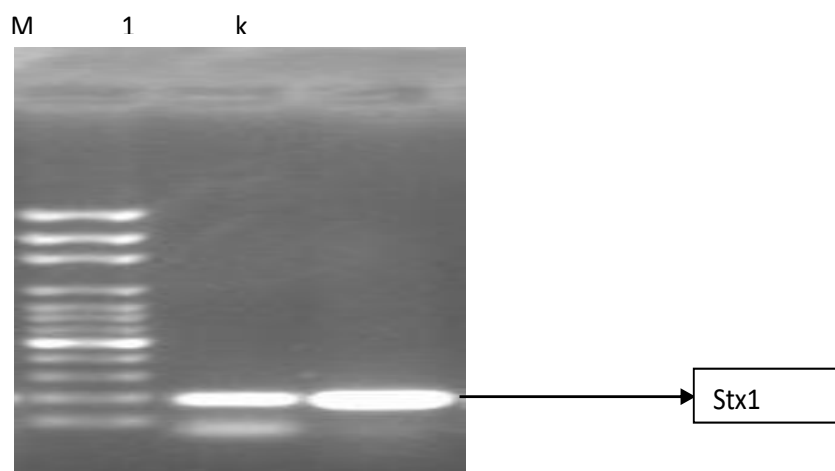
Plate 4. Electrophoretic gel plate of amplified gene for type shiga toxin virulence factors in two of the *E. coli* isolates.

Representative gels for PCR amplification of DNA extracted from *E. coli* isolated from human urine (samples 4 and 7) showing the presence of virulence genes *stx*1 Lane M (100bp) = DNA size marker; Lane 1 = *E. coli* Lane K = Control, respectively.

Plasmid profile result

Plate 5. Electrophoretic gel plate of amplified gene for plasmid resistance factors in eleven of the *E. coli* isolates. (Note: Isolate 4 has no resistant plasmid encoded in it).
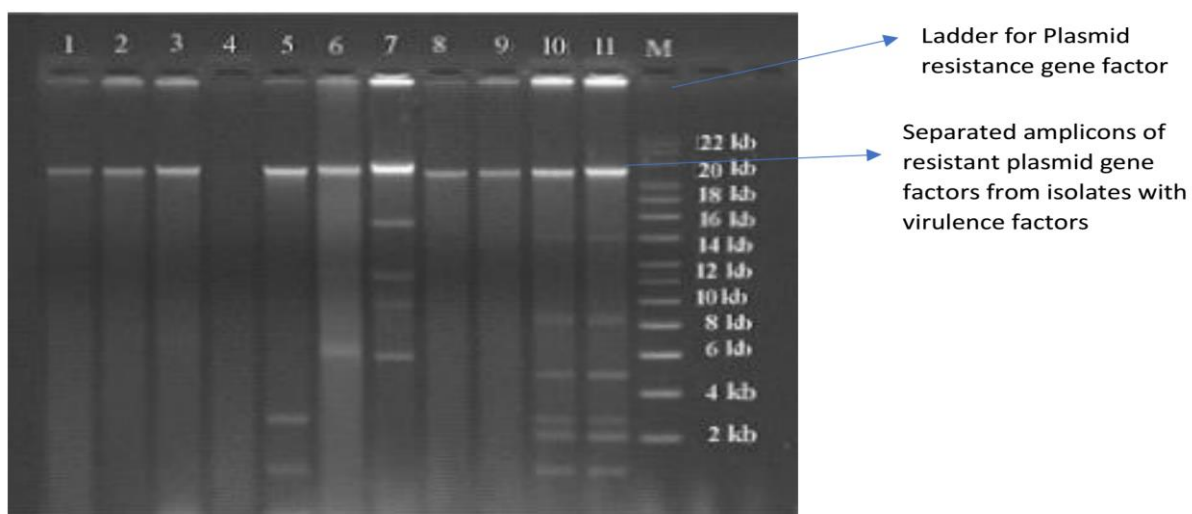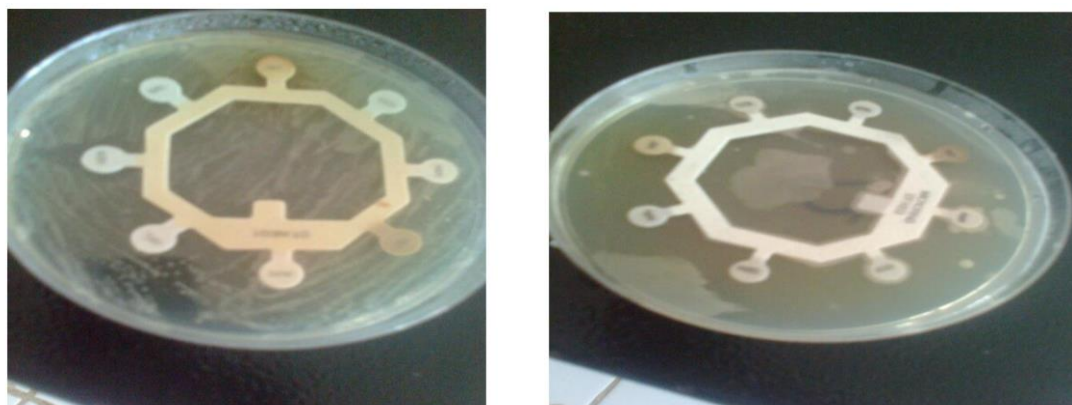

Plate 6. Photograph of *E. coli* susceptibility test before and after plasmid curing.

Discussion

The results of the isolation showed that 47 out of the 300 students (women) whose urine were tested yielded significant bacteriuria giving a prevalence rate of 10.5% asymptomatic bacteriuria. This figure is similar to the result obtained by [16] in which the result of bacteriuria in pregnant women in Oshogbo recorded a prevalence rate of 11%. Two hundred and twenty-two (72.2%) showed no or insignificant growth while 77 (17.3%) yielded mixed growth. The mixed growth showed that *Staphylococcus aureus (S. aureus) was* the most common bacteria. This result is in agreement with the work [7] in which the most common bacteria was *E. coli* followed by *S. aureus*.

Out of the 47 asymptomatic bacteriuria, 34 (72.4%) were between the age range of 25-34 years. The majority of the patients (19 out of 47 representing 40.4%) with asymptomatic bacteriuria. According to previous research made by [17] and [18], the age of active reproduction is usually highest with positive result for bacteriuria and sexually transmitted diseases. This result was equally in agreement with the results obtained by [10] in which women with asymptomatic bacteriuria in Mother and Child hospital in Ondo town of Ondo State were between the ages of 20-34 years.

Majority (97.9%) of significant growth were christians while only 2.1% were muslims. Most of the asymptomatic bacteriuria were found among the well-educated (300-500 level) population as 83.0% of the significant culture were students of the tertiary institution and almost half 48.9% were staff of the university. This result agrees with the result obtained by [19].

Escherichia coli was the most frequent bacteria (44.7%), followed by *S. aureus* (25.5%). Other isolates found in this study included *Klebsiella aerogenes* (12.8%), *Proteus mirabilis* (8.5%) *Enterococcus faecalis* (6.4%) and *Enterobacter sp* (2.1%) **(Table 3)**. According to [20], several reasons may be responsible for the prevalence of *E. coli* among which are the fact that it is common flora of the gastro-intestinal tract of humans and most women after using the toilet practice downward wash thereby moving the bacteria from the anal region to the urethra.

The prevalence of asymptomatic significant bacterial found in this study among 300 students (women) was 10.5%. [21] reported that the prevalence of asymptomatic bacteriuria varies from 4-7%. [22] reported a slightly higher prevalence of 12.2% from this same location. This is also similar to 12% reported in Ibadan and 14.1% in Ile-Ife [23] from Southwest Nigeria. A lower prevalence of 7.0% and 7.3% [21] were reported from Ethiopia and Ghana respectively. The results of this study further affirm that certain biosocial variables affect the frequency of UTIs in women. This study showed that 72.4% of significant bacteriuria occurred among 25 – 34-year age group with a mean age of 25.81±6.01. It is believed that subjects within this age group are more sexually active and are therefore more prone to UTI. This also reflects the peak reproductive period in our communities.

The result of this study revealed that *E. coli* (44.6%) was the most predominantly isolated uropathogen followed by *Staphyloccocus aureus* (25.5%). Others include; *Klebsiella aerogenes* (12.8%), *Proteus mirabilis* (8.5%), *Enterococcus faecalis* (6.4%) and *Enterobacter* sp (2.1%).

However, this result revealed a changing trend in the bacterial profile found in asymptomatic bacteriuria. It is important to note that although earlier studies had shown that *E. coli* was implicated in greater than 85% of cases of asymptomatic bacteriuria, more recent studies are showing a reduction in this prevalence and increased prevalence of other uropathgens such as coagulative negative Staphylococci. In fact, [22] reported that *S. aureus* was the predominant bacterium (21.3%). The in vitro antimicrobial susceptibility pattern in this study revealed that most of the common antibiotics used in most hospital in the treatment of UTI in pregnancy exhibited a high degree of resistance. Cefuroxime, a 2nd generation cephalosporin and most commonly used antibiotics in UTIs and other related infection was 100% not susceptible. Quinolones which are equally widely used were highly resistant.

The use of these antibiotics should be discouraged as much as possible in the empirical treatment of UTI. The 3rd generation cephalosporins and the quinolones showed excellent in vitro activities against all the uropathogens isolated in this study. An important finding in this study is that cotrimoxazole (Trimethoprim-sulfamethoxazole) showed excellent antimicrobial susceptibility to all the uropathogens found in this study. It had been observed in earlier studies that there is high resistance to this agent in Nigeria due to the widespread of its usage.

Escherichia coli is a very diverse species of bacteria found naturally in the intestinal tract of all humans and animals. Diarrhoeagenic *E. coli* are classified into six major pathotypes, each with distinct phenotypic and genetic make-up [24]. The diarrhoeagenic pathotypes of *E. coli* are also diverse in terms of disease potential, age and host specificity. *E. coli* can cause a range of human diseases, including UTI, neonatal meningitis and sepsis.

The presence of beta-lactamase enzyme detected in these *E. coli* isolates in this research has shown that this bacterium responsible for UPEC in this locality possess this enzyme. The result obtained in these findings was about 80% which is similar to the result obtained by [25] where about 82% of the *E. coli* from urine sample in Nepal had beta-lactamase enzyme embedded in them.

Urinary tract infections are the most common form of extra-intestinal infections with *E. coli*. It is the most common etiology of UTIs and is responsible for 80–90 % of community-acquired UTIs. UPEC are different from the normal flora of *E. coli* in terms of better adaptability to living within the urinary tract and evading the host's immune response [26]. This adaptability has been proven in this research to be the presence of type 1 fimbriae adhesion virulence factor which help them attach themselves to the urinary tract.

Uropathogenic *Escherichia coli* are very heterogeneous in nature, and the presence of shiga toxin detected in one of the *E. coli* isolates have shown this. According to [24], *E. coli* of the UPEC strains harbour several pathogenicity islands, which may encode adhesins, toxins, iron uptake systems, secretion mechanisms and capsules in order to enable them to develop successful UTI. Therefore, this result is in agreement with the work of [26] who isolated *E. coli* strains that habour three different virulent factors from the same site.

Uropathogenic *Escherichia coli* has multiple molecular pathways (for example, biofilm formation and urothelial cell invasion) that may contribute to pathogenesis. The plasmid profiling showed that all, except one, isolate contained at least one plasmid. A band of approximately 20 kb was seen in 10 isolates. Although *E. coli* has been reported to be MDR by possessing the antibiotic resistant genes in its transferable R-plasmids, detection of this feature in UTI isolates from University of Ado-Ekiti teaching hospital has not been done. Therefore, this result is a pointer to the

fact more research needs to be carried out on in this area to help physicians curtail the issue of resistance and the spread of UPEC within the Ado-Ekiti community and in Nigeria at large.

The antimicrobial susceptibility pattern of the bacteria isolated in this study revealed that Ofloxacin was the most effective antibiotic followed by ceftriazone, ceftaxidime, cotrimoxazole and ciprofloxacin. The least effective antimicrobial agents were Cefuroxime Sodium, Amoxycillin, Amoxicillin-clavulanic acid and Nitrofurantoin **(Table 4)**. The result of the antibacterial susceptibility pattern showed ofloxacin could be used as a drug of choice. Although, the susceptibility result is similar to the result obtained by [27] in which ceftriazone exerted the highest antibacterial activity against bacteria isolated from urine, the result however does not conform to the result obtained by [18] in which nitrfurantoin exerted the highest antibacterial effect.

The twenty-six isolates subjected to beta-lactamase enzyme production test of which fifteen of them were positive (i.e. showed the presence of the enzymes) indicates that the resistance to cephalosporin was due to the production of the enzyme. According to [20], this enzyme breaks the beta-lactam ring of the antibiotic thereby rendering it inactive against the bacteria it is meant to attack. Eleven others tested positive for type 1fimbriae adhesin virulence factor. This factor according to [1] is usually plasmid related and difficult to eliminate from bacteria. However, the fact that temperature of 42^0C was able to remove or eliminate it from the bacteria confirmed that it was plasmid coded.

Conclusion

This research has shown that there has not been a significant change in the prevalence of asymptomatic bacteriuria in females in the recent years and recommends that antibiotic use policy should be formulated to prevent indiscriminate use of antibiotics to prevent the current high level of antibiotic resistance of bacteria which is a threat to the present and future antibiotics. Possibly, some antibiotic resistance genes may not be located in the plasmid but may be on the bacterial chromosome. In order to prove the relationship between the plasmid and its resistance, additional studies such as plasmid curing as shown in this work has shown that temperature (at 42˚C incubation) can remove the plasmid gene responsible for the resistance.

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