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Prevalence Of Extended Spectrum Beta-Lactamases (ESBLs)-Producing *Escherichia Coli* Isolated From UTI   Patients Attending some Selected Hospitals In Minna, Nigeria

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Abstract

Globally, urinary tract infections are   one of the most common infections in   need of urgent clinical attention. The prevalence of extended spectrum beta-lactamases (ESBL)-producing *Escherichia coli* isolated from urine samples of some UTI patients and   s of apparently healthy individuals in Minna, Nigeria, is investigated. Standard microbiological techniques were used to conduct this study. A total of 170 catch midstream   urine samples submitted to the Medical Microbiology Laboratories of 4 different hospitals (and samples from healthy individuals) were randomly collected for 5 months and examined for microbial growths. Female patients (65.9%) submitted more urine samples for UTI test than their male counterpart (34.1%). The   age   ranges   of 21 -30 (26.5%) and 31 - 40 (25.3%) had the highest percentages of infection rate while those within the ages 1- 10 (3.5%) and ≥ 71 (2.3%) were the least infected. This study observed a prevalence of 23.5% of *E. coli* in Minna metropolis and a significant number (30%) of healthy individuals (HI) was   observed to harbor the *E. coli* in their urine. The isolates were highly susceptible to Gentamicin (65%), Ofloxacin (65%), Tetracycline (62.5%), Cotrimoxazole (62.5%), and Streptomycin (57.5%). Mildly susceptible to Pefloxacin (37.5%), Chloramphenicol (37.5%), and Ciprofloxacin (35%). There were significant resistance to most of the beta-lactames tested [Cefuroxime (80%), Amoxicillin (42.5%), Augmentin (40), Cefotaxime (20%) and Ceftaxidime (7.5%)]. Two of the isolates were resistant to all the 13 antibiotics tested; 70% (28) of the isolates had multiple antibiotics resistance index (MARI) ≥0.3. Multidrug resistance was expressed in 37.5% of the isolates tested. The study showed a vast resistant pool in the environment. Only 25% of the *E. coli* isolated from the urine samples produced beta-lactamases phenotypically, most of which   expressed resistance to more than 5 of the antibiotics tested and had MARI of ≥ 0.5. Further evaluation showed that 25% (10/40) of the *E. coli* isolated from the UTI patients in Minna, Nigeria, were ESBL-   producers and could harbor one or two of the genes. TEM gene was expressed in 70% (7) of the isolates that produced ESBL phenotypically, 60%

(6) harbored CTXM gene, 20% (2) had the OXA gene while none of the bacteria harbored the SHV gene. The study established a 5.9% ESBL prevalence among the *E. coli* isolated from UTI in the environment studied. This study established that *E. coli* is one of the prevalent bacteri urea majorly isolated from UTI patients in Minna. The prevalent *E. coli* are multidrug resistant and could harbor more than one ESBL gene .

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Introduction

Different studies have defined urinary tract infections (UTIs) as the growth of pathogenic microorganisms in the urinary tract, which might elicit inflammatory disorders resulting in burning sensations while urinating, increase in temperature, dysuria, itching, pain around the pelvic region, development of wounds and inflammation of the genital area, genital and suprapubic pain, and in some cases permanent kidney damage (Prakash and Saxena, 2013; Hoberman *et al.,* 2003). UTIs can result in cystitis, an infection of the upper urinary tract that includes the urethras, renal pelvis and kidneys and pyelonephritis, an infection of the lower urinary tract that includes the infection of urethra and urinary bladder (Lane and Takhar, 2011). With respect to frequency of occurrence of bacteria in UTI, *Escherichia coli*, *Staphylococcus*, *Klebsiella pneumoniae*, *Proteus*, *Pseudomonas*, *Enterococcus*, and *Enterobacter* are the most implicated agents (Mirsoleymani *et al.,* 2014).

Urogenital anomalies occur in individuals who retain urine longer than expected in their bladder (Dougherty and Rawla, 2020). Studies have shown that pregnant women with inflamed intraamniotic balloons, which result to overdistention of the uterus are prone to pyelitis and pyelonephritis with severe consequences (Sani *et al.,* 2019). Martin, *et al.,* (2019) demonstrated that age groups ≤19 years and the elderly, female gender, married individuals, patients with genitourinary tract abnormalities, diabetes, hospitalized patients and those with indwelling catheter >6 days are susceptible to high microbial proliferation in their urinary tracts. Factors such as patients residence, tribe, level of education, marital status, circumcision, pregnancy, hypertension, HIV, abortion, sexual intercourse had no correlation with UTI but can contribute to reinfection. According to the CDC, (2019), within 3 months of birth, male children have more probability of contracting UTI while females become more vulnerable thereafter. UTI could be community acquired or hospital acquired (nosocomial) and expressed in patients as acute, chronic, complicated or uncomplicated and in some cases asymptomatic, depending on an individual's immune status (Nelson and Good, 2015). Other factors of importance in cases associated with UTI are malnutrition, poor hygiene and low socio-economic status, which are common in rural settings (Ahmed & Avasara, 2008). The clinical symptoms of this disease vary depending on the sites of infection, the causative organisms, pregnancy, the severity of the infection and the age of the infected patients (Dias *et al.,* 2010). In 2019, Sani *et al.,* had reported that *E. coli* was the most predominant organism causing UTI in Minna (27.3% prevalence) and females (27.9%) are the most infected. This is followed by *Staphylococcus aureus* (24.6%) and *Pseudomonas species* (1.3%) being the least. According to Pandit *et al.,* (2020), treatment of UTI is becoming difficult due to the emergence, reemergence and spread of multidrug-resistant uropathogens encoding extended-spectrum β-lactamases (ESBLs), which are plasmid mediated. Extended spectrum beta lactamases (ESBLs) are class A β-lactamases produced by the Enterobacteriaceae family of Gram-negative organisms that hydrolyze penicillin, oxyimino-cephalosporins, and monobactams but not cephamycins or carbapenems (Mehrgan and Rahbar, 2008). These types of enzymes are inhibited *in vitro* by clavulanate (Lohani et al., 2020). The most prevalent genes of ESBLs are CTX-M types, TEM, OXA and SHV (Abrar et al., 2019). Other clinically important genes include VEB, PER, BEL-1, BES-1, SFO-1, TLA, and IBC (Jacoby and Munoz-Price, 2005; Falagas and Karageorgopoulos, 2009; Dhillon and Clark, 2012). These genes evolve in bacteria that develop resistance to antibiotics majorly prescribed in an environment

(Lohani et al., 2020). These studies further showed that antibiotics such as imipenem, gentamycin and nitrofurantoin are the best drugs for the treatment of infections linked to ESBL-producing *E coli* isolates from UTI. Study conducted by Baziboroun *et al.,* (2018) further substantiates the fact that high percentage of the UTI isolates did express significant levels of resistance against ceftazidime, cefotaxime, ceftriaxone, cefixime and ciprofloxacin, ranging from 61-100%, while the highest percentage of susceptibilities were observed against meropenem, piperacillin- tazobactam (100%), followed by nitrofurantoin and amikacin (91%). These studies show vast resistance to beta-lactams while other studies have isolated fluoroquinolone-resistant uropathogens (Wu, *et al.,* 2014), carbapenem-resistant Enterobacteriaceae, (Schechner, *et al.,* 2013) and vancomycin-resistant Enterococci (Papadimitriou-Olivgeris, *et al.,* 2014) among UTI patients. This might be linked to factors such as misuse of antibiotics in the society and poultries, re-exposure of infected patients, acquisition of

plasmid mediated ESBL genes, use and administration of drugs without appropriate culture and sensitivity tests for asymptomatic and mildly symptomatic UTI patients (Igwe *et al.,* 2014). This study was hence designed to evaluate the prevalence of *E. coli* in UTI in Minna, investigate the antimicrobial susceptibility patterns of selected antibiotics and also assay for the presence of ESBL.

Materials and Methods

Study Area

Samples were collected from the Medical microbiology laboratories of four hospitals within Minna metropolis: General Hospital, Minna; Ibrahim Badamosi Babangida (IBB) Specialist Hospital, Chanchaga; Top Medical Clinic, Tunga and Standard Hospital, Old airport road. The Map showing the locations of the Four Hospitals in Minna Metropolis, Niger State as captured by Google Earth Image, (2016) is shown below, represented in yellow colour.
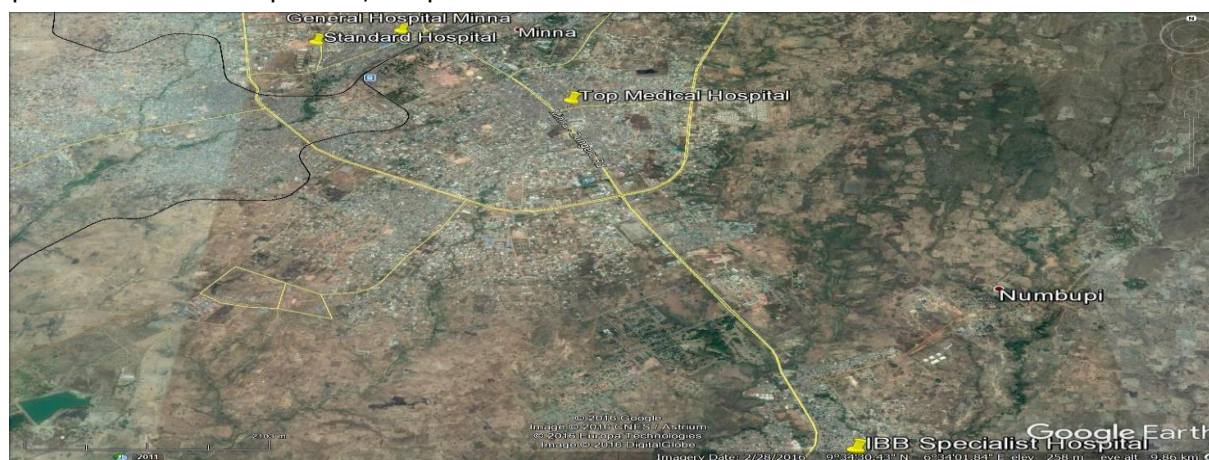
Figure 1: Locations of the Four Hospitals in Minna Metropolis, Niger State

Determination of Sample Size

The sample size was determined using the single proportion method and prevalence of 12.3% as reported by Jombo *et al.,* (2006a).

$$n = \frac{Z^2 \, P \, (1-P)}{D^2}$$

Where n= sample size

Z = 1.96 for Confidence level at 95%

P = Prevalence rate at 12.3%

D = 0.05 for Marginal error at 5% The sample size was calculated as 165, which was rounded up to 170.

Ethical Clearance

Approval for this study was obtained from the Ethical Committee of the General Hospital, Minna, Niger State with the registration number HMB/GHM/STA/136/VOL.II/350, dated 29th October, 2015.

Specimen Collection and Processing

A total of 170 samples of clean-catch midstream urine submitted to the Microbiology Laboratory of the four hospitals in Minna metropolis and 20 samples from healthy individuals were randomly collected within the period of 5 months (May - September, 2015). The samples were transported to the Microbiology Department laboratory, Federal University of Technology (F.U.T), Minna in an ice pack within 40 minutes of collection for further analysis.

Isolation and Identification of Escherichia coli

The *E. coli* isolates were identified and characterized based on their morphological characteristics, culture, metabolic fermentation, Gram's reaction and other biochemical reactions such as indole, methyl red, Voges-Proskauer, urease, citrate utilization, triple sugar iron, motility and lysine decarboxylase tests as described by Cheesbrough (2010).

Antibiotic Susceptibility Test

The confirmed *E. coli* isolates were subjected to antibiotic sensitivity tests against thirteen (13) commonly prescribed antibiotics being used in treating infections caused by *E. coli.* This test was determined according to CLSI (2006) and Cheesbrough (2010). An overnight broth culture of each isolate was inoculated on nutrient agar and incubated at 37°C for 24h. 0.5 McFarland turbidity standard was prepared for standardization of inoculums (1.5×10^8 cfu/ml). McFarland turbidity standard solution (0.5) was prepared by combining 1% solution of anhydrous barium chloride ($BaCl_2$) and 1% solution of sulfuric acid (H2SO4), mixed thoroughly to form a turbid solution. Using a sterile wire loop 3-5 well-isolated colonies of similar appearance from nutrient agar were emulsified in the 5ml sterile saline in a test tube. In a good light match, the turbidity of the suspension was compared to the turbidity standard of 0.5 McFarland and the suspensions were then inoculated on Mueller Hinton agar at angle 60° across the plate using a sterile cotton swab to ensure even distribution and confluent growth. The plates were further allowed to dry for 5 minutes with the plates closed before placing the discs. Furthermore, the plates were exposed to an 18h incubation period at 37°C after a 15 minutes pre-diffusion time. Afterwards, the size of the zones of inhibition were examined and interpreted using the CLSI (2016) (Tab. 1).

Table 1: Clinical Laboratory Standard Institute Interpretation for some Selected Antibiotics

S/N	Beta Lactam Antibiotics	Zone of Inhibition (mm)		
		Susceptible	Intermediate	Resistance
1	Ceftazidime (30µg)	≥18	15- 17	≤14
2	Cefotaxime (30µg)	≥23	15-22	≤14
3	Cefuroxime (30µg)	≥18	15-17	≤14
4	Amoxicillin (30µg)	≥17	14-16	≤13
5	Amoxicillin-clavulanic acid (10/20µg)	≥18	14-17	≤13
6	Cotrimoxazole (7/23µg)	≥16	11-15	≤10

7	Chloramphenicol (30µg)	≥18	13-17	≤12
8	Tetracycline (30µg)	≥15	12-14	≤11
9	Ciprofloxacin (5µg)	≥21	16-20	≤15
10	Gentamicin (10µg)	≥15	14-15	≤12
11	Streptomycin (30µg)	≥15	12-14	≤11
12	Perfloxacin (10µg)	≥21	16-20	≤15
13	Ofloxacin (10µg)	≥16	13-15	≤12

Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of each of the *E. coli* isolates was evaluated according to Krumperman (1983).

MAR Index = Number of Antibiotics to which the isolate is resistant to

Total number of Antibiotics tested

Presumptive Test for Extended Spectrum Beta Lactamase (ESBL) Production

The double disc synergy test described by Dechen *et al.,* (2009) was used to confirm ESBL production among the *E. coli* isolates. *E. coli* isolates that harbored multidrug resistant characteristics were standardized in phosphate normal saline using 0.5 McFarland turbidity standard. A 24h broth culture of the isolates were streaked on nutrient agar and incubated at 37ºC for another 24h. 0.5 McFarland turbidity standard solution was prepared for standardization of inoculums. With the help of a sterile wire loop, 5 good identical colonies from nutrient agar were introduced into 5ml sterile normal saline in a test tube and its turbidity compared to 0.5 McFarland standard. The admixture was then streaked on already prepared Mueller Hinton Agar (MHA). Using a sterile pair of forceps, antibiotic disk containing amoxicillin-clavulanate (20µg/10µg) was placed

in the center of the plate, and ceftazidime (30µg), cefotaxime (30µg), and cefuroxime (30µg) each were placed 20 mm from the amoxicillin-clavulanate disk at the center. The MHA plate was incubated at 37°C for 24 h. For standard control, *E. coli* ATCC 25922 typed culture which is a recommended reference strain for detection of ESBL production was used (CLSI, 2016). An increase in the zone diameter towards amoxicillin-clavulanic acid implies ESBL production or isolates that showed a distinct shape/size with potentiation towards amoxicillin clavulanic disc and a ≥5 mm increase in size for most of the drugs tested compared to when tested alone is an indication for ESBL production.

Molecular Characterization of Extended Spectrum Beta Lactamase Resistant Escherichia Coli

Bacterial Cell Preparation

The method of Dubey (2009) was used in preparing the bacterial cells for molecular analysis. Luria and Bertani broth medium were prepared by dissolving 10g of peptone water, 5g of NaCl, 10ml of 1N NaOH, 5g of yeast extract in 1litre of distilled water. The pH of the solution was increased to 7.0 using NaOH solution and autoclaved. Five overnight cultured identical colonies of the resistant *E. coli* isolates were

transferred into 5ml Luria and Bertani (LB) broth and incubated for 24h. Using an Eppendorff's tube, bacteria cells were then harvested by centrifugation at 4℃, 8000rpm in a refrigerated centrifuge for 30 seconds and the supernatants decanted.

DNA Extraction

DNA extraction was performed using Bioneer Accuprep® GMO DNA Extraction Kit following the manufacturer's instructions   as follows. Bacteria cell suspension in Luria broth was centrifuged at 6000rpm for 1 minute and cell pellets harvested. The pellets were re   suspended in 200 µl of phosphate-buffered saline (PBS) and transferred to a falcon tube containing 20 µl of Proteinase   K and vortexed for 10secs. Thereafter, the tubes were put in a water bath for 10mins at 55°C to lyse the cells. One hundred microlitre (100 µl) of absolute ethanol was added to the suspension in the tubes and allowed to stand for 2mins. Using a pipette, 600 µl of sample (cell lysate) was transferred from the tube into the binding column and centrifuged at   8000rpm for 1min. Supernatant was discarded and the binding column was transferred into another binding column. Five hundred microlitre (500) µl of washing buffer 1 was added and centrifuged for 2 minutes at 8000 rpm. Supernatant was discarded and the binding column was transferred into another column and 500 µl of washing buffer 2 was added and centrifuged at

8000rpm for 1 minute. The column was removed and the supernatant discarded. The   binding column was centrifuged again at 13000rpm (to remove excess water, ethanol). About 50 µl of an elution buffer was added to the binding column and left for 1 min after which it was centrifuged at 10000rpm for 2 min (so that the dissolved DNA flows down to the bottom of the binding column). Extracted DNA was suspended in the elution buffer for further use and preservation (Bioneer Accuprep® GMO DNA Extraction Kit Manual, 2012).

Detection and Characterization of Resistant Genes Using Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) amplification of target genes from isolated plasmid DNA was carried out. Primers which are specific for ESBL genes were used for the PCR amplification (Table 2). This was conducted using Dream   Taq™ PCR master mix (2x), after thawing from ice and centrifuged at 10,000rpm for 30 seconds. An eppendorf tube was placed on an ice and the following components were added to make up 50µl volume reaction: 25µl of Dream   Taq™ PCR master mix, 1.0µl of the forward primer   , 1.0 µl of the reverse primer, 10.0µl of template DNA (plasmid DNA) and 13µl nuclease free water. The admixture was vortexed and spun down, while the conditions in Table 3 were adopted for the PCR.

Table 2: Extended Spectrum Beta lactamases Primers

The nucleotide sequence of the primers and base pair of each of the ESBL genes is stated below.

Gene	Sequence	Amplicon Size (bp)	Reference
SHV	F: 5ʹGCCGGGGTTATTCTTATTTGTCCG3ʹ	868	Igwe *et al.* (2014)
	R:5ʹATGCCGCCGCCAGTCA3ʹ		
TEM	F: 5ʹATTCTTGAAGACGAAAGGGCCTC3ʹ	931	Igwe *et al.*(2014)
	R: 5ʹTTGGTCTGACAGTTACCAATGC3ʹ		
CTX-M	F: 5ʹCGCTTTGCGATGTGCAG3ʹ	593	Monstein *et al.* (2007)
	R: 5ʹACCGCGATATCGTTGGT3ʹ		

OXA	F:5ˈAAGAAACGCTACTCGCCTGC3ˈ	478	Igwe *et al.*(2014)
	R: 5ˈCCACTCAACCCATCCTACCC3ˈ		

Key: SHV (Sulfhydryl variable), TEM (Temoneira), CTX-M (Cefotaxime hydrolyzing), OXA (Oxacillin hydrolyzing), bp (base pair), A (Adenine), C (Cytosine), T (Thymine), G (Guanine)

Table 3: PCR Thermal Cycling Conditions Used

Step	Temperature (^{0}C)	Time	Number of Cycles
Initial denaturation	94	5 minutes	1
Denaturation	94	30sec	35 cycles
Annealing	68 (SHV)	1mins	1
	55 (TEM)		
	60(CTX-M)		
	50(OXA)		
	65(PER)		
	65(VEB)		
Extension	72	1mins	1
Final extension	72	7mins	1

Key: SHV (Sulfhydryl variable), TEM (Temoneira), CTX-M (Cefotaxime hydrolyzing), OXA (Oxacillin hydrolyzing).

Agarose Gel Electrophoresis of the PCR products

One percent (1%) Agarose gel (AG) was used to resolve the genomic DNA extracted from the *E. coli* isolates and documented. Preparation of the AG was carried out using 1g of agarose in 90 ml distilled water and heated for 2 minutes using a microwave. Before the heated AG cools, 2.5ml ethidium bromide (5.0 mg/ml) was added and the solution was swirled so it could mix properly. Using an electrophoretic tank and its comb, the gel was casted and allowed to solidify for 30 minutes, thereafter, the comb was removed and a TAE electrophoresis buffer (1X) added to the tank to cover the AG.

A 5µl bromophenol blue was then mixed with 15µl of the extracted genomic DNA and loaded onto the wells of the solidified gel before the electrodes were connected and ran for 45 minutes at 100mV. The product was removed from the tank and viewed under a 302 nm Transilluminator, Polaroid camera and a gel documentation system.

Results

Specimen Collection

A total of 170 mild stream urine samples submitted to the Medical Microbiology

Laboratories of 4 different hospitals and that of healthy individuals were randomly collected for 5 months and examined for microbial growth as shown in Table 4. General Hospital Minna (67) had the highest urine samples followed by Standard Hospital (30) and IBB Specialist Hospital (30) while the Hospital with the least samples was Top Medical Clinic (23). However, only 20 healthy individuals gave their consent and submitted samples for evaluation.

Table 4: Distribution of Samples collected from Four Hospitals and Healthy Individual

S/N	Hospital	Number of Urine Samples collected
1	ST	30
2	IBB	30
3	GH	67
4	TMED	23
5	HI	20
Total		**170**

Key: ST: Standard Hospital, IBB: IBB Specialist Hospital, GH: General Hospital, TMED: Top Medical Clinic, HI: Healthy Individuals

From the sample distributions, female patients (65.9%) submitted more urine samples for UTI test to the Medical Laboratory of the Hospitals sampled compared to their male counterpart (34.1%) (Table 5). Ages between 21 -30 (26.5%) and 31 - 40 (25.3%) had the highest percentages of infection rate while those within the ages 1- 10 (3.5%) and ≥ 71 (2.3%) were the least infected (Table 6).

Table 5: Gender Distribution of Urine Samples from Four Hospitals and Healthy Individuals.

Hospital	Gender (No and % of Sample Collected)		Total (%)
	Male (%)	Female (%)	100%
ST HOSP	10(33.3)	20(66.67)	30(17.6)
IBB HOSP	10 (33.3)	20(66.67)	30(17.6)
GH	21(31.34)	46(68.66)	67(39.4)
TMED	9(39.13)	14(60.87)	23(13.5)
HI	8(40)	12 (60)	20(11.8)
Total	**58 (34.1)**	**112 (65.9)**	**170(100)**

Keys: ST: Standard Hospital, IBB: IBB Specialist Hospital, GH: General Hospital, TMED: Top Medical, H: Healthy Individuals

Table 6: Age Distribution of Urine Samples Collected from Four Hospitals and Healthy Individual

AGE RANGE	IBB	ST	GH	TMED	HI	Total (%)
1 – 10	0	2	3	1	0	6(3.5)
11 – 20	2	4	6	5	2	19(11.2)
21 – 30	9	6	15	7	8	45(26.5)
31 – 40	5	6	20	8	4	43(25.3)
41 – 50	6	5	6	0	3	20(11.8)
51 – 60	5	5	10	1	1	22(12.9)
61 – 70	3	0	5	1	1	10(5.9)
≥71	0	2	2	0	0	4(2.3)
Total	**30**	**30**	**67**	**23**	**20**	**170 (100)**

Keys: ST: Standard Hospital, IBB: IBB Specialist Hospital, GH: General Hospital, TMED: Top Medical, H: Healthy Individuals

Isolation and Identification of *Escherichia coli*

The prevalence of *E. coli* in Minna metropolis was 23.5%. Significant numbers (30%) of healthy individuals (HI) were observed to also harbor *E. coli* in their urine. IBB Specialist hospital had the highest percentage (36.7%) of *E.coli* isolated from urine samples indicating a high UTI infection rate amongst the patients, followed by Standard Hospital (26.7%) and General Hospital (20.9) while the   hospital with the least number of patients that are infected with UTI was the Top Medical Hospital (Table 7).

**Table 7:** Distribution of *E. coli* Isolated from the Four Selected Hospitals and Healthy Individuals

Hospital	Samples Collected	*E. coli* isolated	Percentage of *E. coli*
ST	30	8	26.7
IBB	30	11	36.7
GH	67	14	20.9
TMED	23	1	4.3
HI	20	6	30
Total	**170**	**40**	**23.5**

Key: ST: Standard Hospital, IBB: IBB Specialist Hospital, GH: General Hospital, TMED: Top Medical, HI: Healthy Individuals

Antibiotic Susceptibility Test

The study observed significant susceptibility to Gentamicin (65%), Ofloxacin (65%), Tetracycline (62.5%), Cotrimoxazole (62.5%), and Streptomycin (57.5%). Mildly susceptible to Augmentin (50%), Amoxicillin (45%), Pefloxacin (37.5%), Chloramphenicol (37.5%), and Ciprofloxacin (35%). High resistant profile was observed against all the betalactams tested [Cefuroxime (80%), Amoxicillin (42.5%), Augmentin (40), Cefotaxime (20%) and Ceftaxidime (7.5%)] (Figure 2).
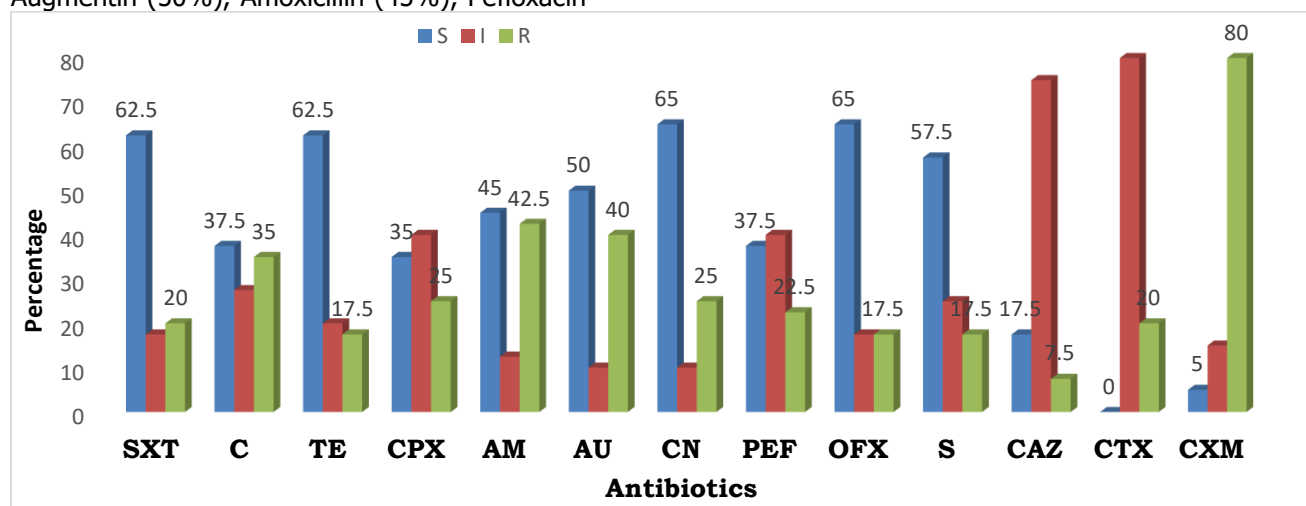
Figure 2: Percentage Antibiotics Susceptibility Profile of *E. coli* Isolated from UTI patients in Minna, Nigeria

Keys: SXT = Cotrimoxazole, C = Chloramphenicol, TE = Tetracycline, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, CN = Gentamicin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin, CAZ = Ceftaxidime, CTX = Cefotaxime, CXM = Cefuroxime, S = sensitive I = intermediate R = resistance

Two of the isolates (U59 and U26) were resistant to all the 13 antibiotics tested; seventy percent (28) of the isolates had multiple antibiotics resistance index (MARI) ≥0.3 while 30% (12) had ≤0.2. The most common resistant pattern observed in 65% (26) of the isolates was simultaneous resistance to Ceftaxidime, Cefotaxime, and Cefuroxime (resistant to the Cephalosporin tested), while 20% (8) were resistant to all the beta lactams tested. Multiple drug resistance (≥3 classes) was expressed in 37.5% of the isolates tested. This study showcases a vast resistant pool in the environment where the study was conducted and possibility of beta lactamase production in the evaluated *E. coli* isolates. Resistance to all the 6 classes of antibiotic tested in 2 isolates from the different patient samples might indicate a plasmid profile encoding diverse genes of resistance or biofilm formation. However, extended spectrum beta-lactamase producing isolates have been reported to develop resistance to arrays of antibiotics commonly consumed in the environment (Table 8).

Table 8: Determination of Resistant Pattern and Multiple Antibiotic Resistant Index of the *E. coli* Isolated from UTI Patients in Minna, Niger State.

S/N	ISOLATE CODE	RESISTANT PATTERN	NCART	NART	MARI

| 1	U123	C,AM,AU,CAZ,CTM	2	5	0.4
2	U19	C,TE,CPX,AM,AU,CN,PEF,OFX,S,CAZ,CTX,CXM	6	12	0.9
3	HU10	CAZ,CXM	1	2	0.2
4	U41	AM,PEF,CAZ,CTX,CXM	2	5	0.4
5	U55	S,CTX,	2	2	0.2
6	U16	C, AM,AU, CAZ,CTX,CXM	2	6	0.5
7	U59	SXT,C,TE,CPX,AM,AU,CN,PEF,OFX,S,CAZ,CTX,CXM	6	13	1
8	U53	C, CAZ,CTX,CXM	2	4	0.3
9	U37	CPX,AM,AU,PEF,CAZ,CTX,CXM	2	7	0.5
10	U26	SXT,C,TE,CPX,AM,AU,CN,PEF,OFX,S,CAZ,CTX,CXM	6	13	1
11	U13	C,CAZ,CTX,CXM	2	4	0.3
12	HU21	AM,AU,OFX	2	3	0.2
13	U50	TE,CPX	2	2	0.2
14	U7	TE, AM,AU,OFX	3	4	0.3
15	U64	AU,CAZ,CTX,CXM	2	4	0.3
16	HU5	AU,CN,CTX,CXM	2	4	0.3
17	U47	C,CPX,AM,AU,CN	4	5	0.4
18	HU18	SXT, CAZ,CTX,CXM	2	4	0.3
19	U70	SXT,AM,CN,CTX,CXM	3	5	0.4
20	U88	SXT,AM,AU,CN, CAZ,CTX,CXM	3	7	0.5
21	U139	C,TE,AM,S,CAZ,CTX,CXM	4	7	0.5
22	U111	SXT, OFX,S,CAZ,CTX,CXM	4	6	0.5
23	U102	AM,AU,CN, S,CAZ,CTX,CXM	4	7	0.5
24	U82	AM,AU,CTM	2	3	0.2
25	U71	SXT,C,AM, CAZ,CTX,CXM	3	6	0.5
26	U134	CN,PEF, CAZ,CTX,CXM	3	5	0.4
27	u65	CAZ,CTX,CXM	1	3	0.2
28	U46	CXM	1	1	0.1
29	U58	CPX,AM, CAZ,CTX,CXM	2	5	0.4
30	U60	PEF, CAZ,CTX,CXM	2	4	0.3
31	U30	CAZ,CTX,CXM	1	3	0.2
32	U44	CAZ,CTX,CXM	1	3	0.2
33	U155	C,PEF, CAZ,CTX,CXM	3	5	0.4
34	U61	CPX,PEF, CAZ,CTX,CXM	2	5	0.4
35	U155	CAZ,CTX,CXM	1	3	0.2
36	U61	SXT,C,CPX,AM,AU,OFX, CAZ,CTX,CXM	4	9	0.7
37	U56	AU,CAZ	1	2	0.2
38	U15	C,TE,CPX,CAZ,CXM	4	5	0.4
39	U63	C, CAZ,CTX,CXM	2	4	0.3
40	U18	CTX,CXM	1	2	0.2

Keys: NCART = number of classes of antibiotics resistant to, NARI = number of antibiotics resistant to, MARI = multiple antibiotics resistance index, SXT = Cotrimoxazole, C = Chloramphenicol, TE = Tetracycline,

CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, CN = Gentamicin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin, CAZ = Ceftaxidime, CTX = Cefotaxime, CXM = Cefuroxime

Plate A shows a significant extended spectrum of activity of amoxicillin/clavulanic acid to the Cephalosporins placed 15mm apart from its center, and all the beta-lactams tested

had ≥5mm zones of inhibition compared to when tested alone. In Plate B there was only an

extended spectrum of the amoxicillin/clavulanic acid to ceftaz idime with reduced zone of inhibition while cefpodoxime and cefotaxime had no zones of inhibitions [Plate I].

*Presumptive Test for Extended Spectrum Beta Lactamase (ESBL) Production*
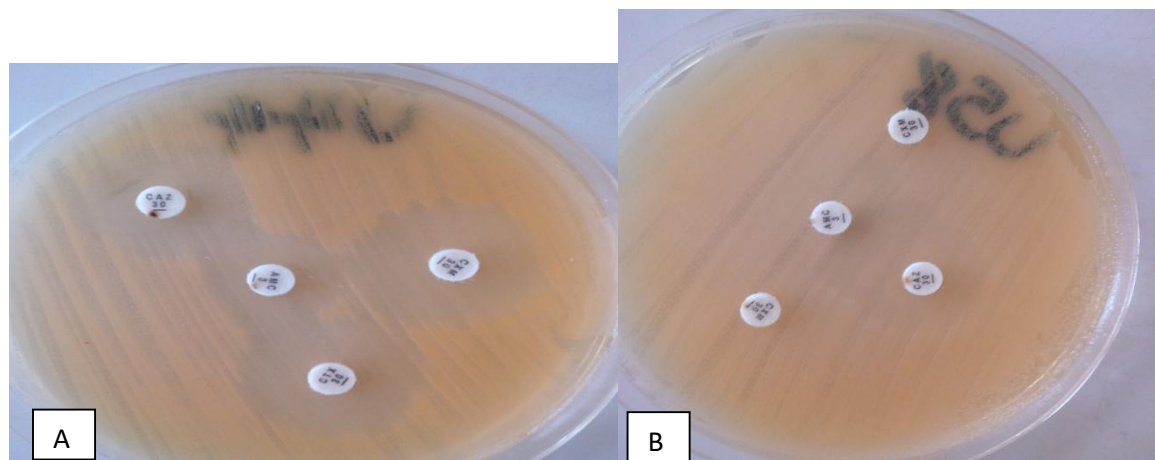
Plate I: Pictorial Representation of Expressed Extended Spectrum β- Lactamase Producing Isolates of *E. coli*.

Keys: Plate A showed the production of ESBL (positive result). Plate B showed no production of ESBL (negative result). - (negative); + (positive); AMC (amoxicillin-clavulanate); CAZ (ceftazidime), CXM (cefuroxime); CTX (cefotaxime), U (urine), HU (healthy urine).

Significant percentage (25%) of the *E. coli* isolated from the Urine samples, expressed the

phenotypic ability to produce beta-lactamases while 75% were none ESBL producers (Figure 3)
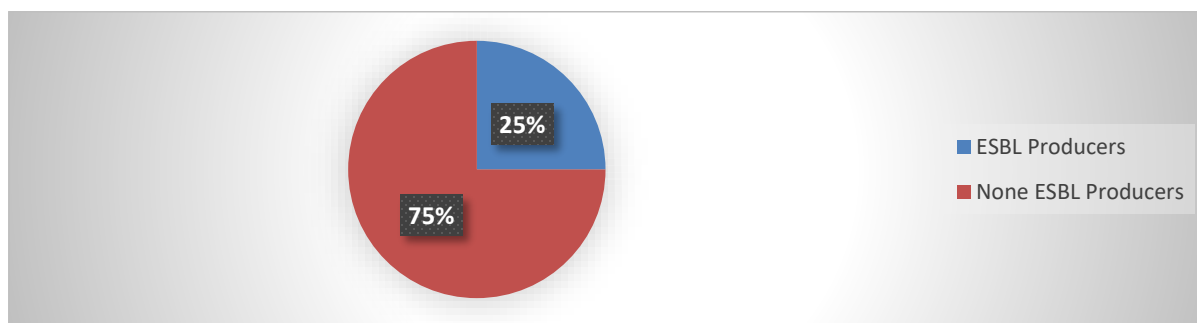
Figure 3: Evaluation for ESBL Production among *E. coli* Isolated from UTI Patients in Minna, Nigeria

Table 9 shows that all the ESBL- producing isolates were resistant to more than 5 antibiotics tested and had a MAR Index of ≥ 0.5, while 90% showed multiple drug resistance (≥ 3 classes).

Further evaluation showed that 25% (10/40) of the *E. coli* isolated from UTI patients in Minna, Nigeria were ESBL producers and could harbor one or two of the genes. This implies that only

5.9% of the urine samples had ESBL- encoding
E. coli with multidrug resistance characteristics.

Table 9: Antibiotics Susceptibility Profile of ESBL Producing *E. coli* isolated from UTI patients in Minna, Nigeria

S/N	ISOLATE CODE	RESISTANT PATTERN	NCART	NART	MARI
1	U19	C,TE,CPX,AM,AU,CN,PEF,OFX,S,CAZ,CTX,CXM	6	12	0.9
2	U59	SXT,C,TE,CPX,AM,AU,CN,PEF,OFX,S,CAZ,CTX,CXM	6	13	1
3	U37	CPX,AM,AU,PEF,CAZ,CTX,CXM	2	7	0.5
4	U26	SXT,C,TE,CPX,AM,AU,CN,PEF,OFX,S,CAZ,CTX,CXM	6	13	1
5	U88	SXT,AM,AU,CN, CAZ,CTX,CXM	3	7	0.5
6	U139	C,TE,AM,S,CAZ,CTX,CXM	4	7	0.5
7	U111	SXT, OFX,S,CAZ,CTX,CXM	4	6	0.5
8	U102	AM,AU,CN, S,CAZ,CTX,CXM	4	7	0.5
9	U71	SXT,C,AM, CAZ,CTX,CXM	3	6	0.5
10	U61	SXT,C,CPX,AM,AU,OFX, CAZ,CTX,CXM	4	9	0.7

Keys: NCART = number of classes of antibiotics resistant to, NARI = number of antibiotics resistant to, MARI = multiple antibiotics resistance index, SXT = Cotrimoxazole, C = Chloramphenicol, TE = Tetracycline, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, CN = Gentamicin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin, CAZ = Ceftaxidime, CTX = Cefotaxime, CXM = Cefuroxime

Molecular Characterization of ESBL Resistant Escherichia coli

The genomic DNA of the 10 samples that were resistant to all the tested beta-lactams and exhibited presumptive ESBL were extracted. The evaluation showed that all the isolates harbor one or two genes harboring the ESBL enzyme. TEM gene of 931bp was expressed in 70% (7) of the isolates, 60% (6) harbored CTXM gene that amplified at 593bp, 20% (2) had the OXA gene which is 478bp while none of the bacteria harbored the SHV gene (Plate II).
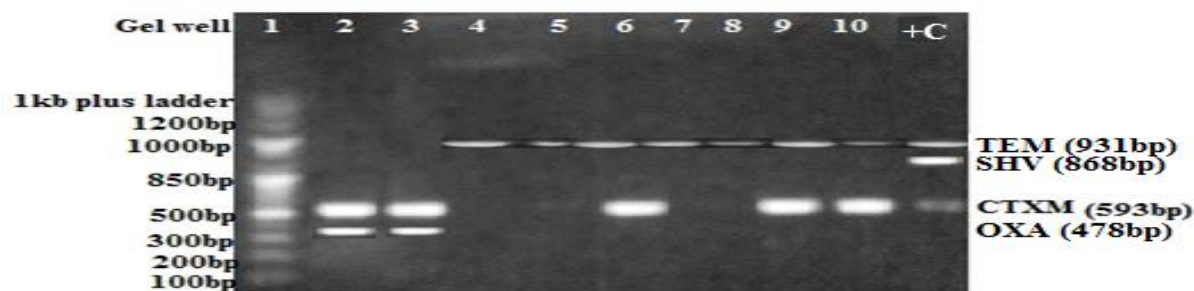
Plate II: Electrophoregraph of ESBL producing *E. coli* isolated from Urine samples of UTI patients in Minna, Nigeria

Keys: 1 = DNA ladder, 2 = U19, 3= U59, 4= U37, 5 = U26, 6= U88, 7 = U111, 8 = U102, 9 = U71, 10 = U61, +C = *E. coli* ATCC25922

Discussion

Studies have shown that UTI is generally caused by bacterial proliferation within the urinary tract in both males and females. *Escherichia coli*-associated UTI accounts for one of the most major causes of hospital visits (Flores-Mireles et al., 2015). This bacterium has also been implicated in most infections, including cholecystitis, bacteremia, cholangitis, and diarrhea (Dong *et al.,* 2018). To achieve effective therapeutic outcome, the causative microorganism must be characterized and an antibiogram conducted to determine the most potent antibiotics (Igwe *et al.,* 2014).

From the sample distribution, female patients (65.9%) suffered more UTI compared to their male counterpart (34.1%). This observation correlates with other reports who also observed that female patients are more prone to UTI than males (Rajalakshmi and Amsaveni, 2012; Sood and Gupta, 2012; Idakwo *et al.,* 2015). This might be due to women anatomy and reproductive physiology i.e. proximity of female urethral opening to the anus. More so, the female urethra is shorter than the male's; this enables easy bacteria access to the bladder. Our study also observed that age ranges of 21 -30 (26.5%) and 31 - 40 (25.3%) had the highest percentages of infection rate while those within the ages of 1- 10 (3.5%) and ≥ 71 (2.3%) were

the least infected. These findings tally with the study conducted by Idakwo *et al.,* (2015) in Minna, of which patients within the age groups of 20 - 29 years were the most infected. According to Dielubenza and Schaeffer, (2011), high sexual activity, the use of a diaphragm with spermicide, childbirth, menopause in such age limit are among the predisposing factors to infections.

The prevalence of *E. coli-* associated UTI in Minna metropolis as observed in this study was 23.5%. This result tallies with the report of Omonigho *et al.,* (2001), who observed a prevalence of 22.3% but lower than the reports of Idakwo *et al.,* (2015), Ebie *et al.,* (2001) and Oladeinde *et al.,* (2011) who recorded 32.5% in Minna, Niger State, 35.3% in Offa, Kwara State and 39.7% in Okada, Edo state, respectively. Comparative evaluation of bacte ria in UTI revealed that *Escherichia coli* prevalence varies from 20% to 85% in most UTI studies (Vasudevan, 2014). This might be attributed to age, parity, gravidity, and other associated

diseases, which might augment the condition (Vasudevan, 2014). Urinary tract infection is not only caused by *E. coli* bacteria but also other pathogens such as *Staphylococcus aureus, Klebsiella spp., Proetus vulgaris, Pseudomonas aeruginosa* and *Salmonella spp*. (Oladeinde *et al.,* 2011).

Significant numbers (30%) of healthy individuals (HI) were observed to also harbor *E. coli* in their urine. This is termed asymptomatic bacteri uria (ABU): a commensal condition where the patients carry >10^5 CFU/ml of the bacteria without any symptom (Salvador *et al.,* 2012). This strain of UTI *E. coli* has been studied to evolve from virulent uropathogenic *E. coli* strains, which had developed significant mechanisms of bacterial adaptation that enables its prototype (ABU strain *Escherichia coli* 83972) to evolve a smaller genome than uropathogenic *E. coli* (UPEC) strains. A study on the bacteria had shown point mutations or deletions in most of the virulence genes encoded by the bacteria. This further highlights that ABU strains are subject to evolutionary transformation within its hosts (Zdziarski *et al.,* 2008). According to Salvador *et al.,* (2012), ABU strains can evolve in 2 to 20% of the population for months or years and it is influenced by factors such as gender and age.

The isolates had varying susceptibility patterns but were more susceptible to Gentamicin, Ofloxacin, Tetracycline, Cotrimoxazole, and Streptomycin. According to Hattewar *et al.,* (2020), most of these antibiotics are considered as appropriate antimicrobials for empirical treatment of urinary tract infections in most clinics, especially in developing countries. The isolates were mildly susceptible to Augmentin, Amoxicillin, Pefloxacin, Chloramphenicol and Ciprofloxacin. High resistance was observed against all the beta-lactams tested [Cefuroxime, Amoxicillin, Augmentin, Cefotaxime and Ceftaxidime]. The differences in drug resistance by the isolates in this study correlate with the findings documented by Onifade *et al.,* (2015) and Lohani et al., (2020). Most of their *E. coli* isolates expressed significant resistance to Trimethoprim, Ceftazidime, Gentamicin, Cefuroxime, Chloramphenicol, Cefixime, Augmentin and Tetracycline but showed mild susceptibility to Quinolones and Nitrofurantoin.

Two of the isolates were resistant to all the 13

antibiotics tested while 70% of the isolates had multiple antibiotics resistance index (MARI) ≥0.3. The isolates had a consistent resistant pattern in 65% of their population. This study showcases a vast resistant pool in the environment where the study was conducted and the possibility of beta-lactamase production in the evaluated *E. coli* isolates. The study conducted by Ochada *et al.,* (2015) further buttresses the fact that *E. coli* from UTI show a pattern of resistance depending on the tested antibiotics. Resistance to all the 6 classes of antibiotics tested in 2 isolates from the different patients sampled might indicate a plasmid profile encoding diverges genes of resistance or biofilms. However, extended spectrum beta-lactamase- producing isolates have been reported to develop resistance to arrays of antibiotics commonly consumed in an environment.

Our study showcases the possibility of better chemotherapeutic outcome of urinary tract infections using Quinolones and Nitrofurantoin even in the presence of multidrug resistance. This is very crucial as total lack of hope on the commonly prescribed antibiotics spontaneously aggravates poor life quality and expectancy, which further materializes to death. However, this study calls for more periodic surveillance on antibiograms as this will influence the use of appropriate antimicrobial agents.

As discussed in other studies, the evaluation of drug resistant isolates are primarily conducted using molecular genotyping and phenotyping techniques, which are deployed in screening and to confirm expression of antimicrobial drug resistance genes within a population (Alyamani *et al.,* 2017). This was carried out among suspected extended spectrum beta- lactamase (ESBL)-producing *Escherichia coli* known to be one of the leading causes of deaths globally among UTI patients, as this strain harbors multidrug resistance and virulent genes (Ramadan *et al.,* 2019). In this study, 25% (5.9% of the total urine samples), expressed the phenotypic ability to produce beta-lactamase enzymes. Examination of these isolates shows that 70% (7) [i.e. 17.5% of the total *E. coli*] harbors the TEM gene; 60% (6) [i.e. 15%] harbored CTXM gene; 20% (2) [i.e. 5%] had the OXA gene while

none of the bacteria had the SHV gene. Further empirical study showed that 90% of the ESBL-producing isolates were multidrug resistant and had MARI of ≥ 0.5. This study is similar to the findings of Ejikeugwu *et al.,* (2013) who reported 27.7% of ESBLs in Enugu and 26.1% in Ile-Ife by Olufunke *et al.,* (2014). However, the prevalence of ESBL is geographically dependent. This is linked to the variability in the use of antimicrobiasl and measures put in place to control infections in these locations (Rapp, 2011). The study conducted by Yasir *et al.,* (2018) also established that most ESBL isolates are multidrug resistant (MDR), especially to 3rd and 4th generation cephalosporins. Most worrisome was the reports of Shakil *et al.,* (2010) and Ny *et al.,* (2017), which revealed that ESBLs are harbored among asymptomatic community dwellers and are often plasmid-associated, with the ability of cross-species dissemination and multidrug resistance genes that encode fluoroquinolones, aminoglycosides, tetracycline's, cotrimoxazole and chloramphenicol.

Conclusion and Recommendations

This study showed a prevalence of 23.5% *E. coli*-associated UTI in Minna, Nigeria and further established the presence of ESBL in 5.9% of the isolates with multidrug resistance characteristics. Drugs of choice which could be used for their treatment were Gentamicin, Ofloxacin, Tetracycline, Cotrimoxazole, and Streptomycin. It further substantiated the importance of good antibiogram evaluation as it establishes a baseline for empirical diagnosis, epidemiological surveillance, drug prescription and infection management.

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