



## **Antimicrobial Resistance Profile of *Escherichia coli* Isolated from Urine of Patients in Nagari Allah Magani Hospital, Keffi, Nigeria**

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### **Authors' contributions**

This work was carried out in collaboration between all authors. Authors YBN and KOE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IHN and RHA managed the analyses of the study. Authors TI and SMJ managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aim:** This study investigated antimicrobial resistance in *E. coli* recovered from urine of patients attending Nagari Allah Magani Hospital, Keffi, Nigeria.

**Place and Duration of Study:** Department of Microbiology, Nasarawa State University, P.M.B. 1022, Keffi, Nasarawa State, Nigeria; between January 2018 and July 2018.

**Methodology:** *Escherichia coli* was isolated and identified from the urine of suspected UTIs patients by culture, microscopy and biochemical tests. Sample: We included 248 patients; (116

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men, 132 women; age range 5-65 years). Antimicrobial Susceptibility Testing of the isolates was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) method.

**Results:** Out of 248 samples, the occurrence of *E. coli* was 43(17.3%). The occurrence of isolates in relation to age of patients was highest in 21-30 year old (26.5%) and lowest in 11-20 year olds (9.5%). The occurrence of the isolates was higher in females (21.2%) than males (12.9%). The isolates were less resistant to imipenem (20.9%), gentamicin (34.9%) and ciprofloxacin (37.3%). The commonest resistance phenotype was Amoxicillin/Clavulanic Acid-Streptomycin-Sulfamethoxazole/Trimethoprim-Cefotaxime-Ceftazidime-Cefoxitin-Ciprofloxacin-Ampicillin with an occurrence of 7.0%. Most of the isolates showed multiple antibiotic resistance (MAR) Index of above 0.2 with the commonest MAR Index being 0.6 (23.8%). Most (95.6%) of isolates were classified into multidrug resistance (MDR), a few (2.3%) were non-MDR or pan drug resistance (PDR), and no extensive drug resistance (XDR) was isolated. The occurrence of classes of antibiotic resistance was of the order: MDR (95.3%) > NMDR = PDR (2.3%) > XDR (0.0%).

**Conclusion:** Resistance was less to imipenem, gentamicin and ciprofloxacin antibiotics. Most isolates originated from an environment where antibiotics are freely available and misused or abused; and are MDR isolates. Further work to detect antibiotic resistance genes in the study location should be carried out.

**Keywords:** *Escherichia coli*; urine; antimicrobial; resistance; multidrug.

## 1. INTRODUCTION

Urinary tract infections (UTI) are a frequent cause of morbidity in the population and a good reason for medical appointments in public health facilities, second only to respiratory infections [1]. Gram-positive species, such as *Staphylococcus* spp and *Streptococcus* spp and *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, are the common etiological agents of UTIs [1,2].

The treatment and control of UTIs are accomplished through therapy with different classes of antibiotics namely  $\beta$ -lactams, fluoroquinolones, aminoglycosides and sulfamethoxazole/trimethoprim, which have greater activity against gram-negative bacteria, the main etiological agents of community-acquired UTI [1]. Resistance developed against these classes of antimicrobial agents and others by bacteria have limited their successful application to manage UTIs. The increasing therapeutic failure observed in empirical treatment using antimicrobial agents has made it important to identify the pattern of susceptibility and resistance of bacterial agents, through *in vitro* antimicrobial susceptibility testing, which can guide the therapeutic approach [1].

Reports from some parts of Nigeria on antimicrobial resistance among *E. coli* from urine of suspected UTIs cases are available [3,4,5]. There is no study on the resistance profile of *E. coli* from UTI cases in the study area that is known to the author. This informed the choice of

the study area as well as the health facility. Understanding the profile of resistance to antimicrobial agents in isolates recovered from this study location will guide the facility managers in their choice of antimicrobials for empirical treatment of their patients.

## 2.1 MATERIALS AND METHODS

### 2.1 Study Location

The study was carried in Nagari Allah Magani Hospital in Keffi metropolis. Keffi is a town found in Nasarawa State, Nigeria. The town is about 138 Km<sup>2</sup>. The postal code of the area is 961. It is situated at 8.84861° (latitude and decimal degrees) and 7.87361° (longitude and decimal degrees) it is 338 meters elevated above the sea level. Keffi has about 85,911 inhabitants making it the second biggest city in Nasarawa. It operates on the WAT time zone [6].

### 2.2 Sample Collection

A total of 248 early morning mid-stream urine samples of patients with suspected cases of UTIs were collected using sterile containers and transported using ice pack to the Microbiology Laboratory, Nasarawa State University, Keffi for analysis.

### 2.3 Isolation and Identification of *Escherichia coli*

*Escherichia coli* were isolated from the urine samples of patients with suspected cases of

Urinary tract infections as follows: With the aid of a wire loop, the urine sample was streaked on MacConkey agar (Oxoid Ltd., Basingstoke, UK) plate and incubated at 37°C for 24 h. Pinkish colonies that grew on MacConkey agar were further inoculated on Eosin Methylene Blue agar (Oxoid Ltd., Basingstoke, UK) and incubated at 37°C for 24 h. Greenish metallic sheen colonies that grew on the Eosin Methylene Blue agar plate were selected as presumptive *E. coli*. Presumptive *E. coli* were identified by microscopical (Gram stain) and minimum biochemical tests for *E. coli* identification namely "IMViC" (Indole, Methyl red, Voges-Proskauer, Citrate) based on method already described [7].

Indole positive, Methyl red positive, Voges-Proskauer negative and citrate negative isolates were further confirmed as *E. coli* using a commercial kit B004HI™ (HiMedia Ltd, India) in accordance with the manufacturer's instructions.

## 2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the confirmed *E. coli* isolates was carried out as earlier described [8]. Briefly, (3) pure colonies of isolated *E. coli* species from urine samples of patients attending Nagari Hospital, Keffi, Nigeria was inoculated in to 5 ml sterile 0.85% (w/v) NaCl (BDH Chemicals Ltd., England) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows; 0.5 ml of 1.172% (w/v) BaCl<sub>2</sub>·2H<sub>2</sub>O (BDH Chemicals Ltd., England) was added into 99.5 ml of 1% (w/v) H<sub>2</sub>SO<sub>4</sub> (BDH Chemicals Ltd., England).

A sterile swab stick was soaked in the standardized bacteria suspension and streaked on Mueller- Hinton agar (Oxoid Ltd., Basingstoke, UK) plates and the antibiotic discs (Oxoid Ltd., Basingstoke, UK) were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were placed in an incubator (Model 12-140E, Quincy Lab Inc.) set at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result of the susceptibility was interpreted in accordance with the susceptibility break point earlier described [8].

## 2.5 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined using the formula: MAR Index = No. antibiotics

isolate is resistant to/No. of antibiotics tested as described [9].

## 2.6 Classification of Antibiotic Resistance

Antibiotic resistance in the isolates were classified into: multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [10].

## 2.7 Statistical Analysis

The data obtained in this study was analysed using chi-square by use of Smith Statistical Package (SSP) version (2.80) and the significance was determine at 95% confidence interval.

## 3. RESULTS AND DISCUSSION

### 3.1 Occurrence of *Escherichia coli*

The cultural, microscopical and biochemical characteristics used to identify the isolates were as shown in Table 1. Pinkish colonies on MacConkey agar that grew with greenish metallic sheen on Eosin Methylene Blue agar and were Gram negative, Indole positive, Methyl red positive, Voges-Proskauer negative, Citrate negative, ONPG positive, Nitrate positive, Lysine positive, Ornithin positive, etc were taken as confirmed *E. coli*.

The occurrence of *E. coli* in the urine was 43(17.3%). The occurrence in relation to age of the patients is as shown in Table 2. The order of occurrence was: 21 - 30 years (26.5%) > 31 – 40 years (16.2%) > 10 years and below (16.0%) > 41 years and above (10.0%) > 11-21 years (9.5%). In relation to gender, the occurrence was higher in females (21.2%) than males (12.9%) as shown in Fig. 1. The occurrence of *E. coli* in urine of the patients in relation to age and gender were statistically insignificant (P>0.05).

### 3.2 Antimicrobial Resistance Profile

The antimicrobial resistance profile of the isolates is shown in Fig. 2. The isolates were more resistant to ampicillin (93.0%), streptomycin (56%), sulfamethoxazole/trimethoprim (86.0%), cefoxitin (60.5%), cefotaxime (72.1%) and

ceftazidime (53.5%) but less resistant to amoxicillin/clavulanic acid (41.9%), ciprofloxacin (37.2%), gentamicin (34.9%), and imipenem (20.9%) respectively.

### 3.3 Antimicrobial Resistance Phenotypes

The isolates were distributed into different antimicrobial resistance phenotypes as shown in Table 3. The commonest resistance of phenotype was AMC-S-SXT-CTX-CAZ-FOX-CIP-AMP occurrence of 7.0%.

### 3.4 Multiple Antibiotic Resistance (MAR) Index

The MAR Index of the isolates is as shown in Table 4. Most (97.7%) of the isolates were MAR isolates and had MAR indices above 0.2. The commonest MAR Index was 0.6 (23.8%).

### 3.5 Classes of Antibiotic Resistance

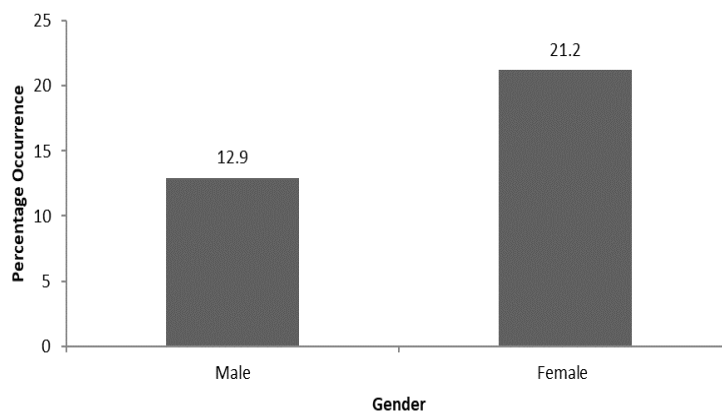
The distribution of the isolates into different classes of antibiotics resistance namely: Multi-drug resistance (MDR), Extensive drug resistance (XDR), Pan-drug (PDR) and Non-Multi drug resistance is as given in Fig. 3. The order of occurrence of the classes of resistance in the isolates was: MDR (95.3%) > PDR = NMDR (2.3%) > XDR (0.0%).

*Escherichia coli* is one of the most common etiological agents of both community and hospital acquired urinary tract infections worldwide [11]. This study focused on antimicrobial resistance in *E. coli* from urine of patients with suspected UTIs

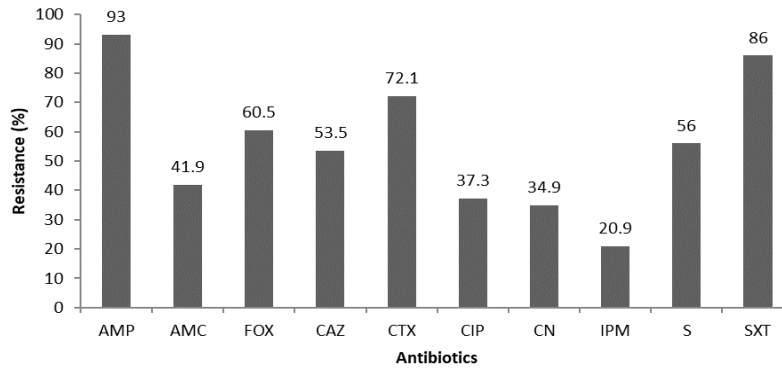
in the study location. The observed percentage occurrences of *E. coli* from urine of the patients was expected, in agreement with the study previously described [12,13,14]. The percentage occurrences of *E. coli* from urine of the patients observed in this study was less than 58.8% and 23.1 % reported [15,16] but greater than 11.5% reported by Ezech et al. 2017. The isolation of *E. coli* from urine of the patients is an indication that this organism may be responsible for the UTIs.

The high occurrence of *E. coli* among the patients of age 21 – 30 years than other age groups observed in this study was in agreement with the study earlier reported [17] and this may be due to the fact that, individuals of this age group may be sexually active and more prone to UTIs although our finding shows that occurrence of isolates in relation to the age of suspected UTIs Patients was statistically insignificant which implies that the age of Patients may be not a necessary factor for occurrence of *E. coli*. In addition, our finding also shows that, the occurrence of *E. coli* was high among females than the male suspected UTIs Patients and this may not be different from the study earlier reported [13,17]. The high occurrence of the isolates in female than male may be due to the differences in the anatomy of the reproductive organs, where the female have short reproductive organ than the male therefore making them more prone to UTIs than the male counterpart.

The high resistance of the isolate to ampicillin, cefoxitin, ceftazidime, cefotaxime, streptomycin and sulfamethoxazole/trimethoprim observed in this study was not surprising and this may be due to

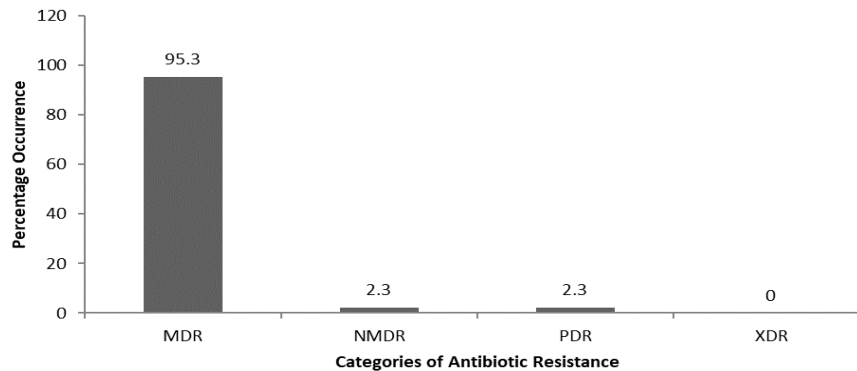


**Fig. 1. Occurrence of *Escherichia coli* from urine in relation to gender of patients attending Nagari Allah Magani Hospital, Keffi, Nigeria**



**Fig. 2. Antibiotics resistance of *Escherichia coli* from urine of patients attending Nagari Allah Magani Hospital, Keffi, Nigeria**

(AMP = Ampicillin; S = Streptomycin; SXT = Sulfamethoxazole/Trimethoprim; CTX=Cefotaxime; CAZ = Ceftazidime; FOX = Cefoxitin; CN = Gentamicin; IPM = Imipenem; CIP = Ciprofloxacin; AMC = Amoxicillin/Clavulanic acid)



**Fig. 3. Occurrence of categories of antibiotics resistance in *Escherichia coli* from urine of patients attending Nagari Allah Magani Hospital, Keffi, Nigeria**

(MDR: multidrug resistance; NMDR = non-multidrug resistance; XDR = extensive drug resistance; PDR = pan drug resistance)

inappropriate use of antibiotics [9]. The percentage resistance of the isolates to cefotaxime and ceftazidime was higher than 40% and 41.6% reported [17]. The resistance of isolates to cefotaxime and ceftazidime observed in this study may be due to the production of Extended Spectrum Beta-Lactamases (ESBL), although the production of ESBL in resistance isolates was not evaluated in this study, but Nkene et al. [18] reported the isolation of urinary *E. coli* resistant to both cefotaxime and ceftazidime. Also, the resistance of the isolates to ampicillin, ceftazidime and cefoxitin observed in this study was also higher than 59.1% and 2.4% previously reported [15].

The low resistance of the isolates to ciprofloxacin, gentamicin and imipenem observed was not different from the study earlier described [17,19].

The percentage resistance of the isolates to gentamicin, imipenem and ciprofloxacin was less than 10.6%, 13.9% and 38.1% reported [17]. The low resistance of isolates to the antibiotics is very effective against the urinary *E. coli* and may be used for effective therapy of UTIs caused by this organism.

The urinary *E. coli* from this study was distributed into different classes of antibiotics resistance and the most common class observed in this study was multi-drug resistance (MDR). The high occurrence of MDR isolates observed in this study is similar with the study earlier reported [20,21]. The percentage occurrence of MDR isolates observed in this study was higher than 56.1% and 64.9% reported [20,21]. The isolates of MDR urinary isolates in this study suggest that the isolates may likely be responsible for UTIs

**Table 1. Cultural, morphological and biochemical characteristics of *Escherichia coli* isolated from patients in Nagari Allah Magani Hospital, Keffi, Nigeria**

Cultural characteristics	Morphological characteristics		Biochemical Characteristics											Inference	
	Gram reaction	Morphology	IND	MR	VP	CT	TDA	ONPG	LYS	ORN	UR	NT	H <sub>2</sub> S		MAL
Pinkish colonies on MCA and Greenish metallic sheen on EMB agar	-	Rod	+	+	-	-	-	+	+	+	-	+	-	-	<i>E. coli</i>

+ = Positive, - = negative, IND = Indole; MR = Methyl red; VP = Voges-Proskauer, CT = Citrate, LYS = Lysine, ORN = Ornithine; ONPG = Ortho-Nitrophenyl-β-galactosidase, UR = Urease, NT = Nitrate, H<sub>2</sub>S = Hydrogen Sulphide, Mal = Malonate, TDA = Phenylalanine deaminase

**Table 2. Occurrence of *Escherichia coli* from urine in relation to the age of patients attending Nagari Allah Magani Hospital, Keffi, Nigeria**

Age (years)	No of Samples	No. (%) <i>E. coli</i>
≤10	50	8(16.0)
11 – 20	42	4(9.5)
21 – 30	68	18(26.5)
31 – 40	68	11(16.2)
≥41	20	2(10.0)
Total	248	43 (17.3)

**Table 3. Antibiotic resistance phenotypes of *Escherichia coli* from urine of patients attending Nagari Allah Magani Hospital, Keffi, Nigeria**

Antibiotic resistance phenotypes	No. (%) resistance isolates (n=43)
FOX	1(2.3)
SXT,FOX, AMP	1(2.3)
S, FOX, AMP	1(2.3)
SXT,CTX,FOX,AMP	1(2.3)
S,SXT,FOX,AMP	2(4.7)
S,CAZ,FOX,AMP	1(2.3)
SXT,FOX,CN,AMP	1(2.3)
S,SXT,CIP,AMP	1(2.3)
S,SXT,CTX,AMP	1(2.3)
AMC,S,SXT,AMP	1(2.3)
AMC,S,SXT,CIP,AMP	1(2.3)
S,SXT,CTX,CAZ,FOX	1(2.3)
S,SXT,CTX,CN,AMP	2(4.7)
S,SXT,CTX,CAZ,AMP	1(2.3)
S,SXT,CTX,CAZ,FOX,AMP	1(2.3)
AMC,S,CTX,FOX,IPM,AMP	1(2.3)
S,SXT,CAZ,FOX,CIP,AMP	1(2.3)
S,SXT,CTX,FOX,IPM,AMP	1(2.3)
AMC,S,SXT,CTX,FOX,AMP	1(2.3)
S,SXT,CTX,FOX,CIP,AMP	1(2.3)
S,SXT,CTX,CAZ,CN,AMP	1(2.3)
S,SXT,CTX,CN,CIP,AMP	1(2.3)
AMC,SXT,CTX,CAZ,CIP,AMP	1(2.3)
S,SXT,CTX,CAZ,CIP,AMP	1(2.3)
S,CTX,CAZ,FOX,CN,IPM,AMP	1(2.3)
AMC,S,SXT,CTX,CAZ,FOX,AMP	2(4.7)
S,SXT,CTX,FOX,CN,IPM,AMP	1(2.3)
AMC,S,SXT,CTX,CN,CIP,AMP	1(2.3)
S,SXT,CTX,CAZ,CN,CIP,AMP	2(4.7)
AMC,SXT,CTX,CAZ,CN,IPM,AMP	1(2.3)
AMC,S,SXT,CTX,CAZ,FOX,CIP,AMP	3(7.0)
AMC,S,SXT,CTX,CAZ,FOX,IPM,AMP	1(2.3)
AMC,S,CTX,CAZ,FOX,CN,CIP,AMP	1(2.3)
AMC,S,SXT,CTX,CAZ,CN,CIP,AMP	2(4.7)
AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,AMP	1(2.3)
AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP	1(2.3)

AMP = Ampicillin; S = Streptomycin; SXT = Sulfamethoxazole/Trimethoprim; CTX=Cefotaxime; CAZ = Ceftazidime; FOX = Cefoxitin; CN = Gentamicin; IPM = Imipenem; CIP = Ciprofloxacin; AMC = Amoxicillin/Clavulanic acid

**Table 4. Multiple antibiotics resistance (MAR) index of *Escherichia coli* from urine of patients attending Nagari Allah Magani Hospital Keffi, Nigeria**

No. of Antibiotic Resistance to (a)	No. of Antibiotics Resistance tested (b)	MAR Index (a/b)	No. (%) MAR isolate (n= 42)
10	10	1.0	1(2.4)
9	10	0.9	1(2.4)
8	10	0.8	7(16.7)
7	10	0.7	8(19.0)
6	10	0.6	10(23.8)
5	10	0.5	5(11.9)
4	10	0.4	8(19.0)
3	10	0.3	2(4.8)
2	10	0.2	0(0.0)

that may be difficult to be treated using conventional or common antibiotics usually prescribed for treatment of UTIs since MDR Enterobacteriaceae have been reported to cause life threatening UTIs that is difficult to be treated [20].

The resistance of the isolates to ciprofloxacin observed in this study appears to be low and this is similar with the study earlier reported [13,17,22] but different from the study earlier reported [23,24]. The percentage occurrence of Ciprofloxacin resistance isolates observed in this study was higher than 35.0% reported [13] and less than 82.4% reported [24].

#### 4. CONCLUSION

*Escherichia coli* is one of the bacterium associated with cases of urinary tract infection in Nagari Allah Magani Hospital in Keffi metropolis. The isolates showed high resistance to commonly available antimicrobial agents; but very low resistance to ciprofloxacin. Most isolates are multidrug resistant strains.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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