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Antimicrobial Resistance Profile of *Escherichia coli* Isolated from Urine of Patients in Selected General Hospitals in Abuja Municipal, Nigeria

M. O. Eghieye[1], S. M. Jodi[2], B. E. Bassey[3], I. H. Nkene[1], R. H. Abimiku[1] and Y. B. Ngwai[1*]

[1]Department of Microbiology, Nasarawa State University, P.M.B. 1022, Keffi, Nasarawa State, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]World Health Organization, Nigeria Country Office, UN House, Plot 617/618, Diplomatic Drive, Central Business District, P.M.B. 2861, Garki, Abuja, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors YBN and MOE 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 SMJ and BEB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the antimicrobial resistance profile of *Escherichia coli* from urine of patients with suspected urinary tract infections (UTIs) in selected general hospitals in Abuja Municipal, Nigeria. Four Hundred and Thirty urine samples were collected between September 2017 and May 2018 from patients attending Asokoro General Hospital (AGH), Garki Hospital Abuja (GHA) and Wuse General Hospital (WGH); and *E. coli* was isolated and identified by culture, microscopy and biochemical tests. The overall occurrence of *E. coli* was 52 (12.1%). The occurrences in relation to the hospitals were of the order: GHA (14.7%) > WGH (12.6%) > AGH (9.0%). The highest (50%) occurrence was at age 41-50 years in WGH, and the lowest (4.3%) was at age 31-40 years in

**Corresponding author: Email: ngwaiyb@yahoo.com, ngwaiyb@nsuk.edu.ng;*

AGH. More females than males harboured the bacteria in all the hospitals. Isolates from AGH showed highest (100.0%) resistance to Sulphamethoxazole/Trimethoprim but least (0.0%) resistance to Ciprofloxacin. Isolates from GHA showed the highest resistance to Cefotaxime and Streptomycin (95.2%) but least (23.8%) to Gentamicin and Imipenem. Isolates from WGH showed highest (88.8%) resistance to Amoxicillin/Clavulanic Acid but least (16.7%) to Sulphamethoxazole/Trimethoprim. The commonest antibiotic resistance phenotype in AGH was Amoxicillin/Clavulanic Acid-Streptomycin-Cefotaxime-Ceftazidime-Imipenem-Ampicillin (3.9%); in GHA was Amoxicillin/Clavulanic Acid-Streptomycin-Sulphamethoxazole/Trimethoprim-Cefotaxime-Ceftazidime-Ampicillin (7.7%); and in WGH was Amoxicillin/Clavulanic Acid-Streptomycin-Cefotaxime-Cefotaxime-Ceftazidime-Imipenem-Ampicillin (3.9%). All the isolates had MAR indices above 0.2; the most common index in AGH was 0.4 (at 30.8%), GHA was 0.7 (at 33.3%) and WGH was 0.7 (at 27.8%). The commonest class of antibiotic resistance was MDR with the order of occurrence as: GHA (92.2%) > WGH (77.7%) > AGH (76.6%). Ciprofloxacin, gentamicin and imipenem were the most effective antibiotics in the study location. However, MAR indices in this study have shown that the isolates originated from an environment where antibiotics are freely available and misused/abused. Hence, there is a need for greater monitoring of antibiotic supplies and use.

Keywords: Escherichia coli; urine; antimicrobial; resistance.

1. INTRODUCTION

Escherichia coli, a member of the bacteria family Enterobacteriaceae, is widely reported as one of the major causes of urinary tract infections (UTIs) in both outpatients and hospitalised patients [1,2]. Treatment of *E. coli* and other bacterial UTIs is by the use of antibiotics [3,4]. However, the antimicrobial resistances in this bacterium have posed a serious threat to their continued successful use in the management of UTIs [4,5,6,7]. Urinary tract infections due to multi drug resistant (MDR) *E. coli* increases the cost of treatment, morbidity and mortality especially in developing countries [8,9].

Antimicrobial resistance in *E. coli* from UTI patients has been reported globally [6,10,11,12, 13,14,15,16]. In Nigeria, several studies have reported increasing resistance [17,18,19,20,21, 22]. There is a dearth of information on *E. coli* resistance in the study area. This study thus investigated the antimicrobial resistance profile of *E. coli* from the urine of patients with suspected UTIs attending some major public general hospitals in the municipal area of Abuja, the Nigerian Federal Capital Territory. Monitoring resistance through the use of an antibiogram is important, to support clinical decision-making, public health and safety.

2. MATERIALS AND METHODS

2.1 Study Locations

The sampling locations for the study were Asokoro General Hospital (AGH), Garki Hospital Abuja (GHA) and Wuse General Hospital (WGH). These hospitals, located in Abuja Municipal, are the oldest public general hospitals in the city.

2.2 Sample Collection

A total of 430 early morning mid-stream urine samples from patients with suspected UTIs were collected between September 2017 and May 2018 using sterile container 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 was isolated from the urine samples of the patients using streak plate method. The urine sample was streaked on MacConkey agar (Oxoid Ltd., Basingstoke, UK) plate and incubated at 37℃ for 24 h. Pinkish colonies that grew on MacConkey agar were further streaked on Eosin Methylene Blue (EMB: Oxoid Ltd., Basingstoke, UK) agar and incubated at 37℃ for 24 h. Greenish metallic sheen colonies that grew on the EMB agar were selected as presumptive *E. coli.*

The presumptive *E. coli* was identified by microscopy (Gram staining) and the minimal biochemical tests for *E. coli* identification called 'IMViC' (Indole test, Methyl red Voges-Proskaeur, Citrate) as earlier described [23] and further identified using KB003HI25™ identification kit following the manufacturer's instructions.

2.4 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by CLSI [24]. Briefly, (3) distinct colonies of *E. coli* isolated from urine samples of patients were inoculated into 5 ml sterile 0.85% (w/v) NaCl (normal saline). Thereafter, the turbidity of the bacteria suspension will be 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) $BaCl_2.2H_2O$ (BDH Chemicals Ltd., England) was added into 99.5 ml of 1% (w/v) H_2SO_4 (BDH Chemicals Ltd., England).

A sterile swab stick was soaked in the standardised 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.) 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 by Clinical and Laboratory Standards Institute [24].

The Multiple Antibiotics Resistance (MAR) index was determined as described previously [25] using the formula:

$$\text{MAR Index} = \frac{\text{No. of antibiotics isolate is resistant to}}{\text{No. of antibiotics tested.}}$$

2.5 Classification of Antibiotic Resistance in the Isolates

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) [26].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Escherichia coli*

Table 1 shows the cultural, morphological and biochemical characteristics of *E. coli* isolated from urine of patients in the selected hospitals. Pinkish colonies on MacConkey agar that grew with greenish metallic sheen on EMB agar and were Gram negative rods with biochemical reactions namely: indole-positive, methyl red-positive, Voges-Proskauer-negative, citrate-negative, ONPG-positive, among others, indicated *E. coli*.

3.2 Occurrence of *Escherichia coli*

Out of 430 urine samples, the occurrence of *E. coli* isolates was 12.1% (52/430). The occurrences of the isolates in relation to selected hospitals are as given in Fig. 1. The order of occurrence in relation to selected hospitals was: GHA (14.7%) > WGH (12.6%) > AGH (9.0%).

The occurrence of *E. coli* isolates in relation to the age of patients is as shown in Fig. 2. The highest occurrence in AGH was observed at 21-39 years (25%) and the lowest at 31-40 years (4.3%). In GHA, the highest occurrence was observed at 31-40 years (26.3%) and the lowest at 21-30 years (10.0%). Similarly, in WGH, the highest occurrence was observed in 41-50 years (50%) and the lowest at 11-20 years (5.0%). The occurrences of *E. coli* from the urine of suspected UTIs patients at the selected hospital in relation to age were statistically insignificant (P=.05).

Gender-related occurrence of *E. coli* is as shown in Fig. 3. The occurrence was higher in females than males in all the selected hospitals. The order of occurrence observed in females in the selected hospitals was: WGH (13.6%) > GHA (12.2%) > AGH (11.0%) and the order of occurrence observed in males in the selected hospitals was: WGH (11.3%) > GHA (11.1%) > AGH (4.5%). The occurrences of *E. coli* isolates in relation to gender of patients in the selected general hospitals were statistically insignificant (P=.05).

3.3 Antibiotic Resistance of the Isolates

The antibiotic resistance of *E. coli* isolated from urine of the patients from the selected general hospitals is as shown in Table 2. In AGH, the isolates were more resistant to sulphamethoxazole/trimethoprim (100.0%), ampicillin and streptomycin (92.3%) and ceftaxidime (76.9%) but less resistant to gentamicin (15.3%) and imipenem (38.5%) and none was resistant to ciprofloxacin. In GHA, the isolates were more resistant to cefotaxime and streptomycin (95.2%) but less resistant to gentamicin and imipenem (23.8%).

Table 1. Cultural, Morphological and Biochemical characteristics of *Escherichia coli* isolated from patients with suspected urinary tract infections in selected general hospitals in Abuja Municipal, Nigeria

Cultural characteristics	Morphological characteristics		Biochemical Characteristics												Inference
	Gram reaction	Morphology	IND	MR	VP	CT	TDA	ONPG	LYS	ORN	UR	NT	H_2S	MAL	
Pinkish colonies on MCA and Greenish metallic sheen on EMB agar	-	Rod	+	+	-	-	-	+	+	+	-	+	-	-	*E. coli*

+ = 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_2S = Hydrogen Sulphide, Mal = Malonate, TDA = Phenylalanine deaminase

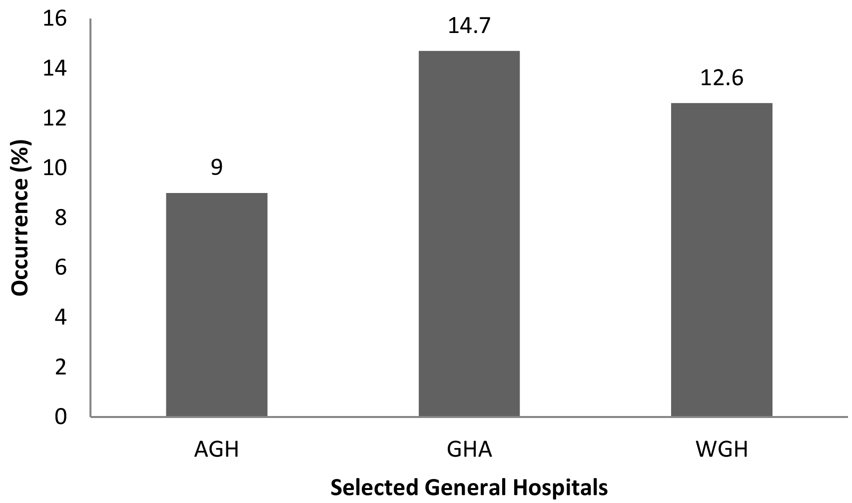

Fig. 1. Occurrence of *Escherichia coli* from urine of patients with suspected urinary tract infections in the selected hospitals in Abuja Municipal, Nigeria

(AGH= Asokoro General Hospital; GHA= Garki Hospital Abuja; WGH- Wuse General Hospital)

In WGH, the isolates were more resistant to ampicillin (88.8%) and ceftazidime (66.7%) but less resistant to amoxicilin (16.7%) and sulphamethoxazole/ trimethoprim and gentamicin (22.2%).

3.4 Antibiotic Resistance Phenotypes of the Isolates

The antibiotics resistance phenotypes of *E. coli* are as given in Table 3. The commonest phenotype in AGH was AMC-S-CTX-CAZ-IPM-AMP (3.9%); in GHA was AMC-S-SXT-CTX-CAZ-AMP (7.7%); and in WGH was AMC-S-SXT-CTX-CAZ-IPM-AMP (3.9%).

3.5 Multiple Antibiotic Resistance (MAR) Index

All the *E. coli* isolated were MAR isolates. The MAR indices are as shown in Table 4. All the MAR isolates had MAR indices of ≥ 0.2. The commonest index in AGH was 0.4 (at 30.8%); in GHA it was 0.7 (at 33.3%); and in WGH it was 0.7 (at 27.8%).

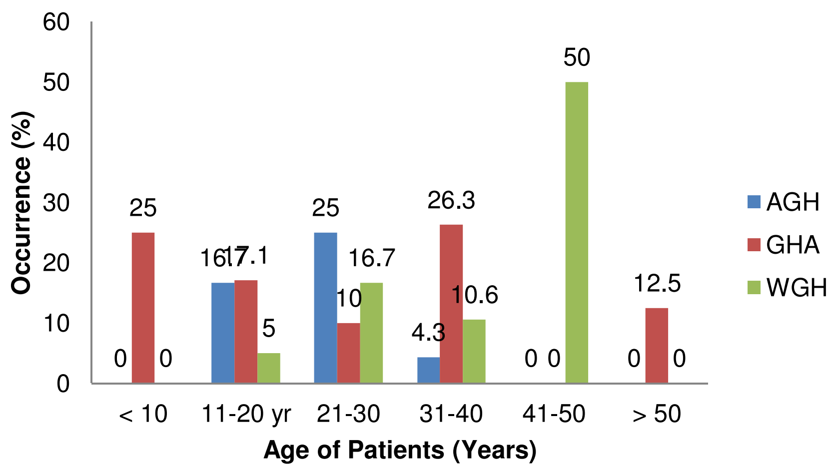

Fig. 2. Occurrence of *Escherichia coli* from urine in relation to age of patients with suspected urinary tract infections in selected general hospitals in Abuja Municipal, Nigeria
(AGH= Asokoro General Hospital; GHA= Garki Hospital Abuja; WGH= Wuse General Hospital).

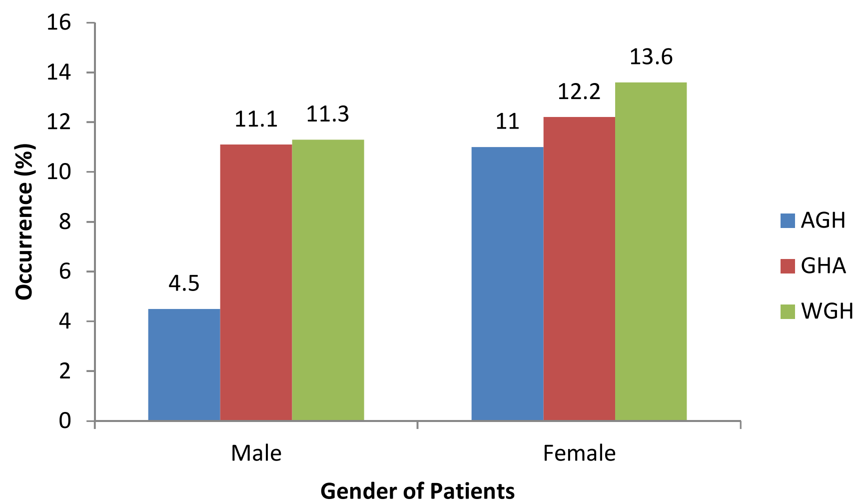

Fig. 3. Occurrence of Escherichia coli from urine to gender of patients with suspected urinary tract infections in the selected general hospitals in Abuja Municipal, Nigeria
(AGH= Asokoro General Hospital; GHA= Garki Hospital Abuja; WGH= Wuse General Hospital)

3.6 Classification of Antibiotic Resistance

The classification of *E. coli* isolates into multidrug resistance (MDR), non-multidrug resistance (NMDR), extensive drug resistance (XDR) and pan drug resistance (PDR) are as shown in Table 5. The order of occurrences of the resistant types was MDR: GHA (92.2%) > WGH (77.7%) > AGH (76.6%); XDR: GHA (19.0%) > AGH (7.7%) > WGH (0.00); PDR: GHA (4.8%) > AGH (0.00%) > WGH (0.00%) and NMDR: AGH (7.7%) > WGH (5.6%) > GHA (0.00%).

Escherichia coli is one of the most common organism associated with both hospital and community acquired infection [27,28]. From this study, we observed the lowest occurrence of *E. coli* from urine of suspected urinary tract infections patients at 12.1% (52/430) than the one reported in a similar research conducted in India which was 54.0% and 54.5% [29]; but higher than 1.2% reported in a study conducted in Norwa [30]. The occurrence of *E. coli* from urine of patients observed in this study suggests that the organism may likely be responsible for the urinary tract infection, since *E. coli* has widely been reported by researchers as the most common cause of urinary tract infections [31,32,33].

The occurrence of *E. coli* in urine of patients in relation to age was higher in age 21-30 years at AGH. This finding is in agreement with a study earlier reported [34]; but not with another study [19] which observed highest yield in neonate (46%) and the above 64 year old with 32.4%, while the age group of 25-32 years had the lowest. This may have been due to the fact that they are sexually active and may have likely been infected with uropathogenic *E. coli*.

The occurrence of *E. coli* in urine of patients in relation to gender was higher in female than male in this study was expected. This is in agreement with the study earlier reported [35,36], this might be due to the fact that the female rectum lies close to the urine outlet which allows the bacteria to enter the urinary tract easily. Also, the urethra is shorter than the males and thus the bacteria can quickly reach the bladder, thereby causing urinary tract infections [32].

The resistance of *E. coli* isolates to ciprofloxacin observed in this study was lower than 91.0% and 41.0% reported by Namrathan [29], Rai et al. [32], but was greater than 1.2% reported by Grude et al. [30]. The low resistance to ciprofloxacin observed in this study disagrees with a study reported by Olson et al. [10] that resistance to ciprofloxacin particularly common among women with a history of prior UTI (11.8%). The low resistance in our study could be that, ciprofloxacin may not have been misused or abused in the study location.

The occurrence of MDR isolates observed in this study was not different from the study described [15,36]. The percentage occurrences of MDR isolates were higher than 37.0% and 58.0% reported [15,36]. The isolation of MDR isolates from the urine of patients may have public health implication since MDR isolates have been reported to cause complicated UTIs which are difficult to treat, in addition to being responsible for increased cost of treatment, morbidity and mortality [8,9,15,36].

Table 2. Antibiotic resistance of *Escherichia coli* isolated from urine of patients with suspected urinary tract infections in selected general hospitals at Abuja Municipal, Nigeria

Antibiotics	Disc content (µg)	No. (%) Resistance			Total
		AGH n =13	GHA n =21	WGH n =18	
AMC	30	7(52.8)	13(61.9)	3(16.7)	23(44.2)
AMP	30	12(92.3)	20(55.2)	16(88.8)	48(92.3)
CTX	30	10(76.9)	20(95.2)	5(27.5)	25(67.3)
CAZ	30	8(61.50	8(38.1)	12(66.7)	28(53.8)
CIP	5	0(0.0)	8(38.1)	0(0.0)	8(15.4)
FOX	30	12(92.3)	10(47.6)	10(55.6)	32(61.5)
CN	10	2(15.3)	5(23.8)	4(22.2)	11(21.2)
IPM	30	5(38.5)	5(23.8)	6(33.3)	16(30.8)
S	30	12(92.3)	20(95.2)	11(61.1)	43(82.7)
SXT	25	13(100.0)	20(95.2)	4(22.2)	37(71.2)

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

Table 3. Antibiotics resistance phenotypes of *Escherichia coli* isolated from urine of patients with suspected urinary tract infections in selected general hospitals in Abuja Municipal, Nigeria

Antibiotic resistance phenotypes	No. (%) *E. coli* isolates		
	AGH	GHA	WGH
AMC,AMP	1(1.9)	0(0.0)	0(0.0)
CTX, AMP	0(0.0)	1(1.9)	0(0.0)
S,SXT,AMP	0(0.0)	1(1.9)	0(0.0)
AMC,CTX,AMP	0(0.0)	0(0.0)	1(1.9)
CAZ,CTX,FOX	0(0.0)	0(0.0)	1(1.9)
S,FOX,CN	0(0.0)	1(1.9)	0(0.0)
SXT,CTX,CAZ,FOX	1(1.9)	0(0.0)	0(0.0)
S,SXT,CTX,AMP	2(3.9)	1(1.9)	1(1.9)
S,CTX,CAZ,FOX	0(0.0)	1(1.9)	0(0.0)
AMC,S,CTX,AMP	1(1.9)	0(0.0)	0(0.0)
S,CTX,FOX,AMP	0(0.0)	1(1.9)	0(0.0)
S,FOX,CN,CIP,AMP	0(0.0)	1(1.9)	0(0.0)
AMC,CAZ,FOX,IPM,AMP	1(1.9)	2(3.9)	1(1.9)
S,SXT,CAZ,FOX,AMP	0(0.0)	2(3.9)	0(0.0)
AMC,SXT,CTX,CAZ,AMP	1(1.9)	0(0.0)	0(0.0)
S,CTX,CAZ,FOX,CIP,AMP	0(0.0)	0(0.0)	1(1.9)
S,SXT,CTX,CAZ,CIP,AMP	0(0.0)	0(0.0)	1(1.9)
S,SXT,CTX,CN,CIP,AMP	1(1.9)	0(0.0)	0(0.0)
AMC,S,SXT,CTX,CAZ,AMP	1(1.9)	4(7.7)	1(1.9)
S,SXT,CTX,CAZ,FOX,CIP,AMP	1(1.9)	0(0.0)	0(0.0)
S,SXT,CTX,CAZ,FOX,IPM,AMP	1(1.9)	0(0.0)	0(0.0)
AMC,S,SXT,FOX,CN,CIP,AMP	0(0.0)	1(1.9)	0(0.0)
AMC,S,SXT,CTX,CAZ,FOX,AMP	1(1.9)	1(1.9)	0(0.0)
S,SXT,CTX,CAZ,CN,IPM,AMP	1(1.9)	0(0.0)	0(0.0)
AMC,S,SXT,CTX,CAZ,IPM,AMP	2(3.9)	4(7.7)	2(3.9)
AMC,S,SXT,CTX,CAZ,FOX,CIP,AMP	0(0.0)	1(1.9)	0(0.0)
AMC,S,SXT,CTX,CAZ,FOX,CN,AMP	0(0.0)	1(1.9)	0(0.0)
AMC,S,SXT,CTX,CAZ,FOX,IPM,AMP	0(0.0)	0(0.0)	1(1.9)
S,SXT,CTX,CAZ,FOX,CN,CIP,AMP	0(0.0)	0(0.0)	1(1.9)
AMC,S,SXT,CTX,CAZ,FOX,IPM,AMP	1(1.9)	0(0.0)	0(0.0)
AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP	0(0.0)	1(1.9)	0(0.0)

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

Table 4. Multiple antibiotic resistance index of *Escherichia coli* isolated from urine of patients with suspected urinary tract infections in selected general hospitals in Abuja Municipal, Nigeria

No of antibiotics Resistance (a)	No of antibiotics tested (b)	MAR Index (a/b)	No. (%) MAR isolates		
			AGH (n =13)	GHA (n=21)	WGH (n=18)
10	10	1.0	0(0.0)	1(4.8)	0(0.0)
9	10	0.9	0(0.0)	1(4.8)	0(0.0)
8	10	0.8	1(7.8)	3(14.3)	1(5.6)
7	10	0.7	2(15.4)	7(33.3)	5(27.8)
6	10	0.6	1(7.8)	5(23.8)	3(16.7)
5	10	0.5	2(15.4)	4(19.0)	2(11.1)
4	10	0.4	4(30.8)	0(0.0)	4(22.2)
3	10	0.3	3(23.1)	0(0.0)	0(0.0)
2	10	0.2	0(0.0)	0(0.0)	3(16.7)

AGH= Asokoro General Hospital; GHA= Garki Hospital Abuja; WGH= Wuse General Hospital; No. = Number

Table 5. Classification of antibiotics resistance in the *Escherichia coli* from urine of patients with suspected urinary tract infections in selected general hospitals in Abuja Municipal, Nigeria

Classes of antimicrobial resistance	No. (%) *E. coli*			Total (%)
	AGH (n=13)	GHA (n=21)	WGH (n=18)	
MDR	10(76.6)	20(95.2)	14(77.7)	**44(84.6)**
XDR	1(7.7)	4(19.0)	0(0.0)	**5(9.6)**
PDR	0(0.0)	1(4.8)	0(0.0)	**1(1.9)**
NMDR	1(7.7)	0(0.0)	1(5.6)	**2(3.8)**

NMDR = Non-multi-drug resistance; MDR = Multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR = Extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR=Pan drug resistance (non-susceptible to all antimicrobial listed) [6]

4. CONCLUSION

Escherichia coli is one of the bacteria associated with cases of urinary tract infections in the selected general hospitals. The isolates showed high resistance to commonly available antimicrobial agents; but very low resistance to ciprofloxacin. All isolates originated from an environment where antimicrobials are freely available and misused or abused. Most isolates in all the selected hospitals are multidrug resistant strains and this may likely lead to treatment failures, especially in community and hospital set ups.

CONSENT

The consent of the patients whose urine were sampled was sought.

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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