Original Research Article

Antibiotic Resistance Profile of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infections in Federal Medical Centre, Keffi, Nigeria

.ABSTRACT

Aims: This study investigated the antibiotic resistance profile of *Escherichia coli* from the urine of patients with suspected urinary tract infections in Federal Medical Centre, Keffi, Nigeria.
Study design: Cross-sectional study.
Place and Duration of Study: Sample was obtained from the Federal Medical Center, Keffi and analyzed at Nasarawa State University, Keffi, Nigeria, between January and April 2018.
Methodology: Three hundred and eighty urine samples were collected and *E. coli* was isolated and identified using standard microbiological methods. Antimicrobial Susceptibility Testing for the isolates was carried out and interpreted as described by the Clinical and Laboratory Standards Institute.
Results: The occurrence of the bacterium was 12.9% (49/380). The occurrence in relation to the gender of the patients was higher in the female (15.5%) than the male (9.8%); in relation to age, it was highest at 11-20 years (23.5%) but lowest at > 50 years (2.3%). The isolates were more resistant to ampicillin (81.6%), streptomycin and sulphamethoxazole/ trimethoprime (75.0%) but less resistant to gentamycin (30.6%), and imipenem (22.4%). The occurrences of different classes of resistance were multidrug resistance (MDR) (93.9%) and pan drug resistance (4.2%). Most of the isolates were more resistant to the commonly prescribed antibiotic and were also MDR isolates.
Conclusion: The need to review antibiotic use by the hospital is thus justified.

Keywords: Escherichia coli; urine, antibiotic; resistance

1. INTRODUCTION

Escherichia coli, a member of the Enterobacteriaceae family, has been reported to be one of the most predominant organisms causing urinary tract infections (UTIs) which are very common reasons for consultation and antibiotic prescription in current practice [1]. Urinary tract infection (UTI) is one of the most frequent types of nosocomial infections and probably affects nearly one-half of all people during their lifetimes [1,2]. Antibiotics such as β-lactams and fluoroquinolones as well as other classes are commonly prescribed for treatment of *E. coli* related UTIs [3, 4].

Massive and usually inappropriate use of antibiotics for treatment of UTIs generates a selective pressure that is followed by the rapid emergence and spread of multi-drug resistant bacterial strains [3,4,5]. Nowadays, the resistance of urinary *E. coli* to many antibiotic classes is a very common finding in human medicine and is usually associated with increased medical costs, prolonged hospital stays and frequent therapeutic failure [5].

In addition, several studies showed that antibiotic resistance in *E. coli* related UTIs is increased [2,6]. The emergence of transferable multidrug resistance genes in gram-negative bacteria, particularly *E. coli* is an important health problem throughout the world [2,3,7-10].

Many reports have described and characterized ~~antibiotic~~ antibiotic-resistant urinary *E. coli* isolates in worldwide [11-15] but in the study location this report is limited, hence this study investigates antibiotic resistance profile of *E. coli* from the urine of Patients with suspected UTIs in Federal Medical Centre, Keffi, Nigeria.

2. MATERIAL AND METHODS

2.1 Isolation of *Escherichia coli*

Escherichia coli was isolated from urine samples as follows: a loopful of urine sample was streaked on MacConkey Agar (Oxoid Ltd. U.K.) plate and incubated at 37°C for 24 h. Pinkish colonies that grew on MacConkey agar were further streaked on Eosin Methylene Blue Agar (Oxoid Ltd. U.K.) 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.*

2.1.1 Identification of *Escherichia coli*

The presumptive *E. coli* was Gram-stained~~,~~ and biochemically identified as suspected *E. coli* using IMViC (Indole, Methyl red, Voges-Proskauer and Citrate) tests as earlier described [16]. The suspected *E. coli* isolates (~~Gram~~ Gram-negative, rod shape, indole positive, methyl red positive, citrate negative and Voges-Proskauer negative) were using a commercial biochemical testing kit (KB003 H125TM) following the manufacturer's instruction.

2.2 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by the Clinical and Laboratory Standards Institute [17]. Briefly, three (3) pure colonies of the isolates were inoculated in~~-~~to 5 ml sterile 0.85% (w/v) NaCl (BDH chemical Ltd, England) (normal saline) and 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 chemical Ltd, England) was added into 99.5 ml of 1% (w/v) H_2SO_4 (BDH chemical Ltd, England).

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar (Oxoid Ltd. U.K.) plates and the antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility break-point earlier described by Clinical and Laboratory Standards Institute [17].

2.3 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 previously [18].

2.4 Classification of Antibiotic Resistance

Antibiotic resistance in the isolates ~~were~~ was 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) [19].

Comment [DM1]: Statistical analysis is missing

3. RESULTS

Table 1: Cultural, Morphological and Biochemical characteristics *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infection in Federal Medical Centre, Keffi, Nigeria

Cultural Characteristics	Morphological Characteristics		Biochemical Characteristics											Inference
	Gram stain	Morphology	ONPG	Ornithine	UR	LYS	NT	H_2S	CT	TDA	VP	MR	IND	MAL	
Pinkish colony on MCA and greenish metallic sheen colony EMB agar	-	rod	+	+	-	+	+	-	-	-	-	+	+	_	*E. coli*

MCA = MacConkey agar; EMB = Eosin methylene blue; UR = Urease; LYS = Lysine; H_2S = Hydrogen Sulphide; CT = Citrate; TDA = Phenylalanine deaminase; VP = Voges-Proskauer; IND = Indole; MAL = Malonate; - = Negative; + = Positive

Table 2: Occurrence of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infection in Federal Medical Centre, Keffi, Nigeria in Relation to Gender

Gender	No. of Samples	No. (%) *E. coli*
Male	173	17(9.8)
Female	207	32(15.5)
Total	380	49(12.9)

Table 3: Occurrence of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infection in Federal Medical Centre, Keffi, Nigeria in Relation to age

Age	No. of Samples	No. (%) *E. coli*
≤10	23	9(13.0)
11-20	51	12(23.5)
21-30	94	8(8.5)
31-40	106	20(18.7)
41-50	62	5(8.1)
>50	44	1(2.3)
Total	380	49(12.7)

Table 4: Antibiotic Resistance of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infection in Federal Medical Centre, Keffi, Nigeria

Antibiotics	Disc Content (µg)	No. (%) Resistance (n=49)
Amoxicillin/clavulanic acid (AMC)	30	20(40.8)
Ampicillin (AMP)	30	40(81.6)
Ceftazidime (CAZ)	30	23(46.9)
Cefotaxime (CTX)	30	28(57.1)
Cefoxitin (FOX)	30	26(53.1)
Ciprofloxacin (CIP)	5	28(57.1)
Gentamicin (CN)	10	15(30.6)
Imipenem (IPM)	30	11(22.4)
Streptomycin (S)	30	37(75.5)
Sulphamethoxazole/trimethoprim	25	37(75.5)

Table 5: Antibiotic Resistant Phenotypes of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infection in Federal Medical Centre, Keffi, Nigeria

Antibiotic Resistance Phenotypes	Frequency (%) (n=49)
FOX	1(2.0)
SXT-FOX-IPM	1(2.0)
SXT-FOX-AMP	1(2.0)
S-FOX-AMP	1(2.0)
S-SXT-AMP	1(2.0)
S-SXT-FOX-AMP	2(4.1)
S-CAZ-FOX-AMP	1(2.0)
S-SXT-CIP-AMP	1(2.0)
S-SXT-CTX-AMP	1(2.0)
AMC-S-CTX-FOX-AMP	1(2.0)
S-SXT-CTX-CAZ-FOX	1(2.0)
S-SXT-CAZ-FOX-AMP	1(2.0)
SXT-FOX-CN-CIP-AMP	1(2.0)
AMC-S-SXT-CIP-AMP	2(4.1)
S-SXT-CN-CIP-AMP	1(2.0)
S-SXT-FOX-UPM-CIP-AMP	1(2.0)
AMC-S-SXT-CTX-FOX-AMP	1(2.0)
S-SXT-CTX-FOX-CIP-AMP	1(2.0)
S-SXT-CTX-CN-CIP-AMP	1(2.0)
S-SXT-CTX-CAZ-CN-AMP	1(2.0)
S-SXT-CTX-CAZ-CIP-AMP	2(4.1)
S-CTX-CAZ-FOX-CN-IMP-AMP	1(2.0)

S-SXT-CTX-CAZ-FOX-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-AMP	1(2.0)
AMC- S-SXT-CTX-CN-CIP-AMP	1(2.0)
S-SXT-CTX-CAZ-CN-CIP-AMP	2(4.1)
AMC- S-SXT-CTX-CAZ-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-CN-CIP-AMP	4(8.2)
AMC-S-CTX-CAZ-FOX-CN-CIP-AMP	1(2.0)
S-SXT-CTX-FOX-CN-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-CN-CIP-AMP	2(4.1)
AMC-SXT-CTX-CAZ-CN-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-CN-IPM-CIP-AMP	2(4.1)

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

Table 6: Multiple Antibiotic Resistance (MAR) Index of *Escherichia coli* isolated from Urine of Patients with Suspected Urinary Tract Infections in Federal Medical Centre, Keffi, Nigeria

No. of Antibiotics Resistance (a)	No. of Antibiotic tested (b)	MAR Index (a/b)	Frequency (%)
10	10	1.0	2(4.7)
9	10	0.9	1(2.39
8	10	0.8	9(20.9)
7	10	0.7	7(16.3)
6	10	0.6	7(16.3)
5	10	0.5	7(16.3)
4	10	0.4	5(11.6)
3	10	0.3	4(9.3)
2	10	0.2	0(0)
1	10	0.1	1(2.3)

Table 7: Categories of Antibiotic Resistance in *Escherichia coli* isolated from Urine of Patients with Suspected Urinary Tract Infection in Federal Medical Centre, Keffi, Nigeria

Categories of Antibiotic Resistance	Frequency (%) (n=50)
NMDR	1(2.0)
MDR	46(93.9)
XDR	0(0.0)
PDR	2(4.7)

NMDR=None Multi-drug resistance; MDR=Multi-drug resistance; XDR=Extensive drug resistance; PDR=Pandrug resistance

4. DISCUSSION

The occurrence of *E. coli* from the urine of suspected UTIs patients in the study center was an indication that the organism may be responsible for UTIs and this finding agrees with the study ~~earliar~~earlier reported by Elboumri *et al.* [1] and Tajbahsh *et al*. [1] that *E. coli* ~~in~~ is the most frequent uropathogen that causes UTIs nearly one half of all people during their lifetimes.

This occurrence of *E. coli* from the urine of suspected UTIs patients was in higher in female than male patients in both study centers and this finding, however, is not different from the study earlier described by Shakya *et al*. [14]. The percentage occurrence of *E. coli* from urine of suspected female and male UTIs patients was lower than 78.9% and 21.1% reported by Shakya *et al*. [14].

The high occurrence of *E. coli* in female ~~–~~than male patients may be due to anatomical differences, hormonal effect, and behavioral patterns [14]. Although, our findings also show the difference ~~on~~ in the occurrence of *E. coli* in relation to the gender of suspected UTIs patients in the study center were statistically insignificance and this however shows that gender of an individual may not necessarily be a predisposing factor for UTIs caused by *E. coli.*

The occurrence of *E. coli* from the urine of suspected UTIs patients in this study was not in agreement with the study earlier described by Shakya *et al*. [14]. The high occurrence of *E. coli* in 11-20years of patients in Federal Medical Center Keffi may be due to behavioral pattern of individuals especially their level of hygiene in this age group may be low,

although Shakya *et al*. [14] reported high occurrence of *E. coli* in ~~age~~ the group; 21-30 (26.0%) and also shown that the high occurrence may be due to fact that individual at this age group are sexually active and may be more prone to UTIs.

The resistance of the isolates from both study center to ampicillin, streptomycin, sulphamethoxazole/trimethoprim, cefotaxime, ceftazidime, and cefoxitin observed in this study was not surprising and this finding agrees with the study earlier reported by Polse *et al.* [20], Padilla *et al*. [21] and Alikhani *et al*. [22]. The percentage resistance of isolates both in the study center to ampicillin was less than 100% and 90 % reported by Polse *et al*. [20] and Shakya *et al*. [14]. The resistance of the isolates to cefotaxime and ceftazidime was less than 82.4% reported by Padilla *et al*. [21]. The resistance of isolates to antibiotic mentioned may be due to antibiotic misuses, ineffective empiric antibiotic therapy, poor dosing regimen of antimicrobial agent, and prolong therapy of infection caused by this organism may also likely being the reason for the resistance of antibiotic mentioned [23].

The low resistance of the isolates from both study center to antibiotics such as gentamicin, imipenem, amoxicillin/clavulanic and ciprofloxacin was expected and this finding also ~~justify~~ justifies their use for the treatment of infection caused by ~~gram~~ Gram-negative bacteria. The percentage resistance of the isolates to gentamicin, and imipenem was less than 10.6% and 13.9% and higher 38.08% resistance to ciprofloxacin as earlier reported by Shakya *et al*. [14].

The occurrence of MAR isolates observed in this study was similar ~~with~~ to the study earlier reported by Ngwai *et al*. [18] and Nkene *et al*. [12]. The occurrence of the MAR isolates in the study location was an indication that the isolates may be more common in the environment where the antibiotics are likely misused [18].

The occurrence of MDR resistance isolates in the study location was expected and this finding is also not different from the study earlier reported by Thakur *et al*. [24] and Parajuli *et al*. [25] that MDR *E. coli* responsible for UTIs that is difficult to be treated using antibiotics. The percentage occurrence of MDR isolates observed in this study was higher than 64.9% reported by Parajuli *et al*. [25]. The occurrence of XDR and PDR resisting isolated observed in this study was also similar ~~with~~ to the study earlier described by Parajuli *et al*. [25]. Most of the isolate ~~were~~ was more resistance to commonly prescribed antibiotic and were also MDR isolates. Further studies on molecular characterization of β-lactam fluoroqunolones resistance in the isolates ~~is~~ are ongoing.

5. CONCLUSION

This study recovered 12.9% (49/380) *E. coli* from the urine of patients. The occurrence in relation to the gender of the patients was higher in the female (15.5%) than the male (9.8%); in relation to age, it was highest at 11-20 years (23.5%) but lowest at > 50 years (2.3%). The isolates were more resistant to ampicillin (81.6%), streptomycin and sulphamethoxazole/ trimethoprime (75.0%) but less resistant to gentamycin (30.6%), and imipenem (22.4%). The occurrences of different classes of resistance were multidrug resistance (MDR) (93.9%) and pan drug resistance (4.2%). Most of the isolates were more resistant to the commonly prescribed antibiotic and were also MDR isolates. The need to review antibiotic use by the hospital is thus justified.

Comment [DM2]: A future perspective is required for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this case report.

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.

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