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(RESEARCH ARTICLE)

Antibiotic resistance in *Escherichia coli* from urine of patients with suspected urinary tract infections accessing Dalhatu Araf Specialist Hospital, Lafia, Nigeria

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Abstract

The wide use of antibiotics to treat urinary tract infection (UTIs) caused by bacteria is compromised by the development of resistance mechanisms in bacteria. This study evaluated the antibiotic resistance in *Escherichia coli* from urine of patients with suspected UTI in Dalhatu Araf Specialist Hospital, Lafia, Nigeria. Four hundred urine samples of suspected UTI patients 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 (CLSI). The occurrence of the bacterium was 14.5 % (58/400). The occurrence was higher in female (15.8 %) than the male (12.5 %); and highest at > 50 years (31.8 %). The isolates were more resistant to ampicillin (79.3 %), streptomycin (62.0 %) and cefoxitin (58.6 %) but less resistant to gentamicin (15.8 %), imipenem (18.9 %) and amoxicillin/clavulanic acid (27.6 %). The occurrence of multi-drug resistance (MDR) isolates was 87.9%. The antibiotics namely gentamicin, imipenem and amoxicillin/clavulanic acid were very effective against the isolates and most of the isolates were MDR. There is thus a need for the hospital to limit its antibiotic use in the light of these observations.

Keywords: *Escherichia coli*; Urine; Antibiotics; Resistance

1. Introduction

Escherichia coli are one of the most common clinically relevant and multi-drugs resistant (MDR) bacteria causing both community and hospital acquired Urinary Tract Infection (UTIs) [1]. The UTIs are one of the most common infections diagnosed in hospital setting especially in developing countries [2]. Antibiotics have been successfully used for treatment of UTIs cause by member of family Enterobacteriaceae especially *E. coli* [2] but emergence of strains of the isolates resistance to commonly prescribed antibiotics have been reported worldwide [2,3,4,5,6] and this however have continued to be a threat to public health due to management of this infection caused by MDR strains [2].

The emergence of antibiotic resistant strains of *E. coli* causing UTIs have been reported in several studies worldwide [7, 8]. The MDR strains of this isolates causing UTIs have increase the cost of therapy, morbidity and mortality especially in developing countries [9], hence there is a need for continue surveillance of antibiotic resistant strains of bacteria causing UTIs especially in third world countries for proper management to reduce the risk of morbidity, mortality and economic loss to the patients and nation. This study however investigates the antibiotic resistance in *E. coli* from urine of patients with suspected UTIs in Dalhatu Araf Specialist Hospital Lafia, Nigeria.

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2. Material and methods

2.1. Study location and sample collection

The study location was the Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. A total of four hundred (400) early morning mid-stream urine samples of patients with suspected UTIs attending the health facility were collected using sterile container and transported using ice pack to the Microbiology Laboratory at Nasarawa State University, Keffi for analysis.

2.2. Isolation of *Escherichia coli*

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

2.3. 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 [10]. The suspected E. coli isolates (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.

2.4. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute [11]. Briefly, three (3) pure colonies of the isolates were inoculated in to 5 ml sterile 0.85 % (w/v) NaCl (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) $BaCl_2.2H_2O$ was added into 99.5 ml of 1 % (w/v) H_2SO_4.

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic discs were aseptically placed at the centre 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 millimetre was measured and the result was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute [11].

2.5. Determination of multiple antibiotics 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 [12].

2.6. Classification of antibiotics 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) [13].

3. Results

Table 1 Cultural, Morphological and Biochemical characteristics *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infection in Dalhatu Araf Specialist Hospital, Lafia, Nigeria

Cultural Characteristics		Pinkish colony on MCA and greenish metallic sheen colony EMB agar
Morphological Characteristics	Gram stain	-
	Morphology	rod
Biochemical Characteristics	ONPG	+
	Ornithine	+
	UR	-
	LYS	+
	NT	+
	H_2S	-
	CT	-
	TDA	-
	VP	-
	MR	+
	IND	+
	MAL	_
Inference		*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 Dalhatu Araf Specialist Hospital, Lafia, Nigeria in Relation to Gender

Gender	No. of Samples	No. (%) *E. coli*
Male	160	20(12.5)
Female	240	38(15.8)
Total	400	58(14.5)

Table 3 Occurrence of Escherichia coli from Urine of Patients with Suspected Urinary Tract Infection in Dalhatu Araf Specialist Hospital, Lafia, Nigeria in Relation to Gender in Relation to age

Age	No. of Samples	No. (%) *E. coli*
≤10	48	10(20.8)
11-20	81	18(22.2)
21-30	72	6(8.3)
31-40	127	13(10.2)
41-50	50	4(8.0)
>50	22	7(31.8)
Total	400	58(14.5)

Table 4 Antibiotic Resistance of Escherichia coli from Urine of Patients with Suspected Urinary Tract Infection in Dalhatu Araf Specialist Hospital, Lafia, Nigeria

Antibiotics	Disc Content (µg)	No. (%) Resistance (n=58)
Amoxicillin/clavulanic acid (AMC)	30	16(27.6)
Ampicillin (AMP)	30	46(79.3)
Ceftazidime (CAZ)	30	30(51.7)
Cefotaxime (CTX)	30	33(56.9)
Cefoxitin (FOX)	30	34(58.6)
Ciprofloxacin (CIP)	5	29(50.0)
Gentamicin (CN)	10	9(15.8)
Imipenem (IPM)	30	11(18.9)
Streptomycin (S)	30	36(62.1)
Sulphamethoxazole/trimethoprim (SXT)	25	34(58.6)

Table 5 Antibiotic Resistant Phenotypes of Escherichia coli from Urine of Patients with Suspected Urinary Tract Infection in Dalhatu Araf Specialist Hospital, Lafia, Nigeria

Antibiotic Resistance Phenotypes	DASHL (n=58)
AMP	1(1.7)
FOX-IMP	1(1.7)
SXT-AMP	1(1.7)
FOX-AMP	2(3.4)
S-AMP	1(1.7)
SXT-FOX-AMP	1(1.7)
S-FOX-AMP	1(1.7)
S-SXT-AMP	1(1.7)
CIP-SXT-AMP	1(1.7)
S-SXT-FOX-AMP	3(5.2)
CTX-CAZ-FOX-CIP	1(1.7)
S-FOX-IPM-AMP	1(1.7)
CTX-CAZ-CIP-AMP	1(1.7)
SXT-FOX-CN-CIP-AMP	1(1.7)
CTX-CAZ-FOX-CIP-AMP	1(1.7)
AMC-S-CTX-CAZ-SXT	1(1.7)
CTX-CAZ-CIP-SXT-AMP	1(1.7)
S-CTX-CAZ-FOX-AMP	1(1.7)
AMC-CTX-CAZ-SXT-AMP	1(1.7)
AMC-S-CTX-CN-AMP	1(1.7)
S-SXT-FOX-UPM-CIP-AMP	1(1.7)
AMC-S-SXT-CTX-FOX-AMP	1(1.7)
S-SXT-CTX-FOX-CIP-AMP	1(1.7)
S-SXT-CTX-CAZ-CIP-AMP	8(13.8)
CTX-CAZ-FOX-CIP-SXT	1(1.7)
AMC-S-CTX-FOX-IPM-AMP	1(1.7)
S-SXT-CTX-CAZ-FOX-CIP	1(1.7)
S-SXT-CAZ-FOX-CIP-AMP	1(1.7)
S-CTX-CAZ-FOX-CIP-AMP	1(1.7)
AMC-S-SXT-CTX-CAZ-CN-AMP	1(1.7)
AMC-S-SXTR-CTX-CAZ-CIP-AMP	1(1.7)
S-CTX-CAZ-FOX-CN-IMP-AMP	1(1.7)
S-SXT-CTX-CAZ-FOX-CIP-AMP	2(3.4)
AMC- S-SXT-CTX-CAZ-FOX-CN-CIP-AMP	5(8.6)
AMC-SXT-CTX-CAZ-FOX-IPM-CIP-AMP	1(1.7)
AMC-S-CTX-CAZ-FOX- IPM-CIP-AMP	1(1.7)
AMC- S-SXT-CTX-CAZ-FOX-CN-CIP-AMP	2(3.4)
S-SXT-CTX-CAZ-FOX-CN-IPM-CIP-AMP	4(6.9)
AMC- S-SXT-CTX-CAZ-FOX-CN-IPM-CIP-AMP	1(1.7)

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 Infection in Dalhatu Araf Specialist Hospital, Lafia, Nigeria

No. of Antibiotics Resistance (a)	No. of Antibiotic tested (b)	MAR Index (a/b)	Frequency (%) (n=58)
10	10	1.0	1(2.0)
9	10	0.9	4(8.0)
8	10	0.8	5(10.0)
7	10	0.7	5(10.0)
6	10	0.6	12(24.0)
5	10	0.5	7(14.0)
4	10	0.4	6(12.0)
3	10	0.3	4(8.0)
2	10	0.2	5(10.0)
1	10	0.1	1(2.0)

Table 7 Classes of Antibiotic Resistance in Escherichia coli isolated from Urine of Patients with Suspected Urinary Tract Infection in Dalhatu Araf Specialist Hospital, Lafia, Nigeria

Categories of Antibiotic Resistance	Frequency (%) (n=58)
NMDR	1(1.7)
MDR	51(87.9)
XDR	5(8.6)
PDR	1(1.7)

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

4. Discussion

The isolation of *E. coli* from urine of suspected UTIs patients in the study centre was expected and this agree with the study earlier reported by Rami'rez-Castillo *et al*. [14] and El-Boumri *et al*. [15], that *E. coli* is one of the is the most common agent of UTIs. The percentage occurrence of the isolates in the study centre was similar with the studies earlier reported by El-Boumri *et al*. [15] in India, Eko *et al*. [16] in Keffi, Nigeria, Eghieye *et al*. [2] and Rami'rez-Castillo *et al*. [14] in Mexico. The occurrence of the isolates was higher than 12.1 % reported by Eghieye *et al*. [2] but less than 54.0 % reported by El-Boumri *et al*. [15].

The occurrence of the isolates in relation to the age of suspected UTIs patients was high in > 50 years and these findings is not in agreement with the study earlier reported by Eghieye *et al*. [2] and Rami'rez-Castillo *et al*. [14] who reported high occurrence of the isolates in 41-50 years (50.0 %) and 2-10 years (29.0 %). The high percentage of the isolates in > 50 years may be due to the fact that individual at that age grouped may have low immune status and may be more prone to UTIs.

The high percentage occurrence of the isolates of the isolates in female than male was expected and these findings agree with the study earlier described by Eghieye *et al*. [2], Rami'rez-Castillo *et al*. [14] and Giwa *et al*. [17]. The high occurrence of the isolates in female than the male counterpart may be due to differences in the anatomy of the reproductive organ where female have short reproductive organ than the male counterpart and this may therefore enables the female to be more prone to UTIs (2), although the percentage occurrence of the isolates in relation to gender was statistically insignificant and this implies that gender may not necessarily be a factor for occurrence of the isolates.

Our findings in this study shows that isolates were more resistance to ampicillin, ceftazidime, cefotaxime, cefoxitin, streptomycin and sulphamethoxazole/trimethoprim and this is not different from the study earlier described by Eko

et al. [16] and Eghieye *et al*. [2]. The percentage resistance of the isolates to the antibiotics mentioned was less than 93.0 %, 60.5 %, 53.7 %, 72.1 %, 56.0 % and 86.0 % resistance to ampicillin, ceftazidime, cefotaxime, cefoxitin, streptomycin and sulphamethoxazole/trimethoprim as earlier reported by Eko *et al*. (2018). The resistance of the isolates to antibiotic mentioned may be due to inappropriate use of the antibiotics during therapy of the infection caused by the isolates.

The occurrence of multi-drug resistance isolates in urine of patients with suspected UTIs suggest that the isolates may likely be responsible for UTIs that is difficult to treated since MDR isolates are responsible for life threatening UTIs that is difficult to be treated [18]. The percentage occurrence of the MDR isolates higher than 56.1 % and 64.9 % reported by Parajuli *et al*. [18] and Hashemizadeh *et al*. [19] but less than 93.5 % and 84.6 % reported by Eko *et al*. [16] and Eghieye *et al*. [2].

5. Conclusion

The occurrence of *E. coli* in the urine of suspected UTIs patients was low. The isolates were least resistant to the antibiotics and the antibiotics namely amoxicillin/clavulanic acid, imipenem and gentamicin, and may be useful for empirical treatment of UTIs. In addition, most of the isolates were MDR.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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