

Tetracycline Resistant Genes in *E. coli* Isolated from UTI and Diarrhea Patients in Zaria, Nigeria

Igwe JC^{1*}, Musa A², Olayinka BO³, Ehnimidu JO³ and Onaolapo JA³

¹Department of Medical Biotechnology, National Biotechnology Development Agency, Abuja, Nigeria

²Department of Water Sanitation and Hygiene, Dutse Local Government, Jigawa State, Nigeria

³Department of Pharmaceuticals and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria

*Corresponding author: Igwe JC, Department of Medical Biotechnology, National Biotechnology Development Agency, Abuja, Nigeria, Tel: +234-8069430222; E-mail: igwejames42@yahoo.com

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Abstract

Tetracycline (TC) is one of the widely used antibiotics for the treatment of infections with significant therapeutic effect due to its broad spectrum. But due to the emergence of high percentages of tetracycline resistance and the recent reoccurrence of multidrug resistance isolates in clinical settings, its use in hospitals have drastically reduced. This study evaluates the percentage of TC resistant in clinical isolates of *E. coli* from UTI and diarrhea patients in Zaria, Nigeria. Out of the 86 *E. coli* isolates collected from 4 hospitals for the period of 6 months (April-September, 2014), 68.6% (59) were observed to be resistant to TC using both disc diffusion and MIC (range of ≥ 4 $\mu\text{g/ml}$) methods. The antibiotic susceptibility profile of the isolates showed that the isolates had varied antibiotic resistant profile to the 14 antibiotics tested. Significant percentage (35.6% (21)) of the isolates also exhibited simultaneous resistance to Ciprofloxacin, Gentamicin and Amoxicillin. The isolates were also observed to have high MARI, and there molecular analysis showed that 95% (20) of the MDR isolates had TetA gene while 90.5% (19) had TetB gene. Our results showed that there is a correlation between phenotypic TC resistance and genomic TetA and TetB carriage in *E. coli* isolates from UTI and diarrhea patients in Zaria, Nigeria.

Keywords: Tetracycline resistance; *E. coli*; UTI and Diarrhea; Multidrug resistance

Introduction

Multidrug resistance (MDR) among pathogenic *Escherichia coli* is one of the leading causes of increased mortality and morbidity, which has contributed majorly to public health problems in both developed and developing countries. Pathogenic *Escherichia coli* develop MDR due to their capacity to acquire different genetic markers by horizontal gene transfer especially conjugation [1]. Resistance to Glycylcyclines (Tetracycline) has been associated with multimeric antiporter proteins (Tet proteins A, B, C, L and M), embedded in the bacterial inner membrane, which in exchange for a proton, catalyze the outward transport of tetracycline-Mg²⁺ complexes from the cytosol. Tet proteins actively efflux antibiotics out of the microbial cell membrane and protect the ribosome DNA, which lead to multidrug resistance [2,3]. Tetracycline enters bacterial cells by passive diffusion across the outer membrane through porin channels, which are composed of the OmpF protein. Transport of the antibiotic across the cytoplasmic membrane and into the cytoplasm requires pH or electropotential gradients [4]. These *Tets* genes have been transferred to a large variety of Gram-negative bacteria (*Salmonella enterica* [5], *Acinetobacter baumannii* [6], *Escherichia coli* [7], and *P. aeruginosa* [8]) and Gram positive bacteria (*Lactobacillus* spp. [9]) due to horizontal gene transfer. Two mutations in the largest cytoplasmic loop of the efflux pump, which resulted from a double frame shift in codons 201, 202 and 203 have been reported to increase the MIC of Tigecycline in *Escherichia coli* [10], contributing largely to antibiotics resistance. These mechanisms have incapacitated broad spectrum antibiotics such

as Tetracycline, Tigecycline and minocycline [11]. Isolates that coded for Tetracycline resistance genes have been reported in food sample (tet(M) and tet(L)); rivers, lakes, seawater, catfish, cows and clinical settings (tetA, tetB, tetC,) [3,11-13]. This has indeed generated a public health concern, due to limited number of antibiotics produced by pharmaceutical industries and low innovative research and limitations associated with clinical trials, which influence the availability of antibiotics for clinical use. Hence, surveillance to monitor the development and prevalence of resistance mechanisms against developed antibiotics is necessary. This study therefore evaluates the level of Tetracycline resistance in *Escherichia coli* among UTI and diarrhea patients in Zaria, Nigeria.

Methodology

Sample collection

One hundred and thirty two presumptive clinical isolates of *E. coli* were randomly collected from 4 hospitals within Zaria metropolis for the period of 6 months (April-September, 2014). Using Microbact™ 12E Gram negative identification kit, 87 of the isolates were confirmed as *E. coli*.

Antibiotic susceptibility testing

The antibiotic susceptibility profile of the identified *E. coli* isolates to Tetracycline and some other 14 selected antibiotics were carried out using Kirby-Bauer [14] disc diffusion method and their corresponding results were interpreted using CLSI [15]. Multiple antibiotic resistance index and classification into different subclass of multidrug resistance

were carried out using the methods described by Paul et al. [16] and Magiorakos et al. [17] respectively.

Molecular analysis

The DNA of MDR *E. coli* that were resistant to Tetracycline from UTI and Diarrhea patients in Zaria, Nigeria were extracted using Zymo Research DNA extraction kit with Lot No: ZRC182717 while the PCR was carried out at the Department of Bioscience, International Institute of Tropical, Agriculture, Ibadan using the primers for tetA (Forward: GTAATTCTGAGCACTGTCCG and Reverse: CTGCCTGGACAACATTGCTT) of 937 base pair and tetB (Forward: CTCAGTATTCCAAGCCTTTG and Reverse: CTAAGCACTTGTCTCCTGTT) of 416 base pair for the amplification of the corresponding genes.

Results and Discussion

Occurrence rate of *Escherichia coli* from UTI and Diarrhea patients within Zaria, Nigeria for the period of 6 months (April-September,

2014), were carried out in 4 hospitals, and 132 presumptive *Escherichia coli* were recovered. Significant numbers of the isolates [65.2% (86)] were confirmed to be *Escherichia coli* {active *Escherichia coli* (62.2% (82) and slow lactose fermenting *Escherichia coli* (inactive) [3% (4)]}, while 34.8% (46) were other Enterobacteriaceae (Table 1). Sixty eight point six percent {68.6% (59)} of the isolates were resistant to Tetracycline while 31.4% (27) were not resistant to it. The antibiotic susceptibility profile of the Tetracycline resistant isolates showed that Imipinem (1.2%), Amikacin (5.1%), Ceftriaxone (27.1%), Nitrofurantoin (28.8%), Azetronam (42.4%) and Gentamicin (49.2%) were the most effective antibiotics for the treatment of Tetracycline resistant *Escherichia coli* in Zaria, Nigeria. The isolates were mostly resistant to antibiotics such as Amoxicillin (93.2%), Cefpodoxime (88.1%), Cefotaxime (84.7%), Cefpirome (64.4%), and Sulphamethonidazole-Trimethoprim (61%) while mid resistance was observed against Flouroquinolones (Ciprofloxacin (54.2%) and Ofloxacin (54.2%)) (Table 2). Significant percentages (35.6% (21)) of the isolates were also observed to show resistance against Gentamicin, Ciprofloxacin and Amoxicillin respectively (Table 2).

S/N	Hospitals Sampled (n=4)	Diarrhea (S)		UTI (U)		Total Isolates Collected
		Isolates collected	<i>E. coli</i> (%)	Isolates collected	<i>E. coli</i> (%)	
1	ABUTH	15	11 (73.3)	28	21 (75)	43 (32.6)
2	ABUSB	12	7 (58.3)	22	15 (68.2)	34 (25.7)
3	SLAH	10	8 (80)	19	9 (47.4)	29 (22)
4	HGSGH	9	6 (66.7)	17	9 (52.9)	26 (19.7)
	Total	46	32 (69.6)	86	54 (62.8)	132 (100)

S/N: Serial Number; S: Stool sample of diarrhea patients; U: Urine sample of UTI patients; ABUTH: Ahmadu Bello University Teaching Hospital, Shika; ABUSB: Ahmadu Bello University Sick Bay; SLAH: St. Luke Anglican Hospital, Wusasa; HGSGH: Hajija Gambo Sawaba General Hospital, Kofan-Gayan

Table 1: Occurrence rate of *E. coli* in diarrhea and urinary tract infections in Zaria, Nigeria. The result below showed the percentages of *E. coli* from diarrhea and UTI in each hospital sampled in Zaria, Nigeria.

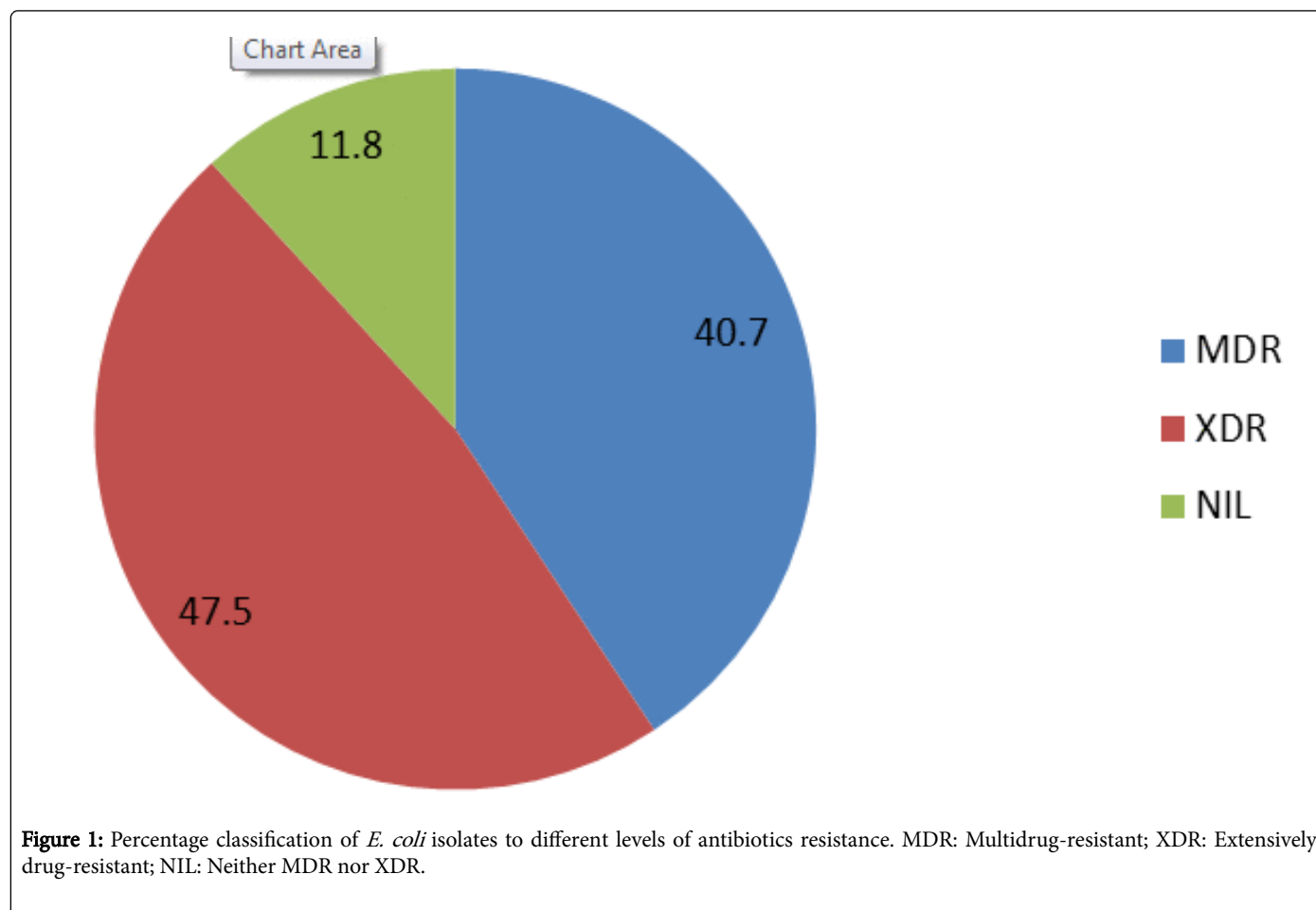
S/N	Isolates Codes	Antibiotic S Resistance Pattern	NA R	Tet. MIC (µg/ml)	Class of Antibiotics Resistance	LOR	GR T	MAR I
1	THU1	OFX, ATM, CN, CPD, CRO, CPO, CTX, SXT, C, AML	11	128	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.8
2	THU10	OFX, ATM, CN, CIP, CRO, CPD, CPO, CTX, SXT, F, AML	11	128	FLU, AMIN, MON, CEPH, MISC, PEN	XDR	6	0.8
3	THU25	OFX, ATM, CN, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, F	12	256	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.9
4	THU27	OFX, CN, CIP, CRO, CPD, CPO, CTX, SXT, AML, F	10	128	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.8
5	THS12	CN, OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, C, AML	11	128	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.8
6	THS1	OFX, CN, CIP, CPD, CPO, CTX, SXT, C, AML	9	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.7
7	THS8	CN, OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, C, AML	11	128	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.8
8	THS15	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6

9	SBS1	CN, OFX, CIP, CPD, CPO, CTX, SXT, C, AML	9	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.7
10	SBS7	CN, OFX, CIP, CPD, CPO, CTX, SXT, C, AML, IPM	10	128	FLU, AMIN, CEPH, MISC, PEN, CAB	XDR	6	0.8
11	SBS11	CN, OFX, CIP, CPO, CTX, SXT, AML	7	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.5
12	SBU2	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6
13	SBU8	CN, OFX, CIP, CPO, CTX, SXT, AML	7	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.5
14	SBU12	CN, ATM, OFX, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, F	12	256	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.9
15	SBU13	CN, ATM, OFX, CIP, CRO, CPD, CPO, CTX, SXT, C, AML	11	128	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.8
16	SBU15	CN, OFX, CIP, CPD, CPO, CTX, SXT, C, AML	9	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.7
17	SBU16	CN, ATM, OFX, CIP, CPD, CPO, CTX, SXT, C, AML	10	128	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.8
18	SBU17	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6
19	SBU20	CN, OFX, CIP, CPD, CPO, SXT, C, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6
20	SLS6	OFX, CIP, CN, CRO, CPO, CTX, AML	7	64	FLU, AMIN, CEPH, PEN	MD R	4	0.5
21	SLU3	OFX, CIP, AK, F, SXT, CXT, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6
22	HGS5	CN, ATM, OFX, F, CPD, CPO, CTX, SXT, AML	10	128	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.8
23	HGS6	CN, ATM, OFX, CIP, F, CPD, CTX, AML	8	64	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.6
24	HGU1	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6
25	HGU16	CN, ATM, OFX, CIP, CRO, CPD, CTX, C, AML	9	64	FLU, MON, AMIN, CEPH, MISC, PEN	XDR	6	0.7
26	SBU22	CPD, CPO, AML	3	16	CEPH, PEN	NIL	2	0.2
27	SLS1	CPO, CTX, SXT, AML	4	16	CEPH, MISC, PEN	MD R	3	0.3
28	SLS2	AML	1	8	P	NIL	1	0.1
29	SLS3	CPD, CPO, CTX, SXT, AML	5	32	CEPH, MISC, PEN	MD R	3	0.4
30	SLS5	OFX, CIP, CN, CRO, CPO, CTX, C	7	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.5
31	SLS8	AK, CRO, ATM, CPO, CXT	5	32	MON, AMIN, CEPH	MD R	3	0.4
32	SLS9	CPO, CTX, AML	3	16	CEPH, PEN	NIL	2	0.2
33	SLU2	CPO, CTX, SXT,	3	16	CEPH, MISC	NIL	2	0.2
34	SLU3	OFX, CIP, AK, F, SXT, CXT, AML	8	64	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.6
35	SLU4	CPD, CPO, C, AML	4	16	CEPH, MISC, PEN	MD R	3	0.3
36	SLU7	CPD, CPO, ATM	4	16	CEPH, MON	NIL	2	0.3
37	SLU8	OFX, CIP, CPD, CPO, ATM, AML	6	32	FLU, MON, CEPH, PEN	MD R	4	0.5
38	HGS2	CN, F, CPD, CPO, SXT, AML	6	32	AMIN, CEPH, MISC, PEN	MD R	4	0.5

39	HGS3	ATM, CPD, CTX, AML	4	16	CEPH, MON, PEN	MD R	3	0.3
40	HGS4	ATM, CPD, CPO, CTX, AML	3	16	CEPH, MON, PEN	MD R	3	0.2
41	HGU4	F, CPO, SXT, AML	4	16	CEPH, MISC, PEN	MD R	3	0.3
42	HGU6	C, CXT, AML	3	16	CEPH, AMIN, PEN	MD R	3	0.2
43	HGU7	ATM, F, CPD, CPO, CTX, SXT, AML	7	64	MON, CEPH, MISC, PEN	MD R	4	0.5
45	HGU9	F, CRO, CPD, CPO, CTX, AML	6	64	MON, CEPH, MISC, PEN	MD R	4	0.3
46	HGU14	ATM, F, CPD, CPO, CTX, AML	6	64	MON, CEPH, MISC, PEN	MD R	4	0.5
47	THU3	ATM, CPO, CTX, SXT, AML	5	64	CEPH, MISC, PEN	MD R	3	0.5
48	THU5	CPO, CTX, SXT, C, AML	5	64	MON, CEPH, MISC, PEN	MD R	4	0.4
49	THU6	ATM, CRO, CPO, CTX, C, AML	6	64	MON, CEPH, MISC, PEN	MD R	4	0.4
50	THU7	OFX, CIP, CPD, CPO, CTX, SXT, C, AML	8	64	FLU, CEPH, MISC, PEN	MD R	4	0.5
51	THU8	OFX, ATM, CIP, CPD, CPO, CTX, AML	7	64	FLU, MON, CEPH, PEN	MD R	4	0.6
52	THU9	CPD, CPO, CTX, AML	4	16	CEPH, PEN	NIL	2	0.5
53	THU13	OFX, ATM, CIP, CRO, CPD, CPO, CTX, C, AML	9	64	FLU, MON, CEPH, MISC, PEN	XDR	5	0.3
54	THU14	ATM, CPO, CTX, C, AML, F	6	32	MON, CEPH, MISC, PEN	MD R	4	0.7
55	THU17	CPO, CXT, F, AML	4	16	CEPH, MISC, PEN	MD R	3	0.5
56	THU18	CPO, CTX, SXT, C, AML	5	32	CEPH, MISC, PEN	MD R	3	0.3
57	THU19	OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, AML, F	10	128	FLU, AMIN, CEPH, MISC, PEN	XDR	5	0.4
58	THU20	CPO, CTX, SXT, C, AML	7	64	FLU, AMIN, CEPH, PEN	MD R	4	0.8
59	THU21	ATM, CN, CPD, CPO, CTX, AML	6	32	MON, AMIN, CEPH, PEN	MD R	4	0.5
								0.5

S/N: Serial Number; NAR: Number of Antibiotics Resistance; Tet. MIC: Tetracycline Minimum Inhibitory Concentration; GRT: Number of groups of antibiotics each isolate of *E. coli* is resistant to; FLU: Fluoroquinolone; MON: Monobactam; AMIN: Aminoglycoside; CEPH: Cephalosporin; MISC: Miscellaneous antibiotics; CAB: Carbapenems; PEN: Penicillin; AK: Amikacin; OFX: Ofloxacin; F: Nitrofurantoin; ATM: Aztreonam; CN: Gentamicin; CIP: Ciprofloxacin; CPD: Cefpodoxime; CRO: Ceftriaxone; CPO: Cefpirome; CTX: Cefotaxime; SXT: Sulphamethonidazole-Trimethoprim; C: Chloramphenicol; IPM: Imipenem; AML: Amoxicillin; MDR: Multidrug-resistant; XDR: Extensively drug-resistant; NIL: Neither MDR nor XDR; MDR: Resistance to at least 4 groups of antibiotics; XDR: Resistance to 5 and above groups of antibiotics tested; PDR: Non-susceptible to all antimicrobial agents listed. PDR was not considered because not all the antibiotics contained in the proposal of Magiorakos et al. [18] are prescribed for infections associated with *E. coli* in A.B.U Teaching Hospital Shika, Zaria

Table 2: Antibiotics susceptibility profile and pattern of Tetracycline resistance *E. coli* from UTI and Diarrhea patients in Zaria, Nigeria.



This study concurs with the report of Okunola et al. [18] in Benin, and Rabasa et al. [19] in Marduguri who reported that *E. coli* associated infections are becoming highly untreatable due to antibiotics resistance, especially to the first line empirical antimicrobials such as Beta-lactames, Sulphanidazole/Trimethoprim, Nitrofurantoin and Nalidixic acid. The sensitivity of *E. coli* to Imipeneme, Amikacin, Ceftriazone, Gentamicin and Quinolones as observed in this study may be due to the fact that the Imipeneme and Amikacin are expensive and not commonly sold over the counter while Quinolones are rarely prescribed for children. The parenteral routes of Ceftriazone and Gentamicin reduce the abuse of these two antibiotics. Also concentration dependent bactericidal activity, extended post-antibiotic effect, and the possibility of reduced nephrotoxicity and ototoxicity also affect the recommendation of Gentamicin [20]. The susceptibility of *E. coli* to Ofloxacin and Ciprofloxacin observed in this study also concurs with the study of Kemebradikumo et al. [21] in Bayelsa, who reported 61.5% *E. coli* sensitivity to Ofloxacin and 75% for Ciprofloxacin. The slight variation in the sensitivity Quinolones observed in this study might be due to high prescription of Ciprofloxacin in this area compared to Ofloxacin. The Tetracycline resistant *E. coli* using disc diffusion method was also observed to have high resistant MIC values against Tetracycline (Table 2). Factors such as low patient compliance, menace of substandard antibiotics which is common in developing countries, self-medication, and potentially sub-therapeutic prescription by health workers are some of the factors influencing multiple antibiotics resistance [22]. The MARI result showed that 23.7% (14) of the isolates have ≤ 0.3 MARI while 76% (45)

had $MARI \geq 0.4$ (Table 2). Classification of the isolates to different levels of resistance showed that 40.7% (24) of the isolates were multidrug resistance (resistance to 4 groups of antibiotics), 47.5% (28) were extensively drug-resistant (resistance to 5 or more than 5 antibiotics groups) while 11.8% (7) were neither MDR nor XDR (Figure 1). The high percentage of *E. coli* having MARI index ≥ 0.4 (Tables 2) in this study, suggests that the isolates originated from a high risk source of contamination where antibiotics are often used [23], while the high percentage of XDR and MDR (Figure 1) might be an indication that a large proportion of the bacterial isolates have been pre-exposed to several antibiotics, and also, a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms might be the cause of this high resistance [24]. To validate that the phenotypic resistance of the isolates to Tetracycline is also coded in the isolates genome, and to substantiate that efflux pumps mechanism which triggers the extrusion of structurally unrelated antibiotics from within the cells into the external environment and encourages multidrug resistance is involved in Tetracycline resistance mechanism, molecular analysis was carried out on the *E. coli* isolates (21) that were simultaneously resistant to Gentamicin, Amoxicillin and Ciprofloxacin using polymerase chain reaction and tetA and tetB primers. The result showed that 95% (20) of the isolates of *E. coli* have tetA gene compared to 90.5% (19) tetB gene. This virulent genes were however higher than that observed by Nahid et al. [25], who reported 49% tet(A) and 51% tet(B) genes, both of which encode for efflux pumping mechanisms. The antibiotics

susceptibility pattern of the isolates also concur that tet(B)-positive strains appeared to be more virulent than tet(A)-positive strains [25]. This is due to the level of antibiotic resistance observed in carriers in this study. Documentary evidence have shown that strains that were tet(B)-or tet(A)-positive might also carry the genes for P fimbriae,

iron-trapping compounds, hemolysin and aerobactin, respectively, more often than susceptible strains [25]. These acquirable virulent characteristics enable extra-intestinal pathogenic *E. coli* to adapt, colonize and persist in adhesion to urinary tract compared to other uropathogens [11,12] (Figures 2 and 3).

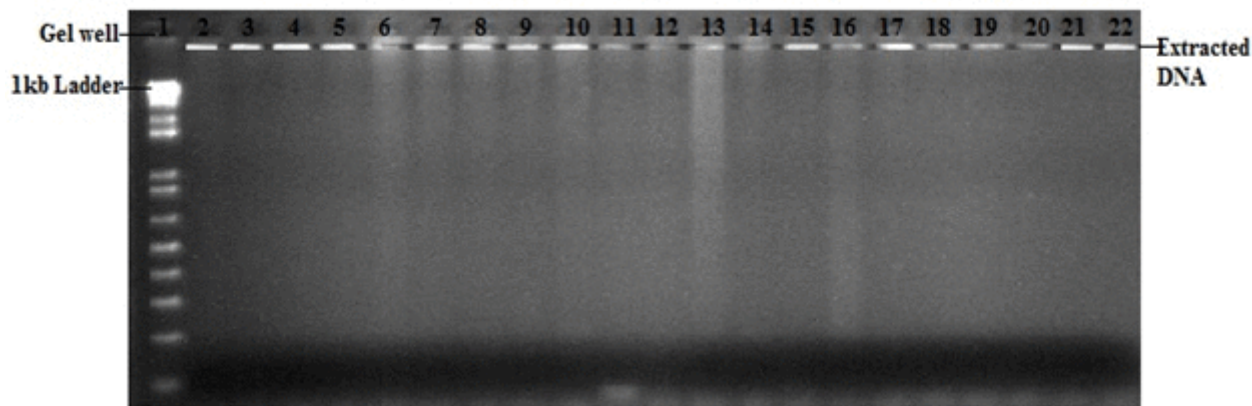


Figure 2: DNA extraction from tetracycline resistant *E. coli* isolated in Zaria, Nigeria.

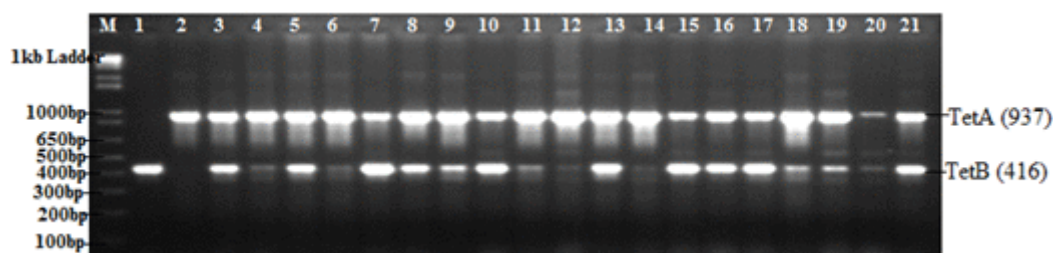


Figure 3: Multiplex Results of TetA and TetB Genes.

Conclusion

This study showed the presence of Tetracycline resistant *E. coli* in UTI and diarrhea patients in Zaria, Nigeria. Resistance to Tetracycline is highly associated with the presence of Tet proteins and isolates with Tet B protein were observed to be more resistant to those with TetA protein. Antibiotic surveillance to monitor the development and prevalence of resistance mechanisms against developed antibiotics is therefore necessary in this environ. Also awareness is important to encourage patient compliance, reduction in substandard antibiotics production in developing countries, reduced self-medication, and potentially sub-therapeutic prescription by health workers are some of the factors that should be curb to reduce multiple antibiotics resistance in Zaria, Nigeria.

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