Novel Research in Microbiology Journal (2022), 6(2): 1543-1556
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/nrmj.2022.231801

Prevalence of multidrug resistant *Escherichia coli* recovered from patients suffering from urinary tract infections

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Received: 8 March, 2022; Accepted: 18 April, 2022; Published online: 19 April, 2022

Abstract

Urinary tract infections are common human bacterial infections caused by *Escherichia coli*, which is one of the most frequent uropathogens. Treatment of such infections has become challenging due to the elevated levels of antimicrobial resistance. The aims of this work were to study the resistance pattern of *E. coli* pathogens recovered from patients with urinary tract infections attending to Minia University Hospital, Minia Governorate, Egypt, and to detect the co-existence of different extended spectrum β-lactamases (ESBLs) and metallo β-lactamases (MBLs) genes. The *in vitro* antibiotic sensitivity was tested using the disc diffusion assay. The production of ESBLs and MBLs was investigated through the combined disk and EDTA-combined disk synergy assays, while the molecular studies were carried out using the conventional polymerase chain reaction (PCR). High prevalence of ESBLs producers (61.6 %) was detected, whereas ESBLs and MBLs production was significantly associated with high multiple antimicrobial resistance index (MARI). The bla-$_{TEM}$ was the most prevalent genotype (94.6 %), while the prevalence of bla-$_{NDM}$ and bla-$_{IMP}$ was 45.9 % and 40.5 %, respectively. Imipenem and amikacin were the most effective antibacterial antibiotics. The current results showed statistically significant association of the tested genes, as 89 % of the *E. coli* isolates harbored more than one gene. This study highlighted a public health concern; as the elevated antibiotic resistance narrowed the therapeutic options and led to the failure of treatment of such bacterial infections.

Keywords: Urinary tract infections, Uropathogenic *E. coli*, ESBLs, MBLs, MDR, XDR

1. Introduction

Urinary tract infections (UTIs) are the most common community and hospital acquired bacterial infections (Ekwealor *et al*., 2016). They are most common among females than males, which is attributed to the female's anatomical structure of the urogenital systems (Tambekar *et al*., 2006). The previous study conducted by Ugwu *et al*., (2020) reported that the majority of uropathogens arise from the host gut microfloras, which pass to the bladder through the urethra; consequently *E. coli* was recorded as the most common uropathogen.

Treatment of UTIs has become more difficult due to the elevated bacterial resistance towards the clinically used antibiotics, which represents a huge health and economic burden (Ekwealor *et al*., 2016). Antibiotic resistance of *E. coli* could take place through efflux pumps; antibiotic-inactivating enzymes, permeability or target modifications, acquired plasmid encoding β-lactamases and\ or through aminoglycosides modifying enzymes (Ruppé *et al*., 2015).

According to the previous study of Dallenne *et al*., (2010), β-lactamases especially Extended spectrum β-lactamases (ESBLs) have the major concern, as they are continuously mutated, transferable and confer resistance to wide variety of antibiotics, which made their production as the main cause of antibiotic therapy failure. ESBLs include different groups, mainly those of class A genes such as bla-$_{TEM}$, bla-$_{SHV}$ and bla-$_{CTXM}$. Meanwhile, Metallo- β-lactamases (MBLs) are class of β-lactamase (class B) genes, which depend on the presence of zinc ions at the active site (i.e. bla-$_{VIM}$, bla-$_{IMP}$, bla-$_{NDM}$ and bla-$_{NDM}$). These genes confer resistance to all the β-lactam drugs such as carbapenems excluding the monobactams (Tooke *et al*., 2019). Co-production of ESBLs and MBLs is a devastating challenge for all the available β-lactam drugs (Reck *et al*., 2018).

The objectives of the current study were to detect the prevalence of uropathogenic *E. coli* and their resistance patterns. Moreover, it detects the production of ESBLs and MBLs, and their correspondences to the elevated MDR resistance. Accordingly, this study provides a local report about the effective therapeutic treatments against the uropathogenic *E. coli*.

2. Materials and methods

2.1. Isolation and identification of the uropathogenic bacteria

One hundred urine samples were collected over 3 months from patients with urinary tract infections attending to Minia University Hospital, EL-Minia, Egypt. All these samples were collected as midstream urine, centrifuged at 3,000 rpm for 20 min. and then the supernatants were decanted. The pellets were streaked on MacConkey agar and Eosin methylene blue (EMB) (Lab M, UK) agar plates. All plates were aerobically incubated at 37°C overnight. The recovered bacterial isolates were identified using both of the microscopical and biochemical assays (Benson, 2002).

2.2. Antibiotic susceptibility assay

The susceptibility of the bacterial isolates to 28 different antibiotics, which cover most of the available antibiotics in the Egyptian markets, was tested using the Kirby-Bauer Disk Diffusion assay according to Kirby, (2009). The tested bacterial isolates were prepared in dilutions equivalent to 0.5 McFarland standards, and then the isolates were streaked individually on Muller Hinton (MH) agar plates using a sterile cotton swab. The swabbed plates were allowed to air dry, and then using a sterile forceps the antibiotic discs were placed on the plate surface. All plates incubated at 37°C for 24 h. All the used antibiotics discs were purchased from Oxoid,

Basingstoke, UK. The tested bacterial isolates were classified as sensitive, intermediate and resistant according to the inhibition zones interpretation standards of the Clinical Laboratory standards Institute (CLSI), in reference to Wayne, (2018). The multiple antimicrobial resistance (MAR) indices of the isolates were calculated as the ratio between the number of antibiotics that an isolate resists and the total number of tested antibiotics. The MAR index greater than 0.2 was interpreted as high risk source of contamination indicating the abuse of the antibiotics (Ayandele *et al*., 2020).

2.3. Phenotypic detection of ESBLs and MBLs

The Extended spectrum β-Lactamases (ESBLs) production was tested using the combined disk test (CDT), in reference to Wayne, (2018). Suspensions of the bacterial isolates equivalent to 0.5 McFarland standards were swabbed on the surface of MH agar plates. Ceftazidime, ceftazidime/ clavulanic, cefotaxime and cefotaxime/ clavulanic discs (Oxoid, UK) combined antibiotics were applied on the surface of the inoculated plates, and then the plates were incubated aerobically overnight at 37°C. The test is considered positive if the difference between the cephalosporin and the cephalosporin/ clavulanic discs inhibition zone diameters is ≥ 5 mm. The MBLs production was investigated using the EDTA-combined disk synergy assay (Lee *et al*., 2001). In this test, 0.5 M EDTA solution was prepared and sterilized by autoclaving. The tested bacterial isolates equivalent to dilutions of 0.5 McFarland were swabbed onto the surface of MH agar plates. A 10 µg imipenem and a 10 µg meropenem discs were placed on the plate's surface, and then 5 µl of EDTA solution was added individually to each disc. After incubation, an increase in the inhibition zone diameter of at least 7 mm around the carbapenem/ EDTA disks was recorded as a positive result.

2.4. PCR amplification

Different ESBLs (bla-$_{CTX-M}$, bla-$_{SHV}$ and bla-$_{TEM}$) and MBLs (bla-$_{NDM}$ and bla-$_{IMP}$) genes were detected through PCR assay. The DNA of the bacterial isolates was extracted by boiling method, as conducted previously by Nobari *et al*., (2014). Few colonies of each bacterial isolate were boiled in 0.5 ml dist. water for 10 min., and then centrifuged at 10,000 rpm for 10 min. The resulting supernatant contains the DNA template used in the PCR assay. The PCR (UnoII Biometra®, Germany) cycling protocol was 95°C for 1 min. as initial denaturation, followed by 30 cycles of denaturation at 95°C for 1 min., extension at 72°C for 1 min., and a final extension at 72°C for 5 min. The annealing temperatures differed according to the type of the tested primers, as shown in Table (1).

2.5. Statistical analysis

Data analysis was performed using SPSS software, version 20 (IBM Corp., USA). To analyze the correlations, the Person's correlation coefficient (r^2) was tested. The *p*-values< 0.05 were considered as statistically significant.

3. Results

3.1. Isolation and identification of the uropathogenic bacteria

Out of 100 collected urine samples, about 60 % of the samples were positive for *E. coli*. In this study, 78 % of the *E. coli* isolates were recovered from the females, while 21.6 % were isolated from the males (the female to male ratio was 3.6: 1). The prevalence of the E. coli isolates with regard to the gender and age distribution are presented in Table (2). Results demonstrated that the infection was higher among the females with the age of 21-40 years.

3.2. Antibiotic susceptibility assay

Results of the susceptibility testing indicated that 73.3 % of the *E. coli* isolates were multidrug resistance (MDR), and 26.66 % of the isolates were extensive drug resistant (XDR). The isolates were highly resistant to the β-lactam antibiotics such as cephalothin (98.3 %) and amoxycillin/clavulanic (95 %). Imipenem showed the highest inhibitory activity

Table 1. List of primers used during this study with different annealing temperatures

Gene	Sequence	Product size	Annealing temp. (°C)	Reference
bla-$_{NDM}$	F 5'GGTTTGGCGATCTGGTTTTC-'3 R 5'-CGGAATGGCTCATCACGATC-3'	621 bp	55	(Nordmann *et al.*, 2011)
bla-$_{CTX-M}$	F 5'-TCTTCCAGAATAAGGAATCCC-3' R 5'-CCGTTTCCGCTATTACAAAC-3'	909 bp	58	(Stürenburg *et al.*, 2004)
bla-$_{SHV}$	F 5'TACCATGAGCGATAACAGCG3' R 5'-GATTTGCTGATTTCGCTCGG-3	450 bp	58	(Ghorbani-Dalini *et al.*, 2015)
bla-$_{TEM}$	F 5'-TCCGCTCATGAGACAATAACC3' R 5'ATAATACCGCACCACATAGCAG3'	296 bp	58	(Ghorbani-Dalini *et al.*, 2015)
bla-$_{IMP}$	F 5'GGAATAGAGTGGCTTAATTCTC3' R 5'-CCAAACCACTACGTTATCT-3	188 bp	56	(Ellington *et al.*, 2006)

Table 2. Prevalence of *E. coli* isolates among different gender and age groups

Age groups (years)	10-20	21-30	31-40	41-50	51-60	Total
Females (No.) (%)*	3 (5 %)	13 (21.6 %)	13 (21.6 %)	10 (16.6 %)	8 (13.3 %)	47 (78.3 %)
Males (No.) (%)*	0 (0 %)	2 (3.3 %)	5 (8.3 %)	3 (5 %)	3 (5 %)	13 (21.6 %)
Total (No.) (%)						60 (100 %)

Where; *indicates the percentage of bacterial infection correlated to the total number of recovered *E. coli* isolates

against the MDR and XDR followed by amikacin. The average number of MDR isolates that showed resistance was 10 (MAR index of 0.38), whereas the number of antibiotics that were inactive against the XDR was 14 (MAR index of 0.51), as presented in Table (3). All the *E. coli* isolates recorded high MAR indices that ranged from 0.18 to 0.96; however, 96 % of the isolates showed MAR indices above 0.2.

3.3. Phenotypic characteristics

It was found that 61.6 % of the *E. coli* isolates were phenotypically positive for ESBLs production; however, 33.3 % of the isolates were recorded positive for MBLs production. Significant and strong positive correlations were observed between MBLs and ESBLs production, where all the MBLs producers were ESBLs producers as well ($p< 0.001$). Furthermore, ESBLs and MBLs production was significantly associated with elevated MAR indices among the *E. coli* uropathogens (Table 4).

3.4. Antibiotic resistance among the ESBL producing *E. coli* isolates

Resistance to most of the tested antibiotics among the ESBL producers was found to be significantly elevated. Most of the E. coli isolates were totally

Table 3. Resistance pattern of the tested *E. coli* isolates against 28 different antibiotics

Antibiotics	Resistant isolates	
	No.	%*
Cephalothin	59	98.3
Amoxycillin/clavulanic	57	95
Cefadroxil	57	95
Cefotaxime	57	95
Ampicillin /sulbactam	53	88.3
Cefpodoxime	51	85
Ceftazidime	48	80
Sulfamethoxazole/trimethoprim	48	80
Doxycycline	45	75
Ofloxacin	42	70
Nalidixic acid	41	68.3
Norfloxacin	40	66.6
Ciprofloxacin	40	66.6
Ceftriaxone	37	61.6
Streptomycin	37	61.6
Levofloxacin	35	58.3
Aztreonam	35	58.3
Cefoperazone	35	58.3
Cefepime	33	55
Meropenem	27	45
Tobramycin	22	36.6
Nitrofurantoin	21	35
Gentamycin	17	28.3
Piperacillin /tazobactam	15	25
Chloramphenicol	14	23.3
Azithromycin	14	23.3
Amikacin	9	15
Imipenem	5	8.3

Table 4. Correlation matrix (r^2) between ESBLs, MBLs phenotypes and associated antimicrobial resistance

	ESBLs production	MBLs production
MAR index	0.448**	0.394**
ESBLs production	-	0.558**

Where; ** *p* value was significant at 0.01 level

resistant to all the β-lactam antibiotics. However, low resistance was observed towards azithromycin, amikacin and imipenem antibiotics, where amikacin and imipenem were the most effective drugs (Table 5). About 21/ 37 of the ESBLs isolates were MDR; recording resistance to 16 antibiotics (MARI: 0.58), whereas 16/ 37) isolates were XDR (resistant to 20 antibiotics) (MARI: 0.73).

3.5. Detection and association of ESBLs and MBLs genotypes using PCR

The ESBLs producers were tested for the presence of different ESBLs and MBLs genotypes. As shown in Table (6), results of PCR amplification recorded that bla-$_{TEM}$ was the most prevalent genotype (94.6 %), followed by bla-$_{CTX-M}$ (51.4 %). The PCR bands recovered for the 5 genes on the agarose gel are indicated in Fig. (1-5).

Furthermore, the present study detected high coexistence of the studied 5 genes; as 89 % of the *E. coli* isolates harbored more than one gene. Overall positive statistical correlations were observed between the studied genes. The bla-$_{TEM}$ gene was significantly associated with all the other detected genes. Statistical significant correlation was recorded between bla-$_{CTX-M}$ and bla-$_{SHV}$ genes, while bla-$_{IMP}$ gene was associated with the bla-$_{NDM}$ gene. Moreover, most of the detected genes were significantly associated with high MAR indices (Table 7).

The current results presented in Table (8) revealed that 37.5 % of XDR *E. coli* isolates harbored about 4 to 5 genes, whereas 9.52 % of the MDR isolates harbored 4 genes; indicating the abundance of resistance genes among the XDR Extended spectrum β-lactamase isolates. Prevalence of the co-harbored genes in the XDR *E. coli* isolates is shown in Table (9), demonstrating that coexistence of the bla-$_{TEM}$ and bla-$_{CTX-M}$ genes was the highest (18.75 %).

4. Discussion

The urinary tract infections (UTIs) are the most common nosocomial or community acquired infections. *E. coli* has been reported as the most etiological agent causing the urinary tract infections (Tabasi *et al*., 2016). During this study, out of 100 urine cultures, 60 cultures were recorded as *E. coli* positive (60 %). This high prevalence of *E. coli* was close to the results demonstrated by Arsalane *et al*., (2015); Angoti *et al*., (2016). However, the previous studies conducted by Mashwal *et al*., (2017); Hassuna *et al*., (2020) reported a lower prevalence of *E. coli*.

The urinary tract infection occurs in all age groups, and is likely to occur in females more than males, which is attributed mainly to the anatomical structure of the female's urogenital tract (Tambekar *et al*., 2006). In this study, the prevalence of UTI's in females was higher than males in all the age groups (female to male ratio 3.6: 1). These results were consistent with Dehbanipour *et al*., (2016) who reported the prevalence of UTI's (68 %) among the females. In contrast to our study, the previous study of Otajevwo and Amedu, (2015) reported higher prevalence of UTI's in males (57.1 %) than in females (42.9 %).

Table 5. Antibiotic resistance pattern among the ESBLs producing *E. coli* isolates

Antibiotics	Resistant	
	No.	%*
Cephalothin	37	100
Amoxycillin/clavulanic	37	100
Cefadroxil	37	100
Cefotaxime	34	91.8
Ampicillin /sulbactam	35	94.5
Cefpodoxime	34	91.8
Ceftazidime	34	91.8
Sulfamethoxazole/trimethoprim	33	89.1
Doxycycline	32	86.4
Ofloxacin	29	78.3
Nalidixic acid	21	56.7
Norfloxacin	27	72.9
Ciprofloxacin	26	70.2
Ceftriaxone	24	64.8
Streptomycin	26	70.2
Levofloxacin	22	59.4.
Aztreonam	26	70.2
Cefoperazone	25	67.5
Cefepime	19	51.3
Meropenem	27	72.9
Tobramycin	14	37.8
Nitrofurantoin	18	48.6
Gentamycin	12	32.4
Piperacillin /tazobactam	13	35.1
Chloramphenicol	12	32.4
Azithromycin	10	27
Amikacin	5	13.5
Imipenem	5	13.5

Table 6. Prevalence of different resistance genotypes among the ESBLs producers

bla-$_{TEM}$	***bla-$_{CTX-M}$***	***bla-$_{SHV}$***	***bla-$_{IMP}$***	***bla-$_{NDM}$***
N (%)*	N (%)*	N (%)*	N (%)*	N (%)*
35 (94.6%)	19 (51.4%)	16 (43.2%)	15 (40.5%)	17 (45.9%)

Where; * represent the percentages correlated to the total number of ESBL producers

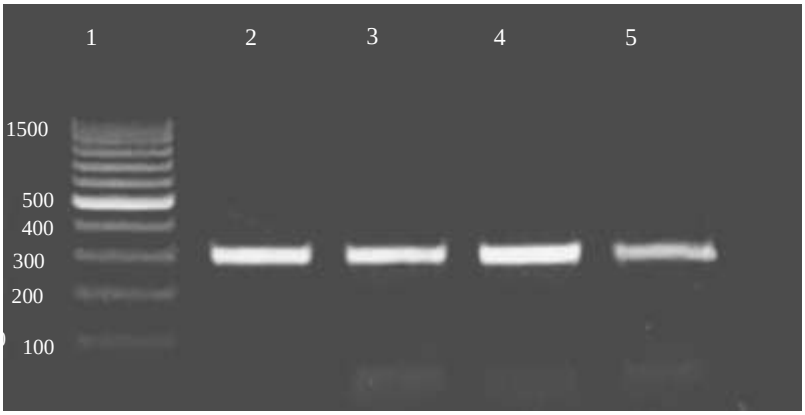

Fig. 1: PCR *bla-$_{TEM}$* gene bands at 296 bp

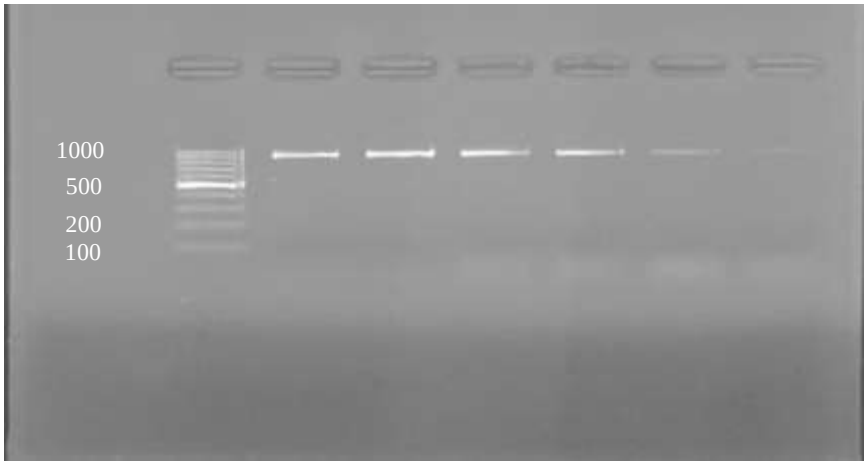

Fig. 2: PCR *bla-$_{CTX-M}$* gene bands at 909 bp

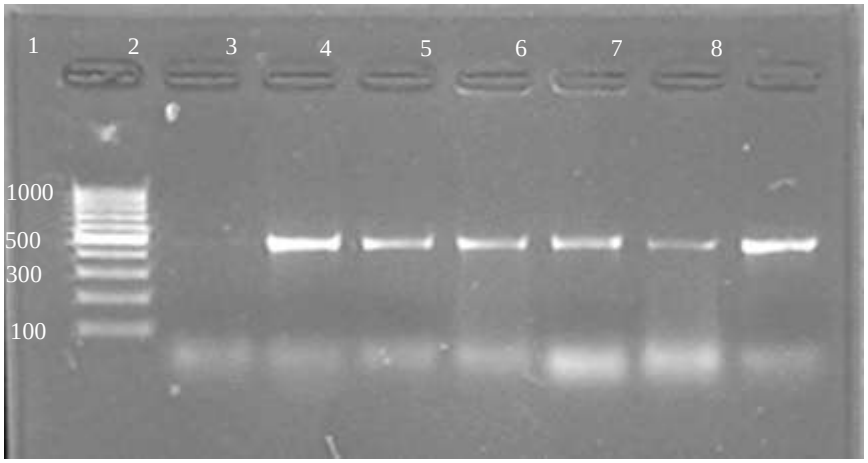

Fig. 3: PCR *bla*$_{SHV}$ gene bands at 450 bp

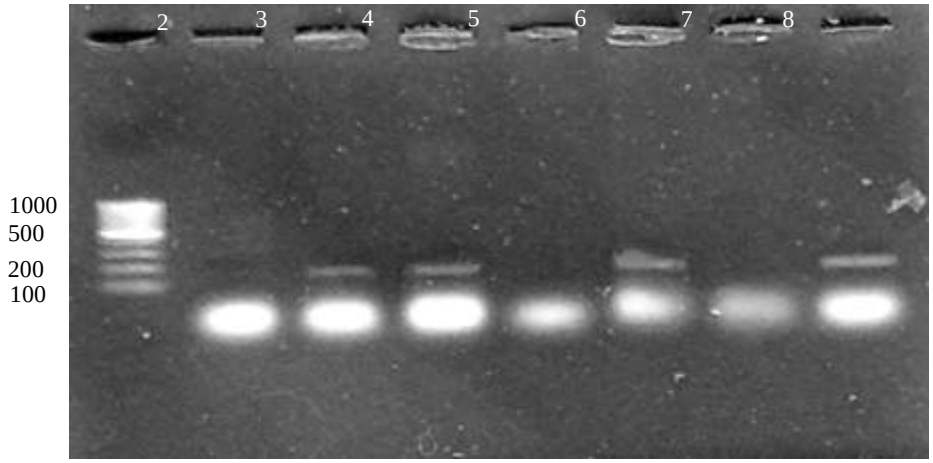

Fig. 4: PCR *bla*$_{IMP}$ gene bands at 188 bp

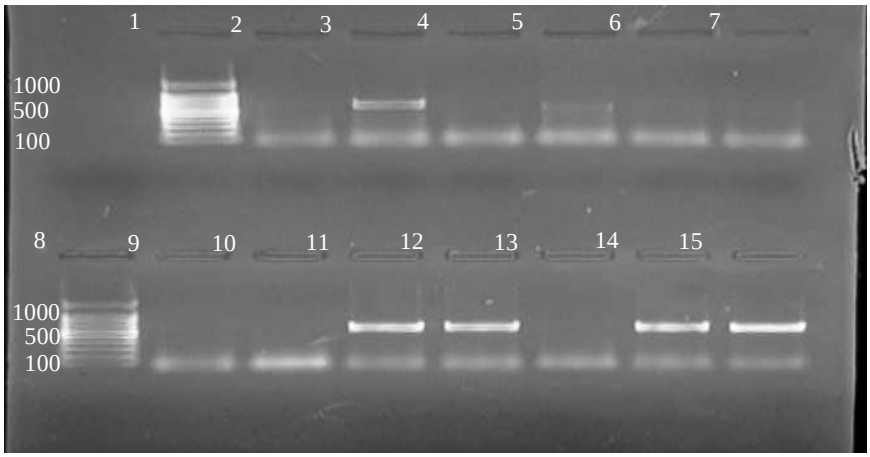

Fig. 5: PCR *bla-NDM* gene bands at 621bp

Table 7. Correlation matrix (r^2) between ESBLs, MBLs genotypes and associated antimicrobial resistance

	bla-TEM	*bla-CTX-M*	*bla-SHV*	*bla-IMP*	*bla-NDM*
MAR index	0.428**	0.350**	0.206	0.328*	0.328*
bla-TEM		0.503**	0.433**	0.488**	0.531**
bla-CTX-M			0.481**	0.186	0.208
bla-SHV				0.174	0.206
bla-IMP					0.406**

Where; $^{*}p$ values were significant at 0.5 level, while $^{**}p$ values were significant at 0.001 level

Table 8. Distribution of the different genes among MDR and XDR Extended spectrum β-lactamases *E. coli* producers

No. of genes	MDR (No.= 21)	XDR (No.= 16)
0	0	1(6.25 %)
1	1(4.76 %)	1(6.25 %)
2	8(38 %)	5(31.25 %)
3	10(47.61 %)	3(18.75 %)
5	2(9.52 %)	4(25 %)
5	0	2(12.5 %)

Where; the percent was correlated to the number of isolates in each category (MDR=21, XDR=16)

Table 9. Distribution of the coexisting genes among the XDR *E. coli* isolates

No. of genes	Coexisting genes	No. of isolates (%)*
1	*Bla-TEM*	1(6.25 %)
2	*bla-TEM, bla-NDM*	2(12.5 %)
	bla-TEM, , blaCTX-M	3(18.75 %)
3	*bla-TEM, bla-SHV, bla-IMP*	1(6.25 %)
	bla-TEM, bla-CTX-M, bla-SHV	1(6.25 %)
	bla-TEM, bla-NDM, bla-IMP	1(6.25 %)
4	*bla-NDM, bla-TEM, , bla-CTX-M, bla-SHV*	2(12.5 %)
	bla-NDM, bla-TEM, , bla-CTX-M, bla-IMP	1(6.25 %)
	bla-TEM, , bla-CTX-M, bla-SHV bla-IMP	1(6.25 %)
5	*bla-NDM, bla-TEM, , bla-CTX-M, bla-SHV, bla-IMP*	2(12.5 %)

Where; *represent the percentage correlated to the total number of XDR *E. coli* isolates

Treatment of the urinary tract infections has become more challenging due to the continuous increase in the bacterial MDR. The present study revealed the high incidence of multidrug resistant (MDR) uropathogenic *E. coli*. About 96 % of the isolates had MAR index greater than 0.2 (average 0.6), which indicated the existence of an environment where antibiotics were widely used or misused (Osundiya *et al*., 2013; Adamus-Białek *et al*., 2018). The current results were consistent with the results reported by Sabir *et al*., (2014) (81 %). On the other hand, Kulkarni *et al*., (2017); Ramírez-Castillo *et al*., (2018); Hassuna *et al*., (2020), reported a fewer MDR incidence of 43 %, 63 % and 62 %, respectively.

In this study, 37 (61.6 %) MDR *E. coli* were recorded to be phenotypically ESBL producers. Similarly, high ESBL production (58 %) was reported by Hassuna *et al*., (2020). Moreover, in Qatar, Eltai *et al*., (2018) reported higher results of 83 %. On the contrary, lower incidence was recorded by Arsalane *et al*., (2015) (6 %); Ugwu *et al*., (2020) (27 %). The MBLs producers represented 33.3 % of the *E. coli* isolates in agreement with the previous results (35 %) reported by Shrestha *et al*., (2020); however, they were higher than the results (16 %) revealed by Jamil *et al*., (2018).

The most prevalent gene was bla-$_{TEM}$ followed by bla-$_{CTX-M}$, which was in agreement with the previous study conducted by Hassuna *et al*., (2020); where bla-$_{TEM}$ and bla-$_{CTX-M}$ were the predominant genes. Moreover, the current study demonstrated the higher coexistence of the studied genes. This is attributed to the presence of circulating plasmids harboring different antibiotic resistance genes, leading thus to the spread of resistance traits (Daini *et al*., 2005; Hassuna *et al*., 2020). Currently, the bla-$_{NDM}$ and bla-$_{IMP}$ genes were significantly coproduced that coincides with the study conducted by Ugwu *et al*., (2020); which detected the co-expression of more than one MBLs gene.

Conclusion

The present study recorded the high prevalence of ESBL producing uropathogenic *E. coli*. Moreover, high prevalence of the co-existed resistance genes with the elevated MAR indices of the *E. coli* isolates indicated the misuse of antibiotics in our community. Imipenem and amikacin antibiotics can be the drugs of choice for treating the

ESBL producing pathogens. This study pointed out the urgent need for continuous surveillance of the antimicrobial resistance, and implementing new protocols of antimicrobial therapy.

Acknowledgement

None.

Conflict of interest

The authors declare no conflict of interests.

Funding source

The current research did not receive fund from any agency.

Ethical approval

The approval code of the Faculty of Pharmacy, Minia University Ethical Committee was provided (HV10/2020). The patient's consents and statement of protection of the patient's privacy are provided.

5. References

Adamus-Białek, W.; Baraniak, A.; Wawszczak, M.; Głuszek, S.; Gad, B.; Wróbel, K.; Bator, P.; Majchrzak, M. and Parniewski, P. (2018). The genetic background of antibiotic resistance among clinical uropathogenic *Escherichia coli* strains. Molecular Biology Reports. 45(5): 1055-1065. https://doi.org/10.1007/s11033-018-4254-0

Angoti, G.; Goudarzi, H.; Hajizadeh, M. and Tabatabaii, Z. (2016). Bacteria isolated from urinary tract infection among patients and determination of the antibiotic susceptibility patterns of the gram negative bacteria in Iran. Novelty in Biomedicine. 4(1): 1-4. https://doi.org/10.22037/nbm.v4i1.6965

Arsalane, L.; Zerouali, K.; Katfy, K. and Zouhair, S. (2015). Molecular characterization of extended spectrum β-lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa. African Journal of Urology. 21(3): 161-166. https://doi.org/10.1016/j.afju.2015.02.005

Ayandele, A.A.; Oladipo, E.K.; Oyebisi, O. and Kaka, M.O. (2020). Prevalence of Multi-Antibiotic Resistant *Escherichia coli* and *Klebsiell*a species obtained from a Tertiary Medical Institution in Oyo State, Nigeria. Qatar Medical Journal. 2020(1): 9. https://doi.org/10.5339/qmj.2020.9.

Benson, H.J. (2002). Microbiological Applications: Laboratory Manual in General Microbiology, 8th Edition, McGraw Hill, New York. 4: 1-478.

Daini, O.; Ogbolu, O. and Ogunledun, A. (2005). Quinolones Resistance and R-plasmids of some gram negative enteric Bacilli. African Journal of Clinical Experimental Microbiology. 6(1): 14-20. https://doi.org/10.4314/ajcem.v6i1.7394.

Dallenne, C.; Da Costa, A.; Decré, D.; Favier, C. and Arlet G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy. 65(3): 490-495. https://doi.org/10.1093/jac/dkp498

Dehbanipour, R.; Rastaghi, S.; Sedighi, M.; Maleki, N. and Faghri, J. (2016). High prevalence of multidrug-resistance uropathogenic *Escherichia coli* strains, Isfahan, Iran. Journal of Natural Science, Biology and Medicine. 7(1): 22-26. https://doi.org/10.4103/0976-9668.175020

Ekwealor, P.A.; Ugwu, M.C.; Ezeobi, I.; Amalukwe, G.; Ugwu, B.C.; Okezie, U.; Stanley, C. and Esimone, C. (2016). Antimicrobial Evaluation of Bacterial Isolates from Urine Specimen of Patients with Complaints of Urinary Tract Infections in Awka, Nigeria. International Journal of Microbiology. 2016(9740273): 1-6. https://doi.org/10.1155/2016/9740273

Ellington, M.J.; Kistler, J.; Livermore, D.M. and Woodford, N. (2006). Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. Journal of Antimicrobial Chemotherapy. 59(2): 321-322. https://doi.org/10.1093/jac/dkl481

Eltai, N.O.; Al Thani, A.A.; Al-Ansari, K.; Deshmukh, A.S.; Wehedy, E.; Al-Hadidi, S.H. and Yassine, H.M. (2018). Molecular characterization of extended spectrum β-lactamases enterobacteriaceae causing lower urinary tract infection among pediatric population. Antimicrobial Resistance Infection Control. 7(1): 1-9. https://doi.org/10.1186/s13756-018-0381-6

Ghorbani-Dalini, S.; Kargar, M.; Doosti, A.; Abbasi, P. and Sarshar, M. (2015). Molecular epidemiology of ESBL genes and multi-drug resistance in diarrheagenic *Escherichia coli* strains isolated from adults in Iran. Iranian Journal of Pharmaceutical Research. 14(4): 1257-1262.

Hassuna, N.A.; Khairalla, A.S.; Farahat, E.M.; Hammad, A.M. and Abdel-Fattah, M. (2020). Molecular characterization of Extended-spectrum β lactamase-producing *E. coli* recovered from community-acquired urinary tract infections in Upper Egypt. Scientific Reports. 10(1): 1-8. https://doi.org/10.1038/s41598-020-59772-z

Jamil, J.; Haroon, M.; Sultan, A.; Khan, M.A.; Gul, N. and Kalsoom (2018). Prevalence, antibiotic sensitivity and phenotypic screening of ESBL/MBL producer *E. coli* strains isolated from urine; District Swabi, KP, Pakistan. Journal Of Pakistan Medical Association. 68(11): 1704-1707.

Kirby, B. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology. 66(208): 1-23.

Kulkarni, S.R.; Peerapur, B.V. and Sailesh, K.S. (2017). Isolation and Antibiotic Susceptibility Pattern of *Escherichia coli* from Urinary Tract Infections in a Tertiary Care Hospital of North Eastern Karnataka. Journal of Natural Science, Biology and Medicine. 8(2): 176-180. https://doi.org/10.4103/0976-9668.210012

Lee, K.; Chong, Y.; Shin, H.; Kim, Y.; Yong, D. and Yum, J. (2001). Modified Hodge and EDTA-Disk Synergy Tests to Screen Metallo-β Lactamase-Producing Strains of *Pseudomonas* and *Acinetobacter* Species. Clinical Microbiology and Infection. 7(2): 88-91. https://doi.org/10.1046/j.1469-0691.2001.00204.x

Mashwal, F.A.; El Safi, S.H.; George, S.K.; Adam, A.A. and Jebakumar, A.Z. (2017). Incidence and molecular characterization of the extended spectrum beta lactamase-producing *Escherichia coli* isolated from urinary tract infections in Eastern Saudi Arabia. Saudi Medical Journal. 38(8): 811-815. https://doi.org/10.15537/smj.2017.8.18578

Nobari, S.; Shahcheraghi, F.; Rahmati Ghezelgeh, F. and Valizadeh, B. (2014). Molecular characterization of carbapenem-resistant strains of *Klebsiella pneumoniae* isolated from Iranian patients: First identification of bla_{KPC} gene in Iran. Microbial Drug Resistance. 20(4): 285-293. https://doi.org/10.1089/mdr.2013.0074

Nordmann, P.; Poirel, L.; Carrër, A.; Toleman, M.A. and Walsh, T.R. (2011). How to detect NDM-1 producers. Journal of Clinical Microbiology. 49(2): 718-721.

Osundiya, O.; Oladele, R. and Oduyebo, O. (2013). Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. African Journal of Clinical Experimental Microbiology. 14(3): 164-168. https://doi.org/10.4314/ajcem.v14i3.8

Otajevwo, F. and Amedu, S. (2015). Community acquired urinary tract infection prevalence in a tertiary institution based in Evbuobanosa, Edo State, Nigeria. Global Journal of Medical Research. 15(3): 52-63.

Ramírez-Castillo, F.Y.; Moreno-Flores, A.C.; Avelar-González, F.J.; Márquez-Díaz, F.; Harel, J. and Guerrero-Barrera, A.L. (2018). An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study.

Annals of Clinical Microbiology Antimicrobials. 17(1): 1-13. https://doi.org/10.1186/s12941-018-0286-5

Reck, F.; Bermingham, A.; Blais, J.; Capka, V.; Cariaga, T.; Casarez, A.; Colvin, R.; Dean, C.R.; Fekete, A. and Gong, W. (2018). Optimization of novel monobactams with activity against carbapenem-resistant Enterobacteriaceae–identification of LYS228. Bioorganic Medicinal Chemistry Letters. 28(4): 748-755. https://doi.org/10.1016/j.bmcl.2018.01.006.

Ruppé, É.; Woerther, P.L. and Barbier, F. (2015). Mechanisms of antimicrobial resistance in Gram-negative bacilli. Annals of Intensive Care. 5(1): 21. https://doi.org/ 10.1186/s13613-015-0061-0.

Sabir, S.; Ahmad Anjum, A.; Ijaz, T.; Asad Ali, M.; Ur Rehman Khan, M. and Nawaz, M. (2014). Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. Pakistan Journal of Medical Sciences. 30(2): 389-392.

Shrestha, A.; Acharya, J. and Amatya, J. (2020). Prevalence of ESBL and MBL producing gram negative uropathogens. International Journal of Infectious Diseases. 101(S1): 52. https://doi.org/10.1016/j.ijid.2020.09.168

Stürenburg, E.; Kühn, A.; Mack, D. and Laufs, R. (2004). A novel extended-spectrum β-lactamase CTX-M-23 with a P167T substitution in the active-site omega loop associated with ceftazidime resistance. Journal of Antimicrobial Chemotherapy. 54(2): 406-409. https://doi.org/10.1093/jac/dkh334

Tabasi, M.; Karam, M.R.; Habibi, M.; Mostafavi, E. and Bouzari, S. (2016). Genotypic Characterization of Virulence Factors in *Escherichia coli* Isolated from Patients with Acute Cystitis, Pyelonephritis and Asymptomatic Bacteriuria. Journal of Clinical and Diagnostic Research. 10(12): DC01-DC07. https://doi.org/10.7860/JCDR/2016/21379.9009.

Tambekar, D.; Dhanorkar, D.; Gulhane, S.; Khandelwa, V. and Dudhane, M. (2006). Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. African Journal of Biotechnology. 5(17): 1562-1565.

Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.; Takebayashi, Y. and Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. Journal of molecular Biology. 431(18): 3472-3500. https://doi.org/10.1016/j.jmb.2019.04.002.

Ugwu, M.C.; Shariff, M.; Nnajide, C.M.; Beri, K.; Okezie, U.M.; Iroha, I.R. and Esimone, C.O. (2020). Phenotypic and Molecular Characterization of beta-Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria. The Canadian Journal of Infectious Diseases & Medical Microbiology. 2020(5843904): 1-9. https://doi.org/10.1155/2020/5843904.

Wayne, P. (2018). CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100. Clinical and Laboratory Standards Institute. 28th Edition.30-102.