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Research Article
Molecular Characterization of Multidrug Resistant Clinical *Escherichia coli* Isolates

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Abstract

Background: Quinolones are used against a broad range of pathogenic bacteria. It was discovered that quinolone resistance was plasmid-mediated by *qnr* genes (*qnrA*, *qnrB* and *qnrS*) and can be simply transferred between different bacterial strains. Also, the emergence of *E. coli* resistance to carbapenem during the therapy with imipenem and meropenem was attributed to carbapenem-hydrolyzing enzymes encoded by bla_{KPC} gene. This work aimed to study the susceptibility and prevalence of multidrug resistant (MDR) *E. coli* isolates and the possible role of quinolone and carpabenem genes in *E. coli* resistance. **Materials and Methods:** Fifty clinical *E. coli* isolates were collected from different diagnostic centers of Mansoura University Hospitals and their antimicrobial susceptibility pattern was tested using Kirby-Bauer disc diffusion method. Out of 50 isolates, 10 MDR *E. coli* candidates were selected for morphological and biochemical identification as well as for the detection of the plasmid-bearing antibiotic resistant genes by PCR and protein profile in the presence of quinolone and carbapenem using SDS-PAGE. **Results:** The results of antimicrobial susceptibility pattern of 50 clinical *E. coli* isolated from urine, wound swab, blood and sputum of patients from Mansoura University Hospitals, revealed that the isolates were cefuroxim resistant (96%), cefotriaxone resistant (92%), cefaclor resistant (90%), ciprofloxacin resistant (76%), meropenem resistant (40%), imipenem resistant (30%) and amikacin resistant (16%). Plasmid profile showed that all MDR strains harbored plasmids of different sizes. Some isolates possess single sized plasmid while other had multiple plasmids with different sizes. The distribution of antibiotic resistant genes in *E. coli* candidates included *qnrA* (0%), *qnrB* (50%), *qnrS* (70%), *qnrB* and *qnrS* (20%) and bla_{KPC} (10%). All isolates were harbored one of quinolone resistant genes. The protein pattern of the MDR *E. coli* with ciprofloxacin was showed 19 bands distributed as; 16 monomorphic and 3 polymorphic. Meropenem case indicated 20 bands distributed as 14 monomorphic, 5 polymorphic and 1 unique. There were 4 bands present in control and disappeared from isolates with molecular mass (122.9, 74.2, 69.8 and 55.08 kDa). **Conclusion:** The antimicrobial resistance is a clinical and public health problem, there is a need for monitoring the microbial trends and antimicrobial resistance patterns.

Key words: Multi-drug resistant, *E. coli*, quinolone, carpabenam, bla_{KPC}, *qnr*, protein pattern

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The bacterial resistance to antibacterial agents (such as antibiotics) is a threat to public health throughout the world. The bacteria which have resistant genes in their genomes or can grow in the presence of two or more antibiotics are called multi-drug resistant (MDR) bacteria[1]. The resistance pattern of microorganisms to antibiotics was correlated with the antibiotics used heavily at the time of the outbreak. The consequences of resistance do not affect only the ability to treat the infection, but also the cost and duration of treatment[2].

The *E. coli* is the first cause of nosocomial gram-negative[3], rod-shaped, harmless bacteria that commonly found in the lower intestine as normal flora of the gut. Most *E. coli* strains produce vitamin K_2 and prevent the establishment of pathogenic bacteria inside the intestine of the host while some *E. coli* serotypes cause serious food poisoning[4] besides the acute urinary tract infections[5]. *Escherichia coli* that has shown an increasing in antimicrobial resistance to most antibiotics was isolated from human[4]. Bacterial susceptibility to antibiotics is variable and depends on growth conditions and treatment time[6].

The β-lactam antibiotics including penicillins (such as methicillin, ampicillin and amoxicilin), cephalosporins (such as cefaclor, ceftriaxone and cefotaxim), carbapenem (such as imipenem, meropenam and ertapenem) may act at several stages to prevent peptidoglycan synthesis[7].

In some sever infected cases, the only effective antibiotic is carbapenem. Carbapenem-hydrolyzing enzymes (carbapenemases) are one type of β-lactam-hydrolyzing enzymes (β-lactamases) that are significantly hydrolysing a wide range of β-lactam antibiotics. Carbapenem resistance as a type of β-lactam resistance has been rarely reported in *E. coli*[8]. There are several types of carbapenemases such as; bla_{KPC}-type carbapenemase that was firstly recognized 2001 in *Klebsiella pneumoniae* in North Carolina (USA) and then spread in New York[9-11]. The bla_{KPC} genes are predominantly found in *Klebsiella pneumoniae*, however, they have also been found in many other Enterobacteriaceae including *E. coli*[12].

The extensive use of the wide spectrum quinolone antibiotics in human and animal infections resulted in the rising of quinolone resistant bacteria[13]. Two mechanisms of quinolone resistance have been established to date: Alterations in the targets of quinolones and decreasing the accumulation of quinolone inside the bacterial cells through membrane impermeability and/or an over expression of efflux pump systems[14].

The resistance of quinolone was correlated to mutations that lead to amino acid changes in the quinolone-resistance determining regions (QRDRs) within the subunits which are included in the synthesis of DNA. These, altered subunits inhibit the DNA gyrase; the topoisomerases II which is responsible for *GyrA* and *GyrB* genes and topoisomerase IV which is responsible for *ParC* and *ParE* genes that end with the survival of the bacteria[15]. Also, quinolone resistance genes (*qnr*) as *qnrA*, *qnrB* and *qnrS* were reported as DNA gyrase inhibitor[16]. This study aimed to investigate if the prevalence of quinolone resistance and β-lactam resistance are correlated with the presence of quinolone genes (*qnrA*, *qnrB* and *qnrS*) and carbapenem genes (bla_{KPC}) in multidrug resistant *E. coli*. Also, the impact of the antibiotics (meropenem and ciprofloxacin) on protein pattern was investigated.

MATERIALS AND METHODS

Collection of samples and identification of *E. coli*: Clinical samples (urine, blood, sputum and wound swab) were collected from different diagnostic Departments of Mansoura University Hospitals (Emergency, Oncology, Pediatric, Surgery and General medicine). These samples were cultured using the standard media (CLED agar for urine samples, blood agar for blood sample and MacConkey's agar for blood, sputum and wound swab samples) and incubated at 37°C overnight[17]. Colonies with the typical colour and appearance of *E. coli* were picked.

The confirmation tests of *E. coli* isolates were done by Gram staining, microscopic examination and biochemical tests including indole, methyl red, voges-proskauer and citrate utilization tests[17]. The *E. coli* isolates were further confirmed by using Analytical Profile Index (API 20E)[18].

Antimicrobial susceptibility test: Antibiotic susceptibilities of *E. coli* isolates were determined following Kirby Bauer disc diffusion method[19] using Mueller-Hinton agar medium including 17 selected antibiotics separate discs namely azteronam, ATM (30 µg); amikacin, AK (30 µg); augmentin, AMC (30 µg); meropenem, MEM (30 µg); gentamicin, CN (10 µg); ciprofloxacin, CIP (5 µg); levofloxacin, LEV (10 µg); cefotaxime, CTX (30 µg); cefuroxim, CXM (30 µg); cefotriaxone, CRO (30 µg); ceftazidime, CAZ (30 µg); timipenem, IPM (10 µg); cefaclor, CEC (30 µg); trimethoprim, SXT (25 µg); norfloxacin, NOR (30 µg); nalidixic acid, NA (30 µg) and ampcillin/sulbactam, SAM (30 µg)[19]. Inhibition zone size was measured and then interpreted using standard recommendation of Clinical Laboratory Standard Institute (CLSI)[20]. The sensitivity and resistance was recorded as (S) and

Table 1: *Escherichia coli* antimicrobial resistant genes and primer sequences used for PCR identification

Antimicrobial agents	Resistance gene	Primer sequence	Target size (bp)	Annealing temperature (°C)	References
Quinolone	*qnrA*	(F)5'-TCAGCAAGAGGATTTCTCA-3' (R) 5'-GGCAGCACTATTACTCCCA-3'	516	46	Robicsek *et al.*[14] and Wang *et al.*[24]
	qnrB	(F) 5'-GATCGTGAAAGCCAGAAAGG-3' (R)5'-ACGATGCCTGGTAGTTGTCC-3'	469	48	Robicsek *et al.*[14] and Wang *et al.*[24]
	qnrS	(F) 5'-ACGACATTCGTCAACTGCAA-3' (R) 5'-TAAATTGGCACCCTGTAGGC-3'	417	42	Robicsek *et al.*[14] and Wang *et al.*[24]
Carbapenem	bla_{KPC}	(F) 5'-ATGTCACTGTATCGCCGTCT-3' (R) 5'-TTTTCAGAGCCTTACTGCCC-3'	893	47	Toupkanlou *et al.*[25]

(R) respectively. Multidrug resistance occurred when the bacteria were resistant to at least one antimicrobial agent in three or more antimicrobial classes[21].

Molecular study on multidrug resistance of *E. coli*: Ten *E. coli* isolates were selected to detect the presence or absence of resistant genes; quinolone resistant genes (*qnr* genes) and carbapenem resistant gene (bla_{KPC}). The protein banding patterns of the multidrug resistant *E. coli* isolates were also investigated.

Plasmid isolation: Plasmids were isolated from *E. coli* isolates according to the manual of extraction kit (Gene Jet Plasmid Miniprep Kit) and resolved by electrophoresis in 1% agarose gel included ethidium bromide[22,23].

Primers and PCR assay: The presence and absence of resistant genes (bla_{KPC}, *qnrA*, *qnrB* and *qnrS*) was carried out by PCR reaction using the primers listed in Table 1. The reaction mixture (20 µL) contained 1 µL DNA tempelete, 2 µL 10x buffer, 2 µL dNTPs (dGTP, dATP, dCTP and dTTP), 0.2 µL Taq DNA polymerase, 1 µL of each primer (forward and reverse) and 12.8 µL water (nuclease free). The samples were gently vortexed and the PCR were performed using the thermal cycling condition including the annealing temperature for each gene.

SDS-PAGE for protein of multidrug resistant *E. coli* isolates: Two groups of *E. coli* were cultured in LB both media for overnight at 37°C, the first one contains ciprofloxacin antibiotic while the second contains meropenem antibiotic. Using liquid nitrogen, total protein from *E. coli* pellets were extracted in 100 mM phosphate buffer with pH 7 and followed by measuring the protein concentration according to Bradford method, 10 µg protein concentrations from each *E. coli* isolate were boiled in 2x sample buffer (10 mL distilled water, 2.5 mL, tris-HCl, pH 6.8, 2 mL glycerol, 4 mL 10% SDS and 1 mL β-mercptoethanol) for 2 min to be ready for loading over acrylamide gel. Acrylamide gel was prepared according to Laemmli[26] from two layers; a stacking gel (4%) on top of separating gel (12%).

After electrophoresis at 100 v for 2 h, overnight staining of gel in commassie brilliant blue R250, destaining in destain solution were performed on shaker for some hours. The gel was documented and analysed using gel analyser programme.

RESULTS

Isolation and identification of *E. coli* isolates: A collection of 50 *E. coli* isolates classified as multidrug resistance (being resistant to three or more different classes of antimicrobial compounds) was obtained out of the 50 isolates, 10 multidrug resistant strains were selected and identified as *E. coli* by morphological and biochemical tests (Table 2).

Antimicrobial susceptibility: Fifty *E. coli* isolates were tested for their resistance to 17 antibiotics. The multidrug resistant *E. coli* isolates showed high resistance to cefuroxim (96%), cefotriaxone (92%), cefaclor (90%) and ciprofloxacin (76%). On the other hand, some multidrug resistant *E. coli* isolates recorded low resistance to amikacin (16%), meropenem (40%) and imipenem (30%) (Table 3).

The clear zones were measured and compared with the standard recommendation of Clinical Laboratory Standard Institute (CLSI)[20]. Ten *E. coli* isolates, that are resistant to quinolone and carpabenem antibiotics were selected for molecular analysis (Table 4).

Plasmid profile of *E. coli* isolates: The result in Fig. 1 showed the plasmid profile of the multidrug resistant *E. coli* isolates which are grown in LB media containing ciprofloxacin and meropenem antibiotics and incubated at 37°C for 24 h. Amongst the isolates the number and size of plasmid varied significantly. On the basis of gel electrophoresis, the plasmid copies were found to vary between 1 and 4. The maximum numbers of plasmid copies of 4 were recorded from a total of 8 *E. coli* isolates (2, 3, 4, 5, 6, 7, 8 and 10). Although, all of

Table 2: Morphological and biochemical characteristics of *Escherichia coli* isolates

Test	*Escherichia coli*
Cell shape	Rod-shaped bacterium
Colony colour	Pink colony growth on MacConkey agar
Gram stain	Gram negative straight rods
API20E Test	*Escherichia coli*
ß-galactosidease (ONPG)	+
Arginine diydrolase (ADH)	-
Lysine decarboxylase (LDC)	+
Ornithine decarboxylase (ODC)	+
Citrate utilization (CIT)	+
H_2S production (H_2S)	-
Urease (URE)	-
Tryptophane deaminase (TDA)	-
Indole (IND)	+
Voges Prosakuer (VP)	-
Gelatinase (GEL)	-
Fermentation of glucose (GLU)	+
Fermentation of mannose (MAN)	+
Fermentation of inosito (INO)	-
Fermentation of sorbitol (SOR)	+
Fermentation of rhamnose (RHA)	+
Fermentation of sucrose (SAC)	+
Fermentation of melibiose (MEL)	+
Fermentation of amygdalin (AMY)	-
Fermentation of arabinose (ARA)	+

Table 3: Antibiotic susceptibility: Sensitivity and resistance for 50 *Escherichia coli* isolates were tested against several antibiotics belonging to different antibiotics groups by disc diffusion method

			Sensitive (S)		Resistance (R)	
Antibiotic groups	Antibiotic	Symbol	N	(%)	N	(%)
Monobactams	Azteronam	ATM	20	40	30	60
Quinolone	Ciprofloxacin	CIP	12	24	38	76
Quinolone	Levofloxacin	LEV	18	36	32	64
Quinolone	Norfloxacin	NOR	12	24	38	76
Quinolone	Nalidixic acid	NA	14	28	36	72
β-lactam (pencillin)	Augmentin	AMC	9	18	41	82
β-lactam (pencillin)	Ampcillin/Sulbactam	SAM	22	44	28	56
β-lactam (cephalosporin)	Cefuroxin	CXM	2	4	48	96
β-lactam (cephalosporin)	Cefotriaxone	CRO	4	8	46	92
β-lactam (cephalosporin)	Ceftazidime	CAZ	17	34	33	66
β-lactam (cephalosporin)	Cefaclor	CEC	5	10	45	90
β-lactam (cephalosporin)	Cefotaxime	CTX	8	16	42	84
Aminoglycosides	Amikacin	AK	42	84	8	16
Aminoglycosides	Gentamicin	CN	13	26	37	74
Carbapenem	Imipenem	IPM	35	70	15	30
Carbapenem	Meropenem	MEM	30	60	20	40
Sulfonamides	Trimethoprim	SXT	7	14	44	88

Table 4: Resistance pattern: Clear zones were measured per millimeter in antibiotic resistance pattern for ten *E. coli* isolates isolated from different sample sources

No. of Isolate	Sample sources	Antibiotic resistant pattern (diameter mm)	No. of antibiotic classes
1	Urine	LEV (8 mm), MEM (10 mm), CAZ (5 mm), CN (7 mm), AK (7 mm)	4
2	Urine	NOR (9 mm), CIP (4 mm), IPM (8 mm), CXM (6 mm), CRO (5 mm)	3
3	Blood	CIP (6 mm), IPM (9 mm), AK (6 mm), CAZ (4 mm), SAM (8 mm), MEM (10 mm)	5
4	Urine	LEV (7 mm), IPM (5 mm), CN (4 mm), ATM (10 mm), AK (4 mm), SXT (5 mm)	4
5	Urine	AK (5 mm), MEM (4 mm), LEV (6 mm), ATM (9 mm), CAZ (3 mm), CXM (6 mm), CEC (4 mm)	5
6	Sputum	CIP (4 mm), NA (9 mm), IPM (10 mm), MEM (5 mm), LEV (6 mm), CAZ (4 mm), CXM (9 mm), SAM (5 mm)	4
7	Urine	CIP (5 mm), IPM (10 mm), MEM (8 mm), ATM (4 mm), CEC (3 mm), CTX (6 mm), AMC (8 mm), NA (8 mm), AK (7 mm)	6
8	Wound	CN (5 mm), LEV (9 mm), CIP (6 mm), CTX (3 mm), CAZ (5 mm), AK (10 mm), MEM (8 mm)	4
9	Urine	CIP (6 mm), IPM (5 mm), AK (8 mm), CAZ (6 mm), SAM (11 mm), MEM (10 mm)	5
10	Urine	CN (9 mm), LEV (10 mm), CIP (7 mm), CTX (3 mm), CAZ (5 mm), SAM (8 mm), AK (4 mm), IPM (5 mm)	5

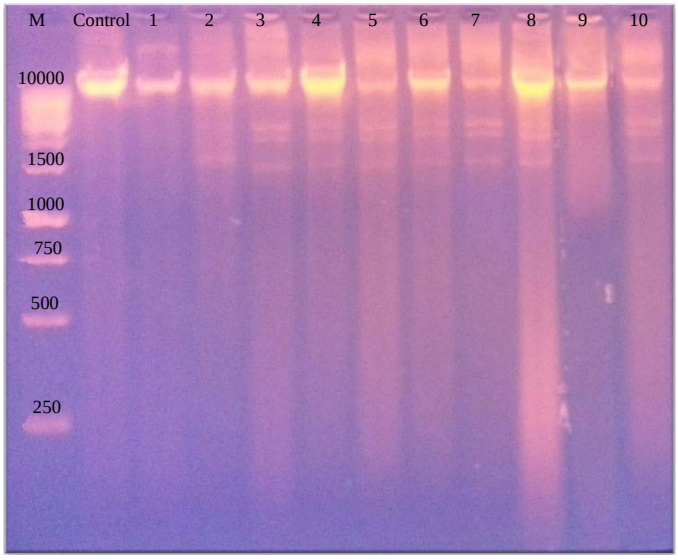

Fig. 1: Plasmid profile: Purifird plasmids from 10 multidrug resistant *E. coli* isolates (1-10) were visualized on 1% agarose gel in lanes from the third lane to the last one, as well as plasmid profile of sensitive *E. coli* (control) in the second lane. The first lane (M) is 50 bp DNA ladder

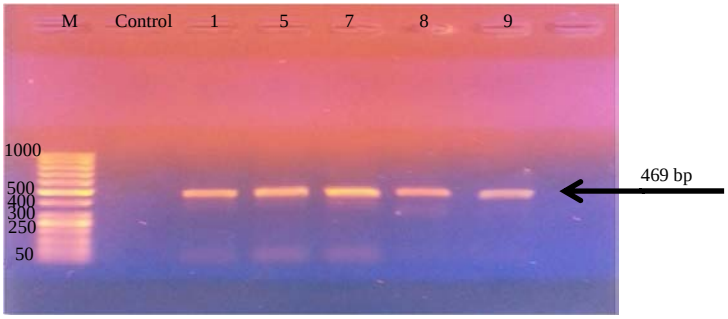

Fig. 2: PCR products of *qnrB* gene, sensitive *E. coli* isolate (control) showed negative result in second lane whereas, resistant *E. coli* isolates (1, 5, 7, 8 and 9) in lanes numbered 1, 5, 7, 8 and 9 showed positive results (469 bp). The first lane (M) is 50 bp DNA ladder

these isolates showed multidrug resistance, the resistance pattern was not the same in all cases (Table 4). The plasmid size of the common large pladmid of all isolates was greater than 10 kb. The plasmid profile of *E. coli* isolates (1 and 9) as well as the sensitive *E. coli* isolate (control) was the same showing only one small plasmid.

Detection of multidrug resistant genes: Ten multidrug resistant *E. coli* isolates that showed resistance to quinolone and carbapenem antibiotic groups were selected to detect the presence of the expected resistant genes using PCR. In PCR, plasmids from the 10 multidrug resistant *E. coli* isolates were used as DNA template to detect the presence or absence of genes responsible for the antibiotic resistance. The results in Fig. 2 showed that, *qnrB* was observed in five *E. coli* isolates (1, 5, 7, 8 and 9) giving the PCR product exhibiting the expected size (469 bp). The results in Fig. 3a and b indicated that, *qnrS* gene was observed in 7 *E. coli* isolates (2, 3, 4, 6, 7, 9 and 10) matching with the expected PCR product size (417 bp). On the other hand, *qnrA* gene was not found in all isolates as well as the control. It was clear from Fig. 4 that carpabenem gene, bla_{KPC} was observed in only *E. coli* isolate (9) exhibiting the expected PCR product size (893 bp). The result in Table 5 summarize quinolone resistant genes (*qnrA, qnrB* and *qnrS*) and the carpabenem bla_{KPC} resistant gene among *E. coli* isolates.

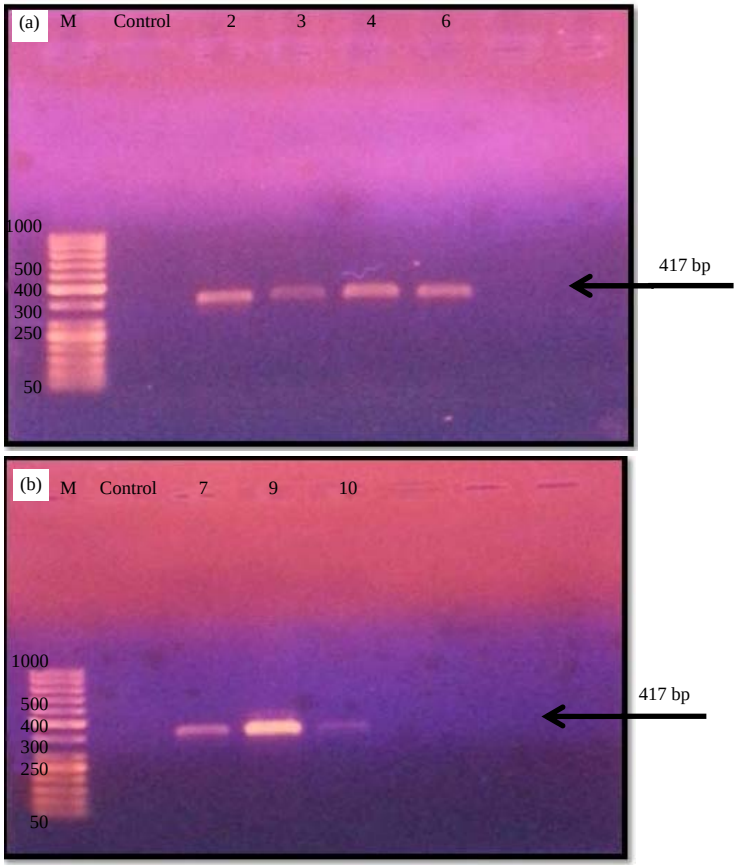

Fig. 3(a-b): PCR products of *qnrS* gene, sensitive *E. coli* isolate (control) showed negative result in second lane whereas (a) Resistant *E. coli* isolates (2, 3, 4, 6) and (b) 7, 9, 10 in lanes numbered 2, 3, 4, 6, 7, 9 and 10 showed positive results (417 bp). The first lane (M) is 50 bp DNA ladder

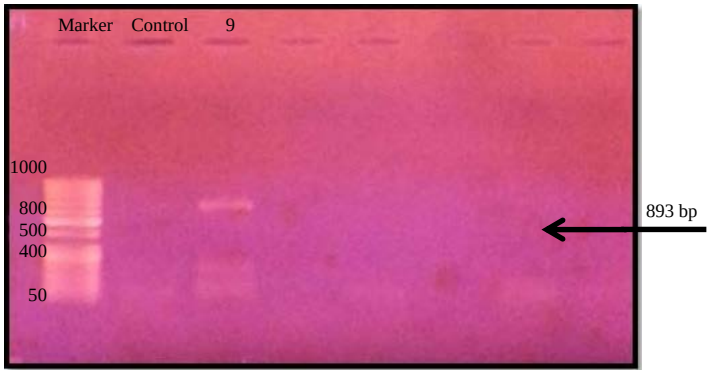

Fig. 4: PCR products of bla_{KPC} gene, sensitive *E. coli* isolate (control) showed negative result in second lane whereas, resistant *E. coli* isolate (9) in the third lane showed positive result (893 bp). The first lane (M) is 50 bp DNA ladder

Protein banding profile of multidrug resistant *E. coli* isolates: Protein banding profile of the multidrug resistant *E. coli* isolates in the peresence of antibiotics quinolone (ciprofloxacin) and carbapenam (meropenam) were illustrated in Fig. 5 and 6, respectively.

Analysis of protein banding pattern of *E. coli* isolates grown on LB containing ciprofloxacin (Table 6) showed that the total bands recorded were 19 bands distributed as; 16 monomorphic (have no change in the protein pattern like the control) and 3 polymorphic bands. In Fig. 5a and b, there

Table 5: Distrbution of the resistant genes (*qnr* and bla_{KPC}) in multidrug resistant *Escherichia coli* isolates as well as sensitive isolate (control)

Quinolone	Primers	1	2	3	4	5	6	7	8	9	10	Control
	qnrA	-	-	-	-	-	-	-	-	-	-	-
	qnrB	+	-	-	-	+	-	+	+	+	-	-
	qnrS	-	+	+	+	-	+	+	-	+	+	-
Carbapenem	*blaKPC*	-	-	-	-	-	-	-	-	+	-	-

Table 6: Analysis of the protein pattern of *Escherichia coli* grown on LB media containing ciprofloxacin antibiotic

No. of band	Molecular weight of bands	Polymorphism	RF	Control	1	2	3	4	5	6	7	8	9	10
1	130.429	Polymorphic	0.138	+	-	-	-	-	-	-	+	+	-	-
2	121.01	Monomorphic	0.226	+	+	+	+	+	+	+	+	+	+	+
3	114.632	Monomorphic	0.254	+	+	+	+	+	+	+	+	+	+	+
4	108	Monomorphic	0.283	+	+	+	+	+	+	+	+	+	+	+
5	102.319	Monomorphic	0.313	+	+	+	+	+	+	+	+	+	+	+
6	94.424	Monomorphic	0.354	+	+	+	+	+	+	+	+	+	+	+
7	90.587	Polymorphic	0.370	+	+	+	+	+	+	+	+	+	+	-
8	85.674	Polymorphic	0.380	-	+	-	-	-	+	-	-	-	+	-
9	75.184	Monomorphic	0.438	+	+	+	+	+	+	+	+	+	+	+
10	70.925	Monomorphic	0.528	+	+	+	+	+	+	+	+	+	+	+
11	65.195	Monomorphic	0.542	+	+	+	+	+	+	+	+	+	+	+
12	60.358	Monomorphic	0.565	+	+	+	+	+	+	+	+	+	+	+
13	56.205	Monomorphic	0.621	+	+	+	+	+	+	+	+	+	+	+
14	52.342	Monomorphic	0.658	+	+	+	+	+	+	+	+	+	+	+
15	47.39	Monomorphic	0.709	+	+	+	+	+	+	+	+	+	+	+
16	43.181	Monomorphic	0.757	+	+	+	+	+	+	+	+	+	+	+
17	40.096	Monomorphic	0.799	+	+	+	+	+	+	+	+	+	+	+
18	37.483	Monomorphic	0.829	+	+	+	+	+	+	+	+	+	+	+
19	33.96	Monomorphic	0.881	+	+	+	+	+	+	+	+	+	+	+
No. of bands/lane			18	18	17	17	17	18	17	18	18	18	16	
Total No. of bands									19					
No. of monomorphic bands									16					
No. of unique bands									0					
No. of polymorphic bands									3					

Table 7: Analysis of the protein pattern of *Escherichia coli* grown on LB media contaning meropenem antibiotic

No. of band	Molecular weight of bands	Polymorphism	RF	Control	1	2	3	4	5	6	7	8	9	10
1	122.92	Unique	0.22	+	-	-	-	-	-	-	-	-	-	-
2	112.9065	Polymorphic	0.267	+	+	+	+	+	-	+	+	+	+	+
3	100.240	Monomorphic	0.332	+	+	+	+	+	+	+	+	+	+	+
4	94.696	Monomorphic	0.363	+	+	+	+	+	+	+	+	+	+	+
5	86.505	Monomorphic	0.414	+	+	+	+	+	+	+	+	+	+	+
6	82.807	Monomorphic	0.444	+	+	+	+	+	+	+	+	+	+	+
7	78.466	Polymorphic	0.472	-	+	-	+	+	-	-	-	-	-	-
8	74.223	Polymorphic	0.496	+	+	+	+	+	-	+	+	+	+	+
9	72.936	Monomorphic	0.510	+	+	+	+	+	+	+	+	+	+	+
10	69.891	Polymorphic	0.531	+	+	+	+	+	+	+	+	+	-	+
11	67.226	Monomorphic	0.554	+	+	+	+	+	+	+	+	+	+	+
12	63.504	Monomorphic	0.591	+	+	+	+	+	+	+	+	+	+	+
13	60.172	Monomorphic	0.616	+	+	+	+	+	+	+	+	+	+	+
14	55.08	Polymorphic	0.656	+	-	+	-	-	+	+	+	+	+	+
15	51.489	Monomorphic	0.710	+	+	+	+	+	+	+	+	+	+	+
16	47.404	Monomorphic	0.745	+	+	+	+	+	+	+	+	+	+	+
17	41.749	Monomorphic	0.810	+	+	+	+	+	+	+	+	+	+	+
18	37.62	Monomorphic	0.870	+	+	+	+	+	+	+	+	+	+	+
19	34.3153	Monomorphic	0.923	+	+	+	+	+	+	+	+	+	+	+
20	30.519	Monomorphic	0.963	+	+	+	+	+	+	+	+	+	+	+
No. of bands/lane				20	18	18	18	18	16	18	18	18	18	18
Total No. of bands									20					
No. of monomorphic bands									14					
No. of unique bands									1					
No. of polymorphic bands									5					

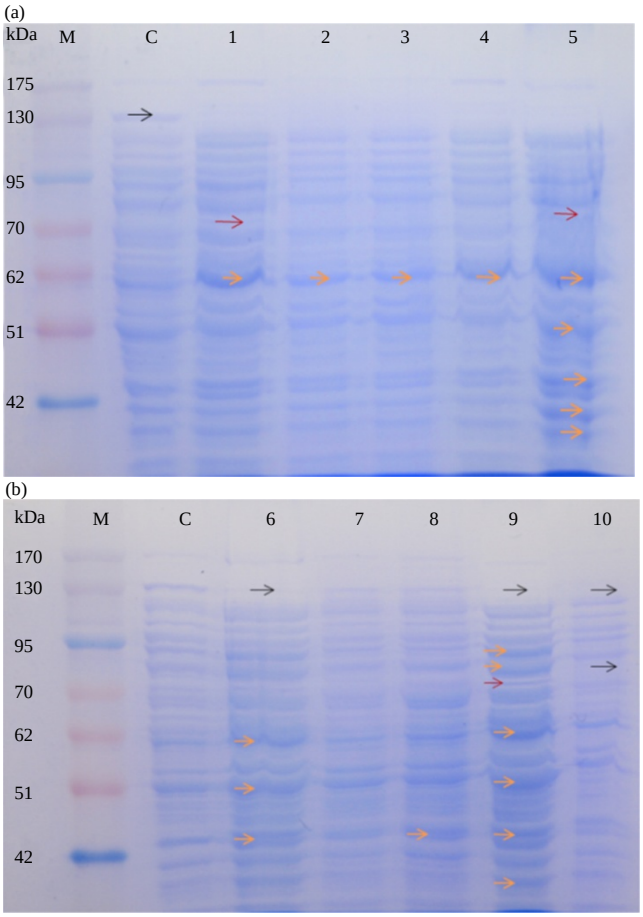

Fig. 5(a-b): Protein profile with ciprofloxacin: Proteins from 10 *E. coli* isolates grown on LB media containing ciprofloxacin were separated on 14% acrylamide gel (a) Gel contained samples from1-5 and (b) From 6-10 Control sample (C) is a protein of *E. coli* grown without antibiotic. Disappeared bands indicated by black arrow, new bands indicated by red arrow and enhanced bands indicated by orange arrow. The first lane (M) is protein ladder

were two bands present in the control and disappeared from the isolates with molecular mass (130.4 and 90.5 kDa) (black arrows). One new band with molecular mass (85.6 kDa) was raised in isolates (1, 5 and 9) (red arrows).

On the other hand, analysis of the protein banding profile of *E. coli* grown on LB media containing meropenem antibiotic (Table 7) indicated that 20 total bands distributed as 14 monomorphic (have no change in the protein pattern like the control), 5 polymorphic and 1 unique bands. In Fig. 6a and b, there were 4 bands present in control and disappeared from isolates with molecular mass (122.9, 74.2, 69.8 and 55.08 kDa) (black arrows). One new band with molecular mass (78.46) rasied in isolates (1, 3 and 4) (red arrows).

DISCUSSION

Resistance to antibacterial is highly prevalent in bacterial isolates worldwide, particularly in developing countries. Normal intestinal flora is a reservoir for resistance genes; the prevalence of resistance in *E. coli* is a useful indicator of antibiotic resistance in bacteria at the community. The correlation between antibiotic resistance and plasmid profile may indicate that the genetic information is plasmid borne[27].

In this study, it is observed that *E. coli* isolates from urine were obtained (58%) of the samples. In other studies, it estimated about 150 million infections worldwide through Urinary Tract Infections (UTI). The most common nosocomial infection occurred in many hospitals is approximately 35%

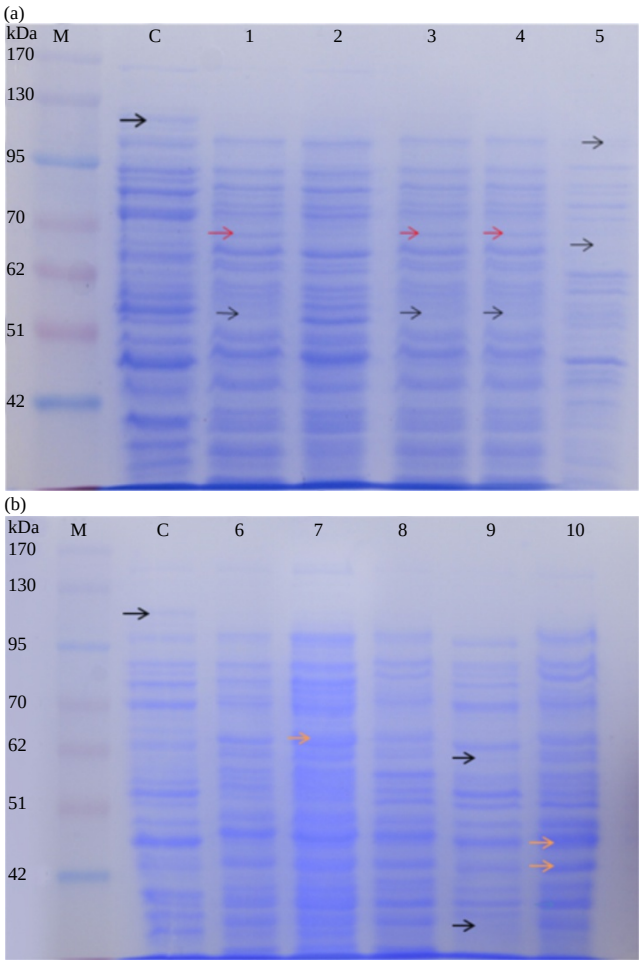

Fig. 6(a-b): Protein profile with meropenem: Protein banding profile, proteins from 10 *E. coli* isolates grown on LB media containing meropenem were separated on 14% acrylamide gel (a) Gel contained samples from 1-5 and (b) From 6-10, Control sample (C) is a protein of *E. coli* grown without antibiotic. Disappeared bands indicated by black arrow, new bands indicated by red arrow and inhanced bands indicated by orange arrow. The first lane (M) is protein ladder

of all hospital acquired infections[28]. The most common etiological agent in UTI is *E. coli* from uncomplicated urinary tract infection isolates[29,30].

The *E. coli* isolates collected from different pathological specimens showed different degree of sensitivity to seventeen different antimicrobials. The *E. coli* isolates were highly resistant cefuroxim (96%), cefotriaxone (92%), cefaclor (90%) and ciprofloxacin (76%). The least resistance of *E. coli* isolate was to meropenem (40%), imipenem (30%) and amikacin (16%).

In another studies, out of 138 *E. coli* strains isolated from urine samples; 62 isolates (44%) were multi drug resistant obtained by antibiotic susceptibility pattern by Kirby-Bauer disc diffusion test. Fourteen clinically prescribed antibiotics were tested, in which high level of resistance was seen to ciprofloxacin (75%), gatifloxacin (68%), ceftazidime (62%), meropenem (51%), imipenem (39%) and nitrofurantoin (20%)[31].

In this study, the *qnr* genes were detected in all isolates. The distribution of *qnr* resistant genes in *E. coli* isolates were *qnrA* (0%), *qnrB* (50%), *qnrS* (70%) and *qnrB* and *qnrS* (20%). In another study, the *qnrA* genes were present in 3.88% of *E. coli* isolates and 7.69% of *Enterobacter cloacae*. The *qnrB* genes were present in 6.20% of *E. coli* isolates and 7.69% of *Enterobacter cloacae*. The *qnrS* genes were present in 2.23% of *E. coli* isolates and 18.92% of *Klebsiella pneumoniae*[32]. The rate of *qnrA* positive *E. coli* isolates is lower than that in Shanghai reports in china[24].

The occurrence of an outer membrane porin deficiency and the expression of a plasmid-mediated class C β-lactamase were reported to be responsible for carbapenem resistance in *E. coli*[8]. The serine carbapenemase bla_{KPC} (*Klebsiella pneumoniae* carbapenemase) has emerged as a β-lactamase capable of inactivating carbapenem antibiotics. The bla_{KPC} is plasmid transmissible among Enterobacteriaceae, which has implications for infection control[33]. In this study, the bla_{KPC} genes were detected in one isolate (10%), although all isolate are resistant to carpabenem antibiotic as detected by disc diffusion method indicating that the presence of another mechanism of resistance. The presence of bla_{KPC} may not always result in carbapenem resistance *in vitro*, thereby impeding detection during routine workup[34]. The bla_{KPC} gene has been found associated with the plasmid-borne transposon Tn4401, which may be responsible for its rapid dissemination[35]. It was observed that the bla_{KPC} resistant gene was detected in 24 and 62% of *Klebsiella pneumoniae* and *E. coli* isolates, respectively[36].

Antibiotic resistance in bacteria has several mechanisms such as; antibiotic inactivating enzymes, extrusion of antibiotics by efflux pumps, ultimate alteration, in metabolic pathways to prevent antibiotic from reaching to their target domains[37].

The β-lactam and quinolone antibiotics enter the bacterial cells at first to induce their effect. Antibiotics pass through porins (proteins in the outer membrane of bacteria). There are several type of porins (*OmpF*, *OmpC*, *OmpD*, *PhoE*, *LamB*, *OmpA* and *OmpK36*)[38]. In *E. coli*, there are cation-selective (*OmpF* and *OmpC*) and anion-selective (*PhoE*) porins, with opposite voltage-dependences for *OmpF* and *PhoE*[39]. Carbapenem resistance can arise through the acquisition of resistance genes encoding metallo-β-lactmases, non metallo-carbapenemases (bla_{KPC}, *GES* or *OXA*-type) and an alteration in the expression of the outer membrane protein (OMP)[40]. Resolustion of three-dimensional structure of the *E. coli ompF* and *Klebsiella pneumoniae* OmpC-like porin (ompK36) has led to the identification of the functional domains of the channels[41]. Recent studies have identified amino acids important in porin structure and function in bacteria[42]. The replacement of these amino acids by mutation may greatly decrease diffusion through the porin in some isolates[43].

In MRD *E. coli*, the upregulation of the acquired genes (bla_{KPC}) encoding β-lactamase and quinolone genes (*qnrA*, *qnrB* and *qnrS*) encoding altered target proteins or efflux pumps might be behind the increase in bands intensity of protein profile or appearance of new protein bands.

Analysis of the protein banding profile of the multidrug resistant *E. coli* isolates treated with antibiotic from the group of quinolone (ciprofloxacin) and carbapenam (meropenam). Indicated that in ciprpfloxacin, there was 19 total bands distributed as 16 monomorphic, 3 polymorphic bands. However, In csae of meropenem the 20 total bands were distributed as 14 monomorphic, 5 polymorphic and 1 unique bands.

CONCLUSION

In conclusion, antibiotics resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the prominent public health concern of the 21st century. Antibiotic resistance as a major medical problem for patient and physician could be due to genetic or physiological factors. There is an urgent need to formulate a policy and put the necessary plan in place to execute a policy targeted at the promotion of rational use of antibiotics as an important element in antibiotic resistant containment. It can be recommended that a combination of traditional and advanced prevention and treatment strategies should be organized to combat the threat of emerging antibiotic resistance among uropathogens.

SIGNIFICANT STATEMENTS

- Survey the presence or absence of MDR *E. coli* in Mansoura University Hospitals
- Providing a step to build up MDR data base in Mansoura, Egypt
- Make an attention towards the confinements of intensive use of antibiotics to avoid high cost and long-term treatment

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