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NEW PLASMID MEDIATED QUINOLONE RESISTANCE GENE (*QNR*C) FOUND IN *PROTEUS MIRABILIS*

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ABSTRACT : A total number of [100] midstream urine samples were collected randomly from patients suspected to be infected with UTIs in Al-Hilla Hospital, Babylon from 15 Oct. to 15 Jan. 2018. These samples were streaked by the sterile loop on MacConkey and EMB agar plates, then incubated aerobically 37C^o for 24 hrs. Detection of the causative agents was performed according to the routine diagnostic steps illustrated in Baily and Scott's diagnostic microbiology *i. e.* colonial morphology and the biochemical reactions. Antibiotic susceptibility patterns were done phenotypically determined by clinical laboratory standard Institute (CLSI) (2014); finally, the disclosure of some resistant genes (*par C*, *qnr C* and *aac(6')-Ib-cr*) were diagnosed by PCR technique. The results of the presents study showing that nineteen isolates (72 %) identified as *E. coli*. The results demonstrated that the highest percentage of UTIs was found in third trimester 44% followed by 29% and 27% for second and first trimesters, respectively. Age group (16-22) yrs. occupied the highest ratio 34%. Antibiotic susceptibility patterns phenotypically (disc diffusion method) illustrated that the high proportion of resistance was 73.7% (14/19) for amoxicillin, while the lowest ratio was 0% (0/19) for gentamycin antibiotic. *bla*-TEM-1 was predominant resistant gene 42.10% succeeded by *bla*-CTX-M 31.57% while another gene frequencies were 26.31% and 21.05% for *aac (6')- Ib-cr* and *sul*-1, respectively.Two isolates (10.52%) appear to be bearing *bla-CTX-M* and *bla-TEM-1*, one isolate bearing *bla-CTX-M+ sul-1*, one isolate bearing *bla-CTX-M+ bla-TEM-1+ sul1*, one isolate bearing *aac (6')-Ib-cr+ bla-TEM-1*, and three isolates bearing *bla-TEM-1+sul1*. In conclusion, the virulence of a bacterium has increased significantly as a result of its acquisition of properties that enable it to resist the third generation of cephalosporin's in addition to other antibiotics.

Key words : *E. coli*, *bla-CTX*-M, *bla- TEM*-1, *aac (6')-Ib-cr*, *sul*-1.

INTRODUCTION

Proteus mirabilis, a gram-negative enteric bacterium, occurs as vegetative swimmer cells and hyper flagellated swarmer cells (Alazzwi *et al*, 2011). It was one of the most common causes of urinary tract infections (UTIs) in persons with long term indwelling catheters, complicated UTIs and bacteremia among the elderly (Hussien, 2013). The medical importance of this bacterium may in its ability to produce a variety of extracellular enzymes such urease, which was responsible for the formation of bladder and kidney stones, the formation of stones around the bacteria prevent antibiotic cure effect. Additionally, the hemolysin was cytotoxic for urinary tract epithelial cell (Jansen, 2003).

Quinolones were potent antibacterial agents that specifically target bacterial DNA gyrase and topoisomerase IV. Widespread use of these agents had contributed to the rise of bacterial quinolone resistance. Previous studies had shown that quinolone resistance arises by mutations in chromosomal genes. Recently, a multi-resistance plasmid was discovered that encodes transferable resistance to quinolones. The gene product Qnr was a 218-aa protein belonging to the penta-peptide repeat family and shared sequence homology with the immunity protein McbG, which was thought to protect DNA gyrase from the action of microcin B17. Qnr had penta-peptide repeat domains of 11 and 28 tandem copies, separated by a single glycine with a consensus sequence of A/C D/N L/FXX.

Plasmid-mediated quinolone resistance was first described for a ciprofloxacin-resistant strain of *Klebsiella pneumoniae* in 1998 (Hata, 2005). The responsible gene, *qnr* (later named *qnrA*) was located on plasmid pMG252, which encodes multidrug resistance proteins.

qnrB and *qnrS* were discovered in 2005 and 2006, respectively and mediated similar levels of ciprofloxacin

Table 1 : The primers were used in this study.

Target Gene	Sequence	bp	Reference
qnrC	F 5'-GGGTTGTACATTTATTGAATC-3' R 5'-TCCACTTTACGAGGTTCT-3'	447	Cremet *et al* (2011)
***par*C**	F 5' - TGTATGCGATGTCTGAACTG -3' R 5' - CTCAATAGCAGCTCGGAATA -3'	264	Antonio *et al* (2007)
aac(6)- Ib-cr	F 5' -TTGCGATGCTCTATGAGTGGCTA -3' R 5' -CTCGAATGCCTGGCGTGTTT-3'	482	Kim *et al* (2009)

resistance (Tran, 2002; Hata, 2005). Qnr proteins belong to the penta-peptide repeat protein (PRP) family and protect DNA gyrase and topoisomerase IV from quinolone inhibition (Tran, 2005; Jacoby, 2008; Cavaco, 2009). *Qnr* genes shows a high level of diversity; there were at least 6*qnrA*, 20*qnrB* and 3*qnrS* alleles reported, with one or more amino acid alterations within each family. More recently, *qnrD* was found in *Salmonella* isolates (Robicsek, 2006). *Qnr* genes are widely distributed in clinical *Enterobacteriaceae* isolates around the world and were usually associated with motile elements (Cattoir, 2007).

Plasmid mediated quinolone resistance (PMQR) genes such as *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, and *aac(62)Ib-cr* had been increasingly reported in bacterial pathogens. The *qnr* type PMQR genes bind to DNA gyrase and topoisomerase to block the action of fluoroquinolones resulting in reduced susceptibility to fluoroquinolones (Tran *et al*, 2005). *qnr*C differs substantially from existing families, with >30% (40 to 68%) differences in comparison to *qnr*A, *qnr*B, *qnr*S, and *qnr*D and also >30% (36 to 58%) differences in derived amino acid sequences (Tran and Jacoby, 2002, Rodríguez-Martínez *et al*, 2008).

qnrC was a new plasmid-borne qnr gene, in addition to three existing families, *qnrA*, *qnrB* and *qnrS*. The name *qnrC* was designated according to the recently published qnr numbering proposal by Jacoby *et al* (2008).

Thus, this study was designed to detect the presence quinolones resistance gene type C *qnr*C gene in *Proteus mirabilis* isolated from UTIs.

MATERIALS AND METHODS

Specimens collection

Urine samples were collected randomly from the 100 recruited patients suspected to be infected with UTIs. These samples were cultured on blood and MacConkey agar plates. *Proteus mirabilis* was diagnosed depend on its properties, further identification steps were performed according to Baily and Scotts' diagnostic microbiology.

Antibiotic susceptibility test

This test was performed according to clinical laboratory standard institute (CLSI, 2014).

Extraction of bacterial DNA

Total DNA of isolates was extracted by boiling to 100°C for 10 min a suspension of the strains in 200 µl of distilled water and centrifugation for 7 min at 13,000 ×g, then the supernatant obtained was stored at -20°C. PCR experiments were performed with these crude lysates.

Molecular characterization of studied genes

Polymerase chain reaction was used to amplify the entire sequence of the genes described by previous investigators as in Table 2. These genes were produced shown in Table 1. The amplified PCR products were detected by gel electrophoresis visualized by staining with ethidium bromide.

Statistical analysis

Statistical analysis was performed by using SPSS computing program version 16 for the analysis of the results.

RESULTS AND DISCUSSION

Microbial and anthropometric results

Urinary tract infections midstream urine examination of the randomly recruited 100 patients 45 (45%) males and 55 (55%) females (figure one) from Babylon province, revealed that only 13 (13%) of patients gave positive results for *P. mirabilis* (Fig. 1). Ten of them (76.92%) donated mixed growth with other bacterial genera and 3 (23.08%) were pure culture (Fig. 2). Most of those patients 90 (90%) were outpatients (OP) P<0.000, and 10 of them (10%) were inpatient.

Distributed patients according to the gender the results revealed that the highest percent of infected patients' (36%) located under the age group 15-35 years old, while the lowest ratio was 12% and it was positioned below the age group less than 15 years old (Table 3).

Outpatient occupied 96.77% underneath the age group 15-30 years old, on the other hand the lowermost

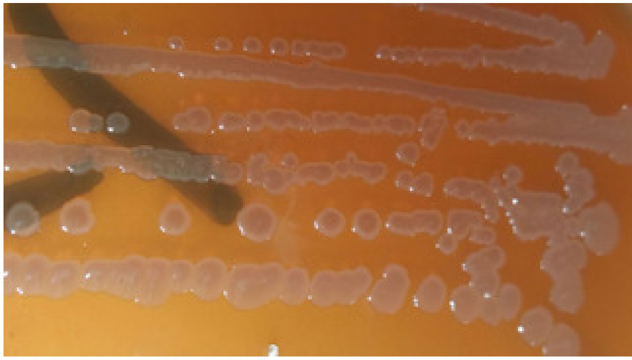

Fig. 1 : *P. mirabilis* isolate growing on MacConkey's agar after 24 hrs. of aerobic incubation.

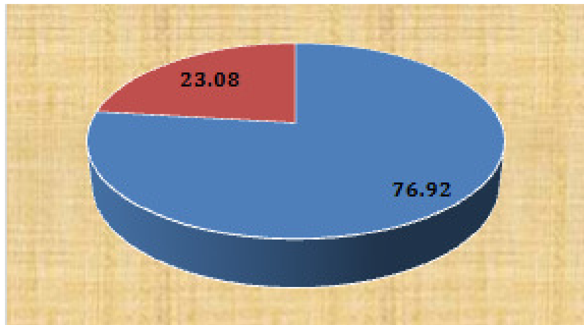

Fig. 2 : Distribution of patients recruited in this study according to the gender.

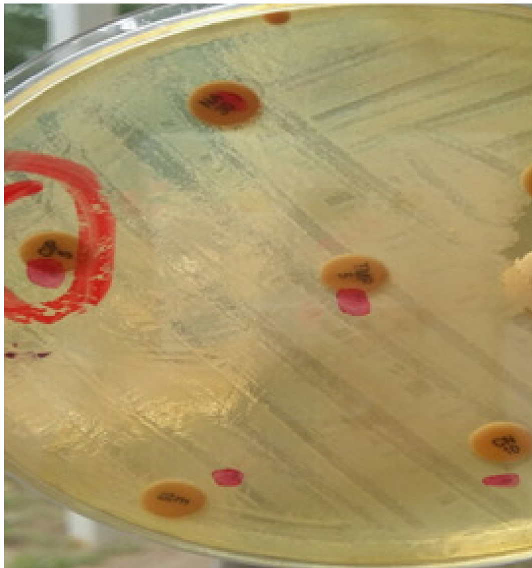

Fig. 3 : Multidrug resistant isolate of *P. mirabilis* isolated from patient with UTIs in this study.

Fig. 4 : Ethidium bromide staining gel electrophoresis product of *par-c* gene (264 bp). Lane 1 (L); 100 bp ladder, lane 1 negative result, lanes, 2, 3, 4, 5, 6, 7, positive *parC* results.

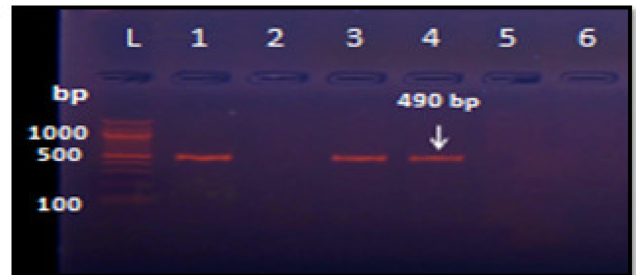

Fig. 5 : .

part was under the age group less than 15 years old.

Antimicrobial susceptibility results

The results in Table 5 revealed that the most effective agent against the bacterial isolates was Imipenem (IPM), the results showed that 11 isolates (84.61%) were sensitive to this antibiotics among 13 isolates, followed by norfloxacin (NOR) (10/13) isolates (76.92%) were sensitive, while the lowest ratio was (2/13) 15.38% for tetracycline (TET). On the other hand, another antibiotics give rise different ratios, there were 61.54% (8/13), for gentamicin (CN), ciprofloxacin (CIP) and Cefoxitin (FOX), 46.15% for Amoxicillin-clavulanate (AMC), 53.85% (7/13) for each of trimethoprim (TMP) and Ceftazidime (CAZ), 69.23% (9/13) for Cefotaxime (CTX), Amikacin (AK) and finally (3/13) 23.08% for nitrofurantoin antibiotic (Table 5) (Fig. 3).

Molecular detection of resistance genes

Detection of the *par-c* gene

par-C gene, which encode for enzymes responsible for catalysis fluoroquinolone antibiotics using PCR technique with specific forward and reverse primers. The results shown that only (6 of 13) 46.15% of the current

Table 2 : Programs of PCR thermo-cycling conditions of primers.

Gene Name	Temperature (°C) / Time					Cycles Number
	Initial Denaturation	Cycling Conditions			Final Extension	
		Denaturation	Annealing	Extension		
qnrC	95°C /5min	95°C /1min	55 °C/1 min	72°C/1 min	72°C/5min	30 cycles
***par*C**	95°C /5min	95°C /30 sec	55.2°C/30sec	72°C/30sec	72°C/5min	30 cycles
aac(6')-Ib-cr	94°C /4 min	94°C /45 sec	55°C/45sec	72°C/45sec	72°C/5min	40 cycles

Table 3 : Age related distribution of patients according to the gender in Babylon province.

		Age (years) (No./%)			
		Under 15	**(15-30)**	**(30-45)**	**More than(45)**
Gender	Male	4(33.33%)	18(50%)	8(38.09%)	15(48.38%)
	Female	8(66.67%)	18(50%)	13(61.91%)	16(51.61%)
Total		12	36	21	31

Table 4 : Distribution of recruited patients according to the age groups, splits by resident in a hospital.

	Age (years)			
	Under 15	**(15-30)**	**(30-45)**	**More than(45)**
OP	10(83.33%)	33(91.66%)	17(80.95%)	30(96.77%)
IP	2(16.67%)	3(8.34%)	4(19.05%)	1(3.23%)
Total	12	36	21	31

Table 5 : Antibacterial sensitivity of selected antibiotics.

No.	Antibiotic:Name (Abbreviation)	Resistance	Sensitive
1	Gentamicin (CN)	5	8
2	Ciprofloxacin (CIP)	5	8
3	Amoxicillin-clavulanate (AMC)	7	6
4	Norfloxacin (NOR)	3	10
5	Tetracycline (TET)	11	2
6	Trimethoprim (TMP)	6	7
7	Cefoxitin (FOX)	5	8
8	Cefotaxime (CTX)	4	9
9	Nalidixic acid (NA)	13	0
10	Nitrofurantoin (F)	10	3
11	Ceftazidime (CAZ)	6	7
12	Amikacin (AK)	4	9
13	Imipenem (IPM)	2	11

study isolates bearing *par-c* gene tested isolates represented in *Proteus mirabilis* (Fig. 4).

Codons 83 and 87 in *gyrA* and codons 80 and 84 in p*arC* gene display the most common alterations in clinical isolates. In addition, expression of high level fluoroquinolone resistance in *Enterobacteriaceae* requires the presence of multiple mutations in *gyrA* and/ or *parC* genes.

Detection of the *aac(6´)-Ib-cr*gene

*aac(6´)-Ib-cr*gene were detected in only three isolates (23.07%) of *P. mirabilis*.

Detection of the *qnr C* gene

Unfortunately, none of the bacterial isolates under study to contain the resistance gene *qnr*C.

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