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Detection of some genes resistance to quinolones in *pseudomonas aeruginosa* isolated from different clinical sources in Al-Diwaniya

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Abstract

Pseudomonas aeruginosa is a clinically important pathogen that causes op-portunistic infections and nosocomial outbreaks. Fluoroquinolonesare an important component of antipseudomonal chemotherapy. The major mechanism of resistance of this bacterium to fluoroquinolones is the modification of type II topoisomerases (DNA gyrase and topoisomerase IV). In this study, we examined the mutations in quinolones resistance-determining regions (QRDR) of gyrA, gyrB, parC and parEgenes. A total of 350 clinical isolates were routinely collected. The most common origin of isolates was 162 ear swab followed by 98 burn swabs, 50 sputum samples and 40 urine samples. The results of culture and biochemical tests showed that 50 isolate was *P. aeruginosa*, which then confirmed by api-20E followed by the genotyping detection by 16S rRNA gene using PCR technique. Antibiotic susceptibility test (by using the Kirby-pour technique) was performed to all these isolates, The results of antibiotic susceptibility test showed that 10 isolate (20%) resistance to at least one type of fluoroquinolones which represented by ciprofloxacin the highest (20%) and norfloxacin (14%) the lowest. The isolates that resistance to at least one type of fluoroquinolones were screened for the presence of genes (gyrA, gyrB, parC and parE) by polymerase chain reaction. The genesgyrA and parE were found in 10(100%) fluoroquinolones resistant isolates, followed bygyrB4 (40%), parC 2(20%). The most repeated combinations, gyrA+ parE and gyrA+gyrB + parE were detected in (50%) and (30%) of isolates, respectively.

Keywords: *P. aeruginosa*; Fluoroquinolones; Resistance; DNAgyrase; Topoisomer-ase IV

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Introduction

*Pseudomonas aeruginosa*is a serious nosocomial bacterial pathogen mainly due to the high level of antibiotic resistance, which is multiple intrinsic and acquired mechanisms of resistance to a wide variety of antibiotics [1, 2]. Fluoroquinolones are bactericidal, rapidly acting antimicrobial drugs with wide spectrums. They are very effective

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against many Gram negative bacterial pathogens [3]. The main mechanisms of resistance are mutations in the target genes, those encoding DNA gyrase (*gyrA*, *gyrB*) [4], and topoisomerase IV (*parC*, *parE*) [5]. Common mutations in fluoroquinolones-resistant isolates occured in the *gyrA*gene [6]. The aim of this study was todetermine the occurrence ofmutations related to fluoroquinolones resistance *(gyrA*, *gyrB*, *parC* and *parE* genes) in *P. aeruginosa* isolated from Al-Diwaniya teaching hospital in Al-Diwaniya province.

Materials and Methods

Collection of Samples

A total of 350 samples were collected during the period from November 2014 to April 2015, Clinical samples were obtained from patients suffering various infections from Al-Diwaniya teaching hospital in Al-Diwaniya city. Samples which were collected by sterile swabs and containers had been cultured on macConkey agar and blood agar, to get pure colonies subculture done on macConkey agar, incubated for overnight at 37C°. The most common origin of isolates were ear swab (46.3), followed by burn (28%), sputum (14.3 %), and urine (11.4%). Isolate confirmations were conducted using conventional biochemical tests and confirmed by the api20E system, followed by the genotyping detection using PCR technique.

Antibiotic Susceptibility Assays

The antibiotic susceptibility pattern of the *P. aeruginosa*isolates was determined using the disk diffusion method according to the modified Kirby-Bauer technique on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. Susceptibility of the selected isolates to two fluoroquinolone antibiotics [ciprofloxacin (5µg), norfloxacin (10µg)]. All drugs were obtained from (Bioanalyse company-Turkey).

Polymerase Chain Reaction Amplification (PCR)

Is used to screen for the presence of the fluoroquinolone genes: *(gyrA, gyrB, parC and parE)* genes. Primers were designed from sequences deposited in the GenBank database Table (1). The total template DNA for the PCR amplification was extracted from the supernatant of a mixture of *P. Aeruginosa* cells produced by salting out method [8]. PCR amplification was performed using 5µl of the template DNA, 2µl of each primer, 10 µl master mix, and 1 µl of Taq DNA polymerase in a total volume of 20µl. A thermocycler (Mastercycler gradient; Eppendorf, Hamburg, Germany) was programmed with the appropriate conditions [8]. Then, 5µl of each PCR product was analyzed by electrophoresis on 1% (w/v) TAE agarose gel containing 0.1 µl/mL ethidium bromide [9]. The amplicon were then visualized on a UV transilluminator and photographed (BioDoc-Analyse; Biometra, Goettin-gen, Germany).

Table 1.

DNA Primer which purchased from Bioneer (Korea) company.

Primer name	Oligosequence (3'-5')		Amplicon	Genbank code
16S rRNA	F	TCAACCTGGGAACTGCATCC	468bp	FM881781.1
	R	ACATCTCACGACACGAGCTG		
gyrA	F	GAGGAACTGGAAGCGGTCAA	315bp	NC002516.2
	R	CGGAATCTCGAAGGTACGCA		
gyrB	F	CCGGAGACCTTCAGCAACAT	403bp	FJ652724.1
	R	TGAATTTCTTCGCCAGGCCT		
ParC	F	CTCTCGGAAAAAGGCTGGGT	458bp	AB003428.1
	R	AACAATAGCAGACGGCCCTC		
ParE	F	GAAATGGCGGACGAACAGC	244bp	AY164481.1
	R	GGACAAGGAATTCCAGGCGA		

*F= Forword Primer. *R = Reverse Primer.

Results

In the current study, a total of 350 clinical samples have been collected, only 50(14.3 %) isolates were belonging to *P. aeruginosa* Table (2), in which 16.7% of them were isolated from ear swab, 13.3% were isolated from burn while 12% and 10% were isolated from sputum and urine respectively.

Table 2.

Incidence of the isolated microorganisms in different clinical sample sites

Source of samples	No. of Samples	No.(%) of *P. aeruginosa* Isolates	No.(%) of Gram positive and negative isolates	No.(%) of no growth or contaminated cultures
Ear	162	27(16.7 %)	55(33.9%)	80(49.4 %)
Burn	98	13(13.3 %)	34(34.7 %)	51(52%)
Sputum	50	6(12%)	15(30%)	29(58%)
Urine	40	4(10%)	10(25%)	26(65%)
Total	350	50(14.3 %)	114(32.6 %)	186(53.1%)

Either of the genetic by Monoplex PCR, using *16S rRNA* gene for diagnose *P. aeruginosa*. The results of current study all isolates of *P. aeruginosa*contian*16S rRNA* gene, which represents a diagnostic gene for these bacteria, Figure (1).

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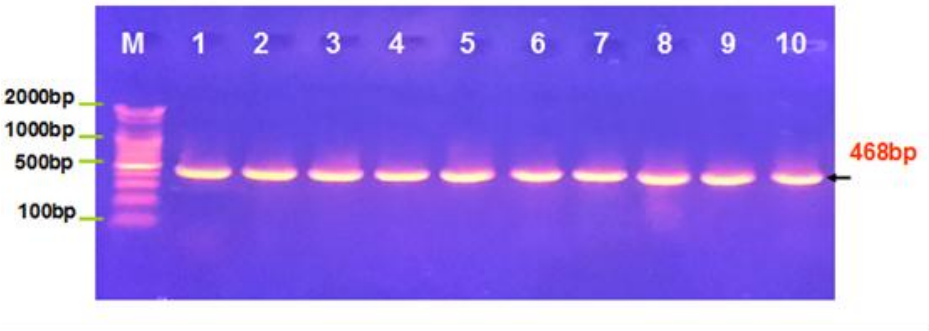

Figure 1.

Ethidium bromide-stained agarose gel (1% agarose) of PCR amplified products from extracted DNA of *P. aeruginosa* amplified with *16S rRNA*gene primer. The electrophoresis performed at 80 volt for 1 hr. Lane(L), DNA molecular size marker (100-2000bp ladder). Lanes (1-10) of isolates show positive results with (468bp).

The isolated bacteria showed variable results of resistance to fluoroquinolones: ciprofloxacin and norfloxacin. Based on the results from susceptibility testing, Table (3).

Table 3.

Incidence of antibiotic resistance in *P. aeruginosa* isolates (*n*=50).

Antibiotic	Sensitive	%	Intermediate	%	Resistant	%
Ciprofloxacin	31	62	9	18	10	20
Norfloxacin	32	64	11	22	7	17

To investigate the mechanism of fluoroquinolones resistance among *P. aeruginosa*, Figure (2). Showed the distribution of fluoroquinolones resistance genes among fluoroquinolones resistance *P. aeruginosa* isolates, the frequency of*gyrA* and *parE* were (100%), while the frequency of *gyrB* was (40%) and *parC* was (20%).

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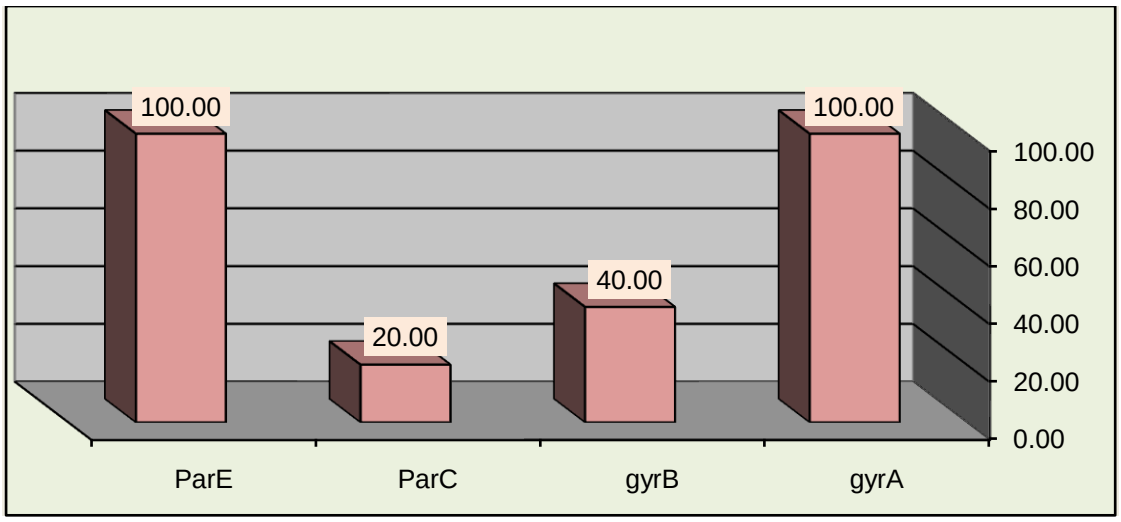

Figure 2.

Show the distribution of fluoroquinolones resistance genes among *P. aeruginosa* isolates.

The results of this study showed that thedistribution of quinolone antibiotics-resistance genes and their combinations isolates of *P. aeruginosa* Table (4).

Table 4.

The distribution of quinolone antibiotics-resistance genes and their combinations isolates of *P. aeruginosa*.

Occurrence of gene	No.(%) of *P. aeruginosa* isolates
gyrA+ pare	5 (%50)
gyrA+parC+ pare	1 (10%)
gyrA+gyrB + parE	3 (30%)
gyrA+gyrB+ parC+ pare	1 (10%)
Total	10 (100%)

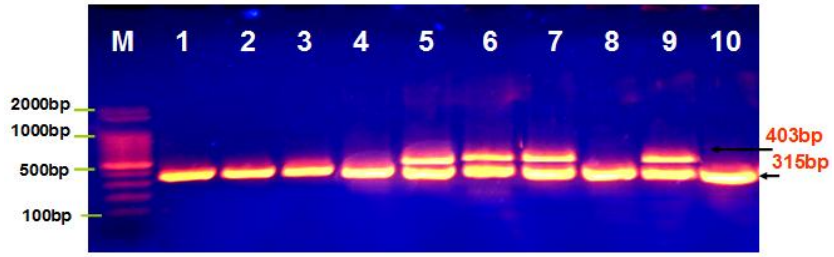

Figure 3.

Ethidium bromide-stained agarose gel (1% agarose) of PCR amplified products from extracted DNA of *P. aeruginosa* amplified with *gyrA* and *gyrB* genes primers. The electrophoresis performed at 80 volt for 1 hr. DNA molecular size marker (100-2000bp ladder). 10of isolates show positive results with *gyrA* (315bp), 4 of isolates show positive results with *gyrB* (403bp).

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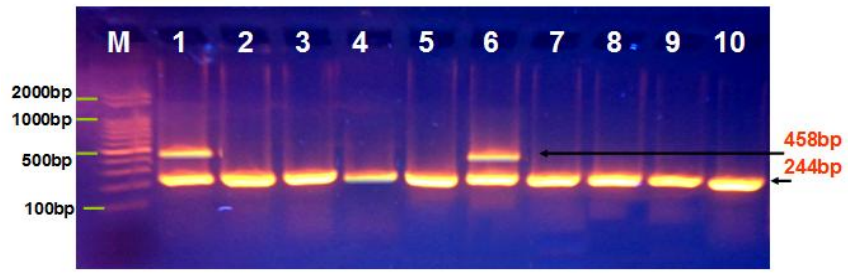

Figure 4.

Ethidium bromide-stained agarose gel (1% agarose) of PCR amplified products from extracted DNA of *P. aeruginosa* amplified with, *parC and parE* genes primers. The electrophoresis performed at 80 volt for 1hr. DNA molecular size marker (100-2000bp ladder). 10 of isolates show positive results with *parE* (244bp),2 of isolates show positive results with *parC* (458bp).

Discussion

Pseudomonas aeruginosa emerged as an important pathogen and responsible for the nosocomial infections that is one of the important causes of morbidity. This might be due to the prolonged stay in hospital following an operation resulting in colonization and subsequent infection [10]. The incidence of *P. aeruginosa* among the examined samples was 50 positive isolates with a percentage of 14.3 %. The result of current study agrees with [11, 12]. Also The result of current study didn't agree with [13]. According to source of infection, the present study revealed that *P. aeruginosa* were most common (16.7 %) in ear infection and this consistent with some previous surveillance studies [14, 15]. The results of the present study showed that out of the entire burn culture positive, 13.3 % exhibited *P. aeruginosa* isolates. Previous studies performed burn hospitals in various cities of Iraq [16] demonstrated high incidence of these isolates, followed by12% isolated from the sputum. This result of the current study comparable with the results of other study [15]. Out of 10% patients with UTI had an established *P. aeruginosa* etiology. This rate was comparable with many publications studies recorded [17]. This study has focused on resistance to two types of fluoroquinolonesin clinically isolates of *P. aeruginosa*from Al-Diwaniya teaching hospital, with emphasis on ciprofloxacin and norfloxacin. In this study the percentage of resistance to ciprofloxacin (20%) followed by norfloxacin (14%). These rates were comparable with previous reports published [18, 19], but this result didn't agree with [15]. Fluoroquinolones resistance among *P.aeruginosa* isolates has increased at an alarming rate due to its extensive use, which severely limits their usefulness [20]. Fluoroquinolones

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resistance can lead to treatment failure in *P. aeruginosa* [21]. The main mechanisms of resistance are mutations in the target genes, those encoding DNA gyrase (*gyrA*, *gyrB*) and topoisomerase IV(*parC*, *parE*) [22] and in regulatory genes for drug efflux pumps [23]. In the present study the fluoroquinolones resistance rate in 10 isolates of *P. aeruginosa*. The most common detected *gyrA* and *parE* (100%).These results are similar to that has been observed in different studies in other countries [24, 25], but these results aren't similar to [26], followed by *gyrB* (40%), *parC* (20%). The result of current study agrees with [27], but the result didn't agree with [28].

Competing interests

The authors declare that there is no conflict of interest .

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