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Detection of Fluoroquinolones Antibiotic Resistant in Pseudomonas Aeruginosa Isolates

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الكشف عن مقاومة المضادات الحيوية الفلوروكوينولون في عزلات بكتريا الزائفة الزنجارية

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ABSTRACT

Background: The Gram-negative bacterium *Pseudomonas aeruginosa* causes widespread morbidity and mortality. It enters the body through any weakness in the basic defenses of the human host.
Materials and Methods: This study designed to assess the correlation between the resistant of fluoroquinolones antibiotics in *P. aeruginosa* clinical isolates bacteria resulting from the excessive use of broad-spectrum antibiotics in hospitals. In the present study, forty clinical isolates (burn seven (17.5%), wound seven (17.5%), ear two (5%), operation room twelve (30%), urine three (7.5%), and nine (22.5%) from an industrial dialysis center were identified as *P. aeruginosa* using bacteriological diagnostic techniques. Nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, lomefloxacin, levofloxacin, and trovafloxacin are the seven antibiotics of the fluoroquinolones family that were used in the Kirby Bauer test to determine the antibiotic susceptibility of each isolate.
Results: Of the forty clinical isolates, three were sensitive and ten resistants to all tested antibiotics, while the other twenty-seven were intermediate, resistant, and sensitive to two or more tested antibiotics.
In conclusion: our findings revealed that highly resistant *Pseudomonas aeruginosa* strains have emerged as a major cause of opportunistic infections in hospitals due to the overuse of broad-spectrum antibiotics in hospitals.

Keywords: Pseudomonas aeruginosa; fluoroquinolones; Antibiotics resistant

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1. INTRODUCTION

The gram-negative bacterium Pseudomonas aeruginosa causes widespread morbidity and mortality. It enters the body through any open wound or other opening and threatens the basic defenses of the human host [1]. It is often very difficult to control the spread of these bacteria in medical facilities, as a result to cause a verity set of serious-opportunistic-infections by secreting the extracellular molecules as exotoxin A, elastase, alkaline protease, phospholipase C, pigments, and other factors which plays an important role in pathogenicity, special in the hospital intensivecare units (ICUs) which is due to cause nosocomialinfections [2]. Most infections occur in the persons with immunodeficiency as a result loss of the physical barrier (mucous membrane), since there are many mechanisms of resistance to most antibiotics [3].

Natural substances known as antibiotics have bactericidal or antibacterial properties that prevent most microorganisms from working. Many types of Gram-negative bacteria can be killed by synthetic antibiotics called fluoroquinolones (FQs) [4]. Humans utilize fluoroquinolones to treat a range of bacterial infections, such as P. aeruginosa and other organisms. In the past few years, there has been a marked increase in fluoroquinolones resistance in clinical isolates, leading to treatment failure for Pseudomonas aeruginosa [5]. Recent studies indicate that the mechanism of fluoroquinolones resistance in Pseudomonas aeruginosa resulting from the excessive use of broad-spectrum antibiotics in most hospitals is a set of molecular mutations occurring in bacterial coding genes [6]. The present study aims to study the resistance of clinical P. aeruginosa isolates bacteria from various clinical sites at Al-Hussein Teaching Hospital in Al-Muthanna province to fluoroquinolones drugs.

2. MATERIALS AND METHODS

2.1 SAMPLES COLLECTION

In all, 258 clinical samples were gathered from November 2018 to February 2019 from a variety of clinical different sites, including teaching laboratories, coworkers and patients at Al-Hussein Teaching Hospital in Samawah City. These specimens were carefully taken utilizing a specific swab from each source, put inside a specific tube with brain heart infusion broth, and then transported to the bacteriological unit at the PublicHealth Laboratory in Al-Muthanna province. They were then incubated at 37°C for 24 h. Burns, wounds, ears, operating rooms, urine, and the Industrial Dialysis Center were the main sources of samples.

2.2 BACTERIAL ISOLATE IDENTIFICATION

All samples were cultured on blood agar and MacConkey agar and, as a first diagnostic step, incubated for 24 h at 37 °C. In order to eliminate bacterial mixture growth and obtain pure colonies from each sample, samples were also re-cultured on MacConkey agar [7]. Pseudomonas aeruginosa was isolated at this step and purified on selective media (Pseudomonas aeruginosa agar and cetrimide agar) to verify that it inhibits the growth of other bacteria and the formation of pigments [8].

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2.3 BACTERIAL IDENTIFICATION BY VITEK₂ COMPACT

All isolates obtained in accordance with the bacteriological diagnostic from a range of samples based on classical methods as an initial diagnostic were identified by VITEK₂ compact system using the gramnegativecard kits (GN-ID) through the colorimetric instrument. The identification assay has been performed in accordance with the manufacturer's instructions (Biomerieux, France).

2.4 ANTIBIOTIC SENSITIVITY TEST

The Kirby-Bauer method was used to determine the antibiotic susceptibility test using the seven fluoroquinolone antibiotics (nalidixic acid (30 µg), ciprofloxacin (10 µg), norfloxacin (10 µg), ofloxacin (5 µg), lomefloxacin (10 µg), levofloxacin (5 µg), and trovafloxacin (10 µg)) purchased from Bio Analyse (Turkey) and carried out in accordance with the Clinical Laboratory Standards Institute's (CLSI, 2018 28th) recommendations to antibiotic susceptibility test in clinical laboratories using the agar disk-diffusion method in the Mueller-Hinton medium [9].

3. RESULTS

In this study, amongst the 258 clinical samples, 40 isolates were obtained as *P.aeruginosa*, isolated from different sources (burn, wound, ear, operationroom, urine, and industrial dialysis center) of Al-Hussein Teaching Hospital in Al-Muthanna province from the November2018 to February2019. Initial identification tests were conducted on all bacterial isolates (gram stain, oxidase, and catalase). All isolates of *P.aeruginosa* were identified using a different set of basic methods (cultural characteristic, biochemicaltests, pigmentproduction, and vitek₂compact). The *P.aeruginosa* isolates bacteria distributed amongst clinical different samples based on their sites (Table 1). All bacterialisolates were showing β-hemolysis on blood agar medium through leaving a clear zone about bacterial growth which gives greyish white smooth colonies. All bacterial isolates were showing non-fermented for lactose on macconkey agar medium, which gives pale smooth colonies with grape-like odor. Bacterial isolates were subcultured on macconkey agar plates for purpose of their activations and get pure single colonies. Whereas all bacterial isolates on nutrient agar medium were produced soluble pigments (Pyocyanin and Fluorescein), which gives large and opaque colonies that have an earthy smell, with irregular shape.

Table 1: Samples and isolates distribution in this study.

ID	Source of samples	No. of samples	Isolates	
			No.	%
1.	Burn	58	7	17.5
2.	Wound	50	7	17.5
3.	Ear	20	2	5
4.	Operation Room	54	12	30
5.	Urine	34	3	7.5
6.	Industrial Dialysis Center	42	9	22.5
7.	Total	258	40	100

In addition, all the isolates were defined as *P.aeruginosa* by VITEK$_2$ Compact system. Meanwhile, the identification was conducted using commercial identification kits, VITEK$_2$ GN ID, (BioMerieux, France). In this context, each kit consisted of 20 cards, particular to conducted 20 test. The probability of identifications for all isolates was excellent within a range from 90 % to 99 %. Concerning fluoroquinolones resistant, the results of antibiotic susceptibility test for all the isolates, taken from different clinical sources were mentioned in Table 2. Meanwhile, antibiotic susceptibility test was conducted by agar-disc-diffusion method with utilizing seven fluoroquinolones antibioticsclass (NA, CIP, NOR, LOM, OFX, LEV, and TRV) as shown in Figure 2. Table 2 illustrated the different levels of responded to tested antibiotics as resistance, intermediate and sensitive according to the percent of each antibiotic (Figure 3). Amongst the 40 clinical isolates, were obtained 10 isolates, from different sources showed veritable resistance for all tested antibiotics in the current study based on the normal standard range (CLSI, 2018), while some of the isolates showed veritable resistance to two or more of tested antibiotics. On the other hand, out of forty clinical isolates, ten were resistant to all tested antibiotics, three were sensitive to them, and twenty seven were moderately resistant to two or more tested antibiotics.

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Table 2: Results of antibiotic susceptibility test for *P.areuginosa* isolates.

Source		Antibiotics						
	Pattern	NA	CIP	NOR	OFX	LOM	LEV	TRV
Burn	R	5	1	2	1	3	1	3
	I	2	1	0	0	1	0	0
	S	0	5	5	6	3	6	4
Wound	R	6	3	3	3	3	3	3
	I	1	0	0	0	0	0	0
	S	0	4	4	4	4	4	4
Ear	R	2	0	0	0	1	0	2
	I	0	0	0	0	1	0	0
	S	0	2	2	2	0	2	0
Operation Room	R	11	4	4	3	6	3	10
	I	0	0	0	0	2	0	0
	S	1	8	8	9	4	9	2
Urine	R	2	2	2	2	2	2	2
	I	0	0	0	0	0	0	0
	S	1	1	1	1	1	1	1
IDC	R	8	2	2	2	3	3	5
	I	0	1	0	1	2	0	1
	S	1	6	7	6	4	6	3
Total	R%	85 %	30 %	32.5 %	27.5 %	45 %	30 %	62.5 %
	I%	7.5 %	5 %	0 %	2.5 %	15 %	0 %	2.5 %
	S%	7.5 %	65 %	67.5 %	70 %	40 %	70 %	35 %

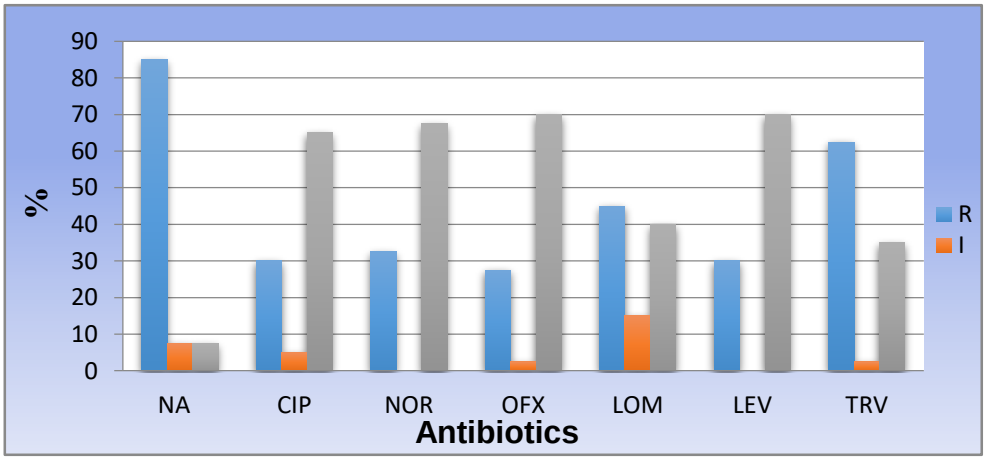

Figure 1: Different levels of responded to tested antibiotics for all isolates follow up with the table (3-3). R= Resistance, S= Sensitive, I= Intermediate. NA= Nalidixic Acid, CIP= Ciprofloxacin, NOR= Norfloxacin, OFX= Ofloxacin, LOM= Lomefloxacin, LEV= Levofloxacin, TRV= Trovafloxacin.

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4. DISCUSSION

Pseudomonas aeruginosa is a gram-negative bacterium that belongs to *Pseudomonadaceae* family. It is a ubiquitous organism, originally present in the environment such as soil, water, human, animals, plants, sewage, and hospitals, which it can be isolated in hospitals from different clinical sources [1]. *P. aeruginosa* produce a variety of soluble pigments such as pyocyanin and fluorescent pigments based on the bacteriological investigation, cultural methods, by using specific selective media with intrinsic and acquired mechanisms to antibiotics resistance due to reduced outer membrane-permeability [2]. The importance of this resistance in multidrug-resistant bacterial strains needs further consideration as the number of antibiotic-resistant bacteria is increasing. One of the most important aspects of treating hospital patients with narrow-spectrum, targeted, and specific antibiotics is to understand the mechanisms that cause this resistance.

In the present study, amongst the 40 clinical isolates, were obtained 10 isolates, from different sources showed veritable resistance for all tested antibiotics in the current study based on the normal standard range (CLSI, 2018), while some of the isolates showed veritable resistance to two or more of tested antibiotics. However, all isolates of *P.aeruginosa* were identified using a variety set of techniques (cultural characteristic, biochemical tests, pigment production, and vitek$_2$ compact). All bacterial isolates were showing non-fermented for lactose on macconkey agar medium, which gives pale smooth colonies with grape-like odor (Figure 1). Whereas all isolates on nutrient agar medium were produced soluble pigments (Pyocyanin and Fluorescein), which gives large and opaque colonies that have an earthy smell, with irregular shape (Figure 1). *P.aeruginosa* isolates bacterium are dispersed among clinical samples according to their source (Table 1). The variation in the percentage of distribution of *P.aeruginosa* isolates reflected the capacity of bacterium to attack different sites in the host (human) through penetrating the tissue by a variety set of virulence factor and entry the blood stream and causes infections [3].

In most hospitals, the pathogenicity of *P. aeruginosa* is very complex and multifactorial, causing many infections, with high morbidity and mortality levels, especially in people with the immunocompromised, with the intrinsic and acquired antibiotics resistance mechanisms as a result to the excessive use of broad-spectrum antibiotics such as fluoroquinolones class [4]. In this study, the results of antibiotic susceptibility test for all the isolates, taken from different clinical sources were mentioned in the Table 2. Meanwhile, antibiotic susceptibility test was conducted by agar-disc-diffusion method on mueller Hinton agar with using seven fluoroquinolones antibiotics class (NA, CIP, NOR, LOM, OFX, LEV, and TRV) as shown in Figure 1. Table 1 illustrated the different levels of responded to tested antibiotics as resistance, intermediate and sensitive based on the percentage for each class (Figure 2). Amongst the 40 clinical isolates, were obtained 10 isolates, from different sources showed veritable resistance for all tested antibiotics in the current study based on the normal standard range (CLSI, 2018), while some of the isolates showed veritable resistance to two or more of tested antibiotics. In contrast, of the forty clinical isolates, ten were resistant and three were responsive to all antibiotics tested, and twenty seven were resistant and intermediately susceptible to two or more antibiotics tested.

However, these results are consistent with other publications on clinical isolates of *P. aeruginosa* by [4, 5, 6, 7].

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Furthermore, according to the percentage of each antibiotic tested, the majority of isolates appeared the highest level of resistance to the testedantibiotics, including ofloxacin (27.5%), ciprofloxacin (30%), levofloxacin (30%), trovafloxacin (62.5%), lomefloxacin (45%), and nalidixic acid (85%) as shown in Figure 2. Previous research suggests that mutational changes in bacterial-encoded genes such as DNA gyrase are one of the key processes behind the emergence of quinolone resistance [8]. In contrast, among the limitations of this study, the prevalence of mutations in DNA gyrase and topoisomerase IV has not yet been investigated and is needed for other studies.

5. CONCLUSION

Our findings revealed that highly resistant *Pseudomonas aeruginosa* strains have emerged as a major cause of opportunistic infections in hospitals due to the overuse of broad-spectrum antibiotics in hospitals. Results from previous research postulate that mutational changes in bacterial-encoded genes such as DNA gyrase are one of the key processes behind the emergence of quinolone resistance. To better understand quinolone resistance caused by *P. aeruginosa*, further investigation is needed.

Conflict of interest.

There are non-conflicts of interest.

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