How to Cite:
Najem, S. A.-A., & Shubbar, E. E. (2022). Fluoroquinolones resistance of Pseudomonas aeruginosa isolated from different clinical samples. *International Journal of Health Sciences*, *6*(S4), 3253–3260. https://doi.org/10.53730/ijhs.v6nS4.9015

Fluoroquinolones resistance of Pseudomonas aeruginosa isolated from different clinical samples

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***Abstract*---**A total of 156 clinical specimens were collected from patients at Al-Sadder Medical City, Al-Furat al-Awsat Hospital, and the burn center between August 2021 and February 2022. According to medical service utilities, Burn center was the highest with total number of samples with 96 samples and the highest recovery rate 17 isolates followed by Alfurat Al-Awsat Hospital with 32 samples and 4 isolates and finally tame Al-Sadder Medical City with only 28 samples and 2 isolates. The results of 23 isolates of *Pseudomonas aeruginosa* revealed that the most effective agent was ciprofloxacin with (60%) resistance which represents regression in the overall potential while Norfloxacin showed high resistance percentage (73%) (The least effective among them) followed by Ofaloxacin (69%) and levofloxacin with (65%).genetically, the result of aac(6´)-Ib-cr gene showed that 21/23 isolates gave positive result with percentage (91.3%).

***Keywords*---**Pseudomonas aeruginosa, resistance, fluoroquinolones, antibiotics.

Introduction

Pseudomonas aeruginosa is a multidrug-resistant (MDR) and extensively drug-resistant bacterial pathogen that infects the respiratory tract, circulatory system, urinary tract, and central nervous system, causing severe morbidity and mortality. *Pseudomonas aeruginosa* strains have emerged as the most common source of nosocomial infections in humans, posing a serious risk to hospitalized

patients with compromised immune systems. (Abdelrahman *et al.*,2020; Pang *et al.*,2018).

P. aeruginosa due to its widespread distribution, ability to thrive in moist environments, and remarkable innate resistance to a wide range of antimicrobials and antiseptics, is a leading cause of nosocomial infections, ranking second among gram-negative pathogens reported to the National Nosocomial Infection Surveillance System (Aboulfotoh *et al.*,2017; Szmolka *et al.*,2013). The presence of virulence components such as hemolysin enzyme, Biofilm production, exotoxin A, proteases, alginate, lipopolysaccharide, flagellum, type IV Pili, and type III Secretion System is ascribed to *P. aeruginosa's* exceptional pathogenic capabilities.(Moradali *et al.*, 2017). Fluoroquinolones are highly effective antibacterial medications used to treat bacterial infections in both people and animals. (Hopkins *et al.*, 2005). *Pseudomonas aeruginosa* has a diverse set of innate and acquired antimicrobial resistance pathways, many of which coexist and result in MDR phenotypes (Horcajada *et al.*,2019). Fluoroquinolones resistance mechanisms in *P. aeruginosa* thought to be the result of changes in the quinolone-resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV (Nouri *et al.*, 2016).

Quinolone targets are protected by qnr proteins from the penta peptide repeat (PRP) family, and quinolones are degraded by the aac (6′)-Ib-cr protein when plasmid-mediated quinolone resistance genes (PMQR) are acquired (Strahilevitz *et al.*,2010; Jacoby ,2005; Muylaert *et al.*, 2013). *Pseudomonas aeruginosa* is an opportunistic bacteria that can cause acute and chronic illnesses.. The broad and diverse arsenal of virulence factors including the formation of biofilms and the production of toxins and enzymes that cause widespread tissue damage and thus enter the bloodstream, allowing the bacteria to spread throughout the body (Salman *et al.*,2017).

Fluoroquinolones are broad-spectrum antibiotics that have a strong affinity for gram-negative bacteria, particularly *Pseudomonas aeruginosa*. When taken orally, these drugs are well absorbed. Because tissue and fluid concentrations often exceed serum drug concentrations, these antibiotics are particularly useful for various conditions, such as pneumonia (KW Garey *et al.*, 1999; GE Stein, 1996). Fluor quinolones are generally well tolerated and have few negative side effects. FQs have a high tissue-to-serum drug concentration ratio and have a high bioavailability, wide dispersion, and strong tissue penetration. They can, however, have major negative consequences (Ragnar Norrby *et al.*,1993; Williamson KS *et al.*,2012; Rocha *et al.*,2019).

Material and Method

A total of 156 clinical specimens were collected from patients at Al-Sadder Medical City, Al-Furat al-Awsat Hospital, and the burn center between August 2021 and February 2022. The participants were of all genders and ages ranging from 6 to 70 years old.

Identification of *Pseudomonas aeruginosa* isolates

In a cross-sectional study, a total of 156 clinical samples of hospitalized patients in Najaf City , from August 2021 to February 2022. *Pseudomonas aeruginosa* isolates were identified and confirmed by conventional microbiological and biochemical tests (Sorkh *et al.*,2017). All specimens were cultured on MacConkey agar and incubated at 37°C for 24 hours in an aerobic state. Bacterial isolates were identified mostly based on the morphologic properties, outline, odor, and color of the colonies. A sterile loop selects one colony to arrange a purified subgroup on nutrient broth and agar prior to biochemical identification using standard methods (Preethirani *et al.*, 2015), as well as VITEK 2 system identification.

Antibiotics Susceptibility Testing

Antibiotic susceptibility test was achieved by disc diffusion technique (Kirby-Bauer) (Zaidan *et al.*, 2005). A full loop of the growth was equally placed in a tube of soy broth and conserved at 37°C for 1 hour. Inoculums were arranged from the pure culture disc. The suspension's concentration was changed to 0.5 McFarland. Mueller-Hinton agar was inoculated with sterile swabs soaked in inoculums. Excess inoculums were eliminated by spinning the swab against the tube's side walls above the liquid level. With each application, the swab was patterned through the medium's surface several times, and the plate was then rotated at a 60° angle while the swab was strapped alongside the agar base side. Each plate is placed on an antibiotic disk using a sterile forceps. To achieve homogeneous contact, the antibiotic disks were carefully strapped into the medium. At 37°C, the dishes were incubated. The diameters of each zone were measured in millimeters after a 24-hour incubation period. The outcome was then analyzed based on (CLSI, 2022). To achieve homogeneous contact, the antibiotic disks were carefully strapped into the medium.

DNA Extraction

Two to three colonies of bacteria were re suspended in a 500-µL of sterile distilled water. Suspensions were heated at 100°C for 15 minutes. Then, they were centrifuged at 3000 g for 10 minutes for the precipitation of cell debris. The supernatant was transferred to a new micro tube and stored at -20°C (Hornef *et al.*,2000; Salas *et al.*,2005).

Results and Discussion

The results revealed that the most effective agent was ciprofloxacin with (60%) resistance which represents regression in the overall potential while Norfloxacin showed high resistance percentage (73%) (the least effective among them) followed by Ofaloxacin (69%) and levofloxacin with (65%) (Table 1, Figure 1).

Table 1: fluoroquinolones resistance profile of *P.aeruginosa* Isolates

Phenotype	Ciprofloxacin CIP	Levofloxacin LEV	Norfloxacin NOR	Ofaloxacin OFX

	No.	%	No.	%	No.	%	No.	%
Resistance	14	60%	15	65%	17	73%	16	69%
Sensitive	9	40%	8	35%	6	27%	7	31%

A recent native study referred to that the proportion of resistance to ciprofloxacin equaled (87.5%) which is relatively higher than present study (Shaker and Al-Hadrawi .,2021). continuously ciprofloxacin multiple studies worldwide had explored its activity. For example in Islamic Republic of Iran a study showed 41% of resistance (farahi *et al.*,2018). Earlier studies exhibited variable outcomes 55%, 35% respectively (Saderi *et al.*,2010; Tohidpour *et al.*,2013). Other countries showed interestingly vast differences in ciprofloxacin resistance than current result, where it was about 33% in United States of America(Jones *et al.*,2004) and less than that in Canada (27%)(Karlowsky *et al.*,2011). Since 1990s through 2000s till recent years a notable increase in the proportion of ciprofloxacin resistance has been proven. Were it was 11% followed by 24% (Neuhauser *et al.*,2003) and 30% (Pitt *et al.*,2003**)**. ,40% (Landman *et al.*,2007). and more recently 72% (Abdulameer and Abdulhassan.,2021). In other study the results of *Pseudomonas aeruginosa* isolates was sensitive to Ciprofloxacin, (34%) and (66%) show reported resistant to Ciprofloxacin **(**Hassan *et al.*,2019**).** According to the pattern of resistance to four fluoroquinolones agents including (Ciprofloxacin , Levofloxacin, Ofaloxacin , Norfloxacin) result showed that nearly half of isolates(11 isolates) had the ability to resist them all. While other patterns exhibited similar percentages.

In table 2 we found that the most prevalent pattern of antibiotic is Ciprofloxacin and Levofloxacin which is most used and most common and the three other pattern with similar percentage and less prevalence pattern is Ciprofloxacin , Levofloxacin ,Norfloxacin and Ofloxacin which is may be difference of exposher to these antibiotic. Current outcomes reflect the high resistance to significant antimicrobial agents within fluoroquinolones group which unfortunately reduces treatment options and healing opportunities.

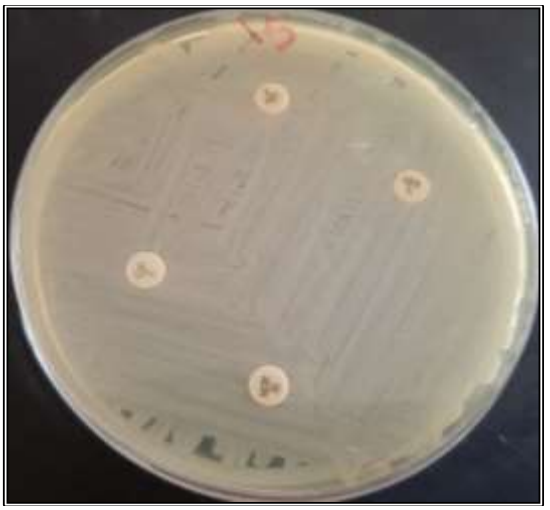
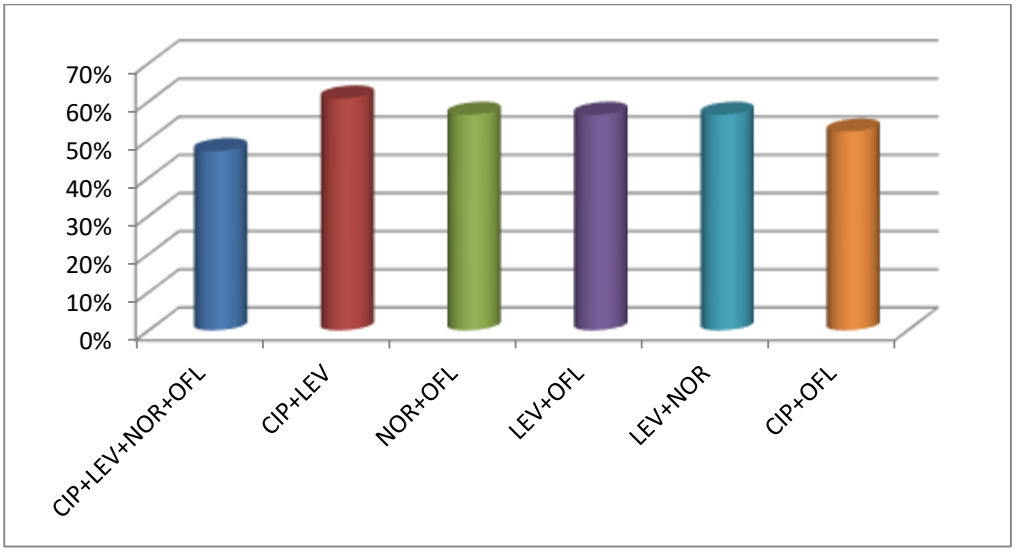


Figure 1: multidrug resistance *P.aeruginosa* in Muller Hinton Aga

Table 2: Fluoroquinolones resistance patterns of *P.aeruginosa* isolates



Genetic Detection of Fluoroquinolones Resistance Genes
Detection of aac(6´)-Ib-cr

Regarding aac(6´)-Ib-cr results showed that 21/23 isolates gave positive result with percentage (91.3%) . the product size was 482pb which is the same of expected size figure(2).

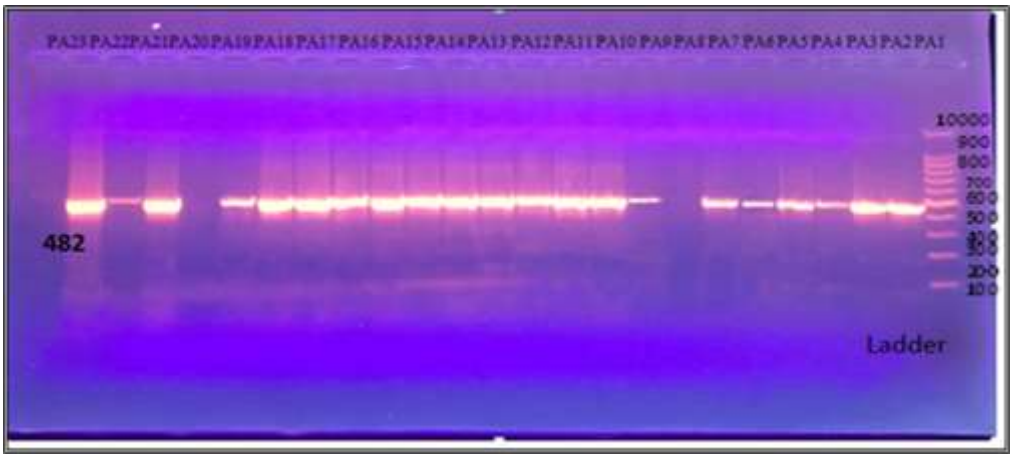


Figure 2: Agarose gel (including agarose powder and TB buffer in appropriate values, in addition to (DSRed Nucleic Acid stain) electrophoresis of *acc-ib 6 ar* gene. A 100 bp plus ladder (L) was included right side of the gel to estimation the gene size

It is necessary to recall that quinolones resistance could be mediated by several mechanisms of which the modifying enzyme including (aac(6´)-Ib-cr) gene which is basically encoding for aminoglycoside resistance as well as quinolones

resistance (Moghadasi *et al.*,2016).in 2017, 60 strains of *P.aeruginosa* were isolated from clinical specimens of patients in Kerman hospitals and result showed that *aac(6)-Ib-cr prevalence was* (33%) (Rajaei, *et al.*,2017). In a study in Saudi Arabia the finding showed that 28/39 quinolone resistant *P.aeruginosa* harboured gene aac(6´)-Ib-cr in (71.8%) percentage of isolates (El-Badawy *et al.*,2019). Interestingly a study in Tehran showed highly discrepancy in their results regarding detection of qnr genes where all isolates showed negative result for the latter , although all isolates possessed the aac(6´)-Ib-cr meanwhile the total number of isolates were resistant to quinolones phenotypically (Molapour *et al.*,2020).

Conclusion

Out of 23 isolates of *P. aeruginosa* revealed that the most effective agent was Ciprofloxacin and Norfloxacin followed by Ofaloxacin and levofloxacin. The result proved that aac(6´)-Ib-cr gene showed with percentage 91.3%.

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