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Effect of Ultraviolet Radiation on Antibiotics Resistance of *Pseudomonas* spp. Isolated from Wastewater in Tikrit City, Iraq

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Abstract

Sixty (60) isolates of *Pseudomonas* belonging to two species (*Pseudomonas fluorescens, and Pseudomonas pyocyanin*) were isolated from Wastewater in Tikrit City, Iraq from April 2010 to February 2011 (four seasons) during this study. The total bacterial count (TBC) were screened. UV irradiation exposure for three period (20, 40 and 60) minutes were used to mutant the isolation to antibiotics. Antimicrobial susceptibility were used in this study within and without UV exposure time compared to National Committee for Clinical Laboratory Standard Guidelines (CLSI). The tested isolates when UV exposure time were highly sensitive to gentamicin, amoxicillin, novobiocin, amoxicillin/clavulanic acid (augmentin) and the batter exposure time was (40) minutes, Whereas the tested isolates in this study exhibited intermediate sensitivity to the cefixime and ampicillin,) and the batter exposure time were (20 and 40) minutes, while *Pseudomonas* species were resistance to ciprofloxacin, tetracycline and vancomycin and no effects of any exposure time to susputable to these antibiotics. The study demonstrated that multiple antibiotics resistant *Pseudomonas* species were quite prevalent in the final effluents of tikrit in Iraq; and this can lead to serious health risk for communities that depend on the receiving waters from Tigres river.

Keywords: Wastewater, UV , *Pseudomonas* spp., Antibiotics Resistance

Introduction

Pseudomonas species are prominent members of this category of emerging waterborne pathogens [1]. The *Pseudomonads* comprises species with ecological, economic and health-related importance [2]. Members of this bacterial group are versatile and able to adapt and colonize a wide variety of ecological environments throughout the World, including water, sewage, soil, plants and animals [3].

Wastewater and drinking water are treated to eliminate pathogenic microorganisms and to prevent waterborne transmission. However, previous study indicated that conventional wastewater treatment does not guarantee their complete elimination[4]. When discharged to environment, untreated or insufficiently treated wastewaters cause several problems, such as eutrophication, oxygen consumption and toxicity[5].

UV disinfection has gained widespread use for municipal wastewater, and more recently, interest in using UV for water reuse applications has increased [6]. UV disinfection has several inherent advantages over all other disinfection methods. In particular, no chemical consumption thereby eliminating large scale storage, no transportation, handling and potential safety hazards, low contact time and reduced space requirements since no contact basin is necessary, no harmful by products are formed as a minimum number of , or no, moving parts nd high reliability and low energy requirements[7].

Fluoroquinolones, β-lactams and aminoglycosides are the main classes of antibiotics used for treatment of *P. aeruginosa* infections. Unfortunately, the multi-drug resistant isolates of *P. aeruginosa* which patients are often exposed to in hospital settings are resistant to one or more of these antibiotic classes [8]. Selective pressure due to excessive exposure of bacteria to antibiotics is generally the origin of such high incidence of resistance in the hospitals

environment. Intensive care units (ICU) and long-term care facilities are also notorious worldwide for harboring multi-drug and pandrug resistant *P. aeruginosa* strains [9].

The present study was therefore designed to investigate the prevalence and antibiogram profiles of *Pseudomonas* species isolated from effluents of main wastewater plant, treatment plants in Tikrit city of Iraq, and to determine the influence of ultraviolet disinfection system (UV-C dose) as a water treatment method on bacterial populations .

Materials and methods

Samples Collection

The sewage water samples were collected from plant at sheshen valley in Tikrit city, Iraq. This plant is located south-west of the city of Tikrit figure (1), it is a great plant for the collection of wastewater and pumped to the treatment plant from seven secondary pumping plants at a period from April 2010 to February 2011 (four seasons). The samples were collected in sterile glass container and transport to laboratory for bacteriological analysis.

Figure (1): Location of wastewater main collection plant in Sheshen valley in Tikrit city

Total bacterial count (TBC)

The total bacterial count (TBC) were screened on nutrient agar by the standard pour plate method [10,11]. Plates were incubated at 37ºC for 24 hours and the grown colonies were calculated.

Isolation of bacteria

The bacterial isolates were grown on Asparagine broth (Hi Media) by the Most probable Number (MPN) method [10], to determine the total count of bacterial isolates [10,12]. The tubes were daily examined for observation of the pigments. The colonies were obtain in form of pure culture and identified on the basis of their morphology and biochemical characters. The shape and colour of the colonies were examined under the microscope after Gram staining. Isolates were bio chemically analyzed for the activities of Oxidase, Catalase and Urease, gelatin hydrolysis, motility, indole production and citrate utilization tests as well as grown in 42ºC and 4ºC. The isolates were identify according to Bergey's Manual of Systematic Bacteriology [13].

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disk diffusion method [14] with Muller-Hinton agar as the growth medium. Antibiotics were selected to represent some major classes of antibiotic and anti-pseudomonal antibiotics used as first line drug for pseudomonal infections. Antibiotics used in the study include: gentamicin (10µg), amoxicillin (25µg), ciprofloxacin (10 µg), tetracycline (10 µg), vancomycin (10µg), cefixime (5µg), novobiocin (30µg), ampicillin (25µg), amoxicillin/clavulanic acid (augmentin) (30µg). Disks were purchased from Bioanalyse (Turke). *Pseudomonas* spp. isolates were identified as susceptible, intermediate or resistant according to the National Committee for Clinical Laboratory Standard Guidelines (CLSI) [15].

UV Irradiation of bacterial isolates

UV irradiation was carried out [16] by subjecting fresh culture of *Pseudomonas* spp. inoculate in 10 ml of Brain heart infusion broth in glass tubes and incubated for 18 hours at 37ºC shaker incubator (10^{-5} to 10^{-6})cells/ml. UV radiation in a dark place using the UV-irradiation device (C75- Chromato- Vue® Large High Performance UV Cabinets. UV irradiation was exposed at 254 nm, 15 watt, 115 volt. The distance between the UV source and irradiated culture was adjusted to be 15 cm. UV irradiation exposure for the following period (20, 40 and 60) minutes. The isolates inoculate after UV irradiation in Muller-Hinton agar, to detect antibiotic sensitivity test as described previously [14].

Results and Discussion

***Pseudomonas* Isolates in sludge wastewater**

All isolates confirmed to belong to the Pseudomonas genus were further screened for three specific species of interest (*P. fluorescens* and *P. Pyocyanin*) selected based on the dominance of these species from the results obtained on the preliminary identification tests. Sixty isolates were identified to belong to the *Pseudomonas* genus, wastewater samples collected in the four seasons (winter, autumn, spring and summer) showed incidences of *Pseudomonas* of 15.4×10^6 cells/g (autumn) and 15.2×10^6 cells/g (spring). Similarly, distribution of *Pseudomonas* with respect to species were 8 isolates for *P. florescence* and 9 isolates for *P. pyocyanin* (autumn). In spring, the distribution was 7 isolates for *P. florescence*, and 10 isolates for *P. pyocyanin* (Table 1). Furthermore, the analysis of sludge wastewater showed incidences of *Pseudomonas* at 13.5 cells/g (summer) and 12.1cells/g (winter) alone, but at the species level, the following were observed; 7 isolates *P. fluorescens* and 7 isolates *P. pyocyanin* during summer, and 5 isolates *P. fluorescens* and 7 isolates *P. pyocyanin* during winter.

Table (1) seasonal changes of *Pseudomonas* spp. prevelance in the primary sludge

Season	T.B.A Cfu/g*	*Pseudomonas* spp.	*Isolates No. of P. florescence*	*Isolatees No. of P. pyocyanin*
Summer	13.5	14	7	7
Autumn	15.4	17	8	9
Winter	12.1	12	5	7
Spring	15.2	17	7	10
Total		**60**	**27**	**33**

***Cfu/g: 10^6×Averge**

The increase in the number of the microorganisms was due to diminution in the concentration of the organic substances, which led to progressive slow-down of the growth rate. At concentration of the organic substances less than 80 mg/l a transition of the microflore to a death phase was observed. The high organic pollution and levels of bacteria

return to temperatures moderation of sludge wastewater appropriate of bacterial activity [17].The incidences of *Pseudomonas* species in the studied sampling sites appeared to be season dependent as variation in seasonal distribution reflected different recovery rates of the bacteria. Nevertheless, it must be appreciated that this recovery rates may not represent the total population of viable *Pseudomonas* species present in the samples, but selective for some species based on the incubation temperature used, especially considering that some *Pseudomonas* species do not grow at temperatures above 30 °C. The decrease in the number of the *Pseudomonas* isolates during winter suggests that the recovered isolates could not strive at low temperature in line with their mesophilic nature. Higher prevalence of *Pseudomonas* isolates were recovered during spring and autumn followed by summer especially in the mixed liquor samples, suggesting that warmer temperature favoured the recovery of these isolates. These variations may be attributed to human activities at various sites of the rivers, however; this explanation will not suffice for mixed liquor, although the limitations on overreliance on one primer pair/species for speciation of the Pseudomonas species must be appreciated. Similarly, the variation of incidence with season needs to be further investigated as it is not clear why season play a role in the occurrence of *Pseudomonas* species [18].

The antibiograms of the *Pseudomonas* species are as shown in figures (2-10). In figure (2) showed all non treated isolates were susceptible to novobiocin (30µg) except two (*P. pyoc.1*, *P. pyoc.3*) were resistant to novobiocin and were compared with Std. (CLSI). The zone of inhibition of novobiocin when treated with UV dose in exposed time (20, 40, 60) minutes was larger than the controls Std.(CLSI), and susceptible significantly compared with non-treated and Std.(CLSI), the increase in the UV treatment time, the rate of degradation increases, resulting in the gradual inactivation of the antibiotic, [19]. The cause of the susceptible of species to novobiocin were not used this antibiotic previously on this species because not have the genes inherited from predecessors resistance to this antibiotic and curing plasmid to lose resistance of this antibiotic.[20]

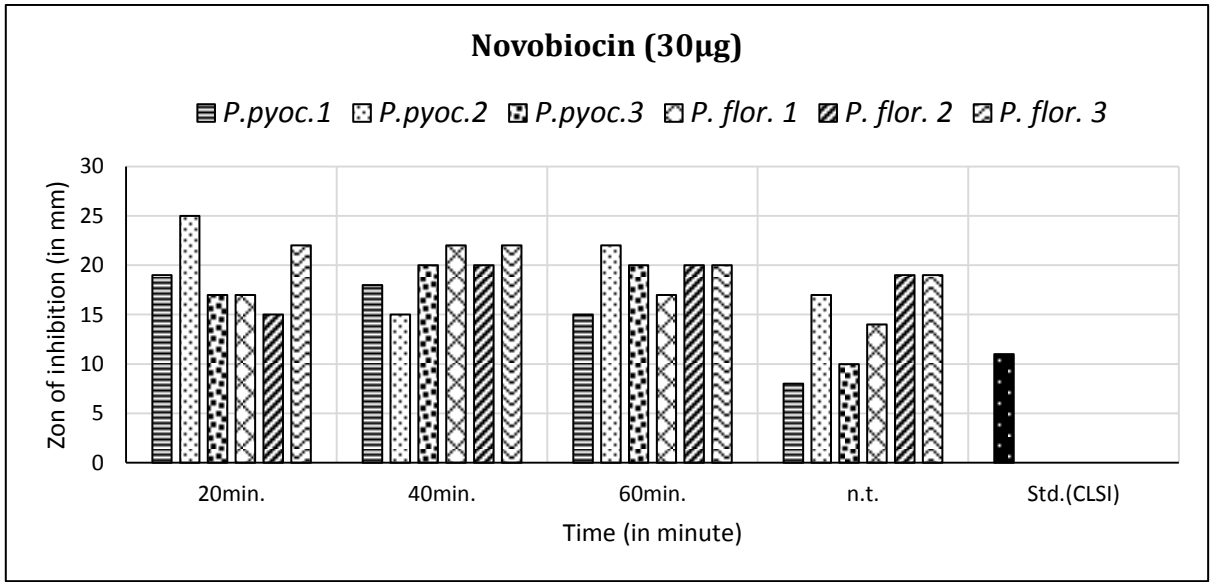

Figure (2): Inhibition zone of novobiocin resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes (n.t.: non treated with UV irradiation; Std.(CLSI): National Committee for Clinical Laboratory Standard Guidelines; *P. pyoc.1,2,3*: *Pseudomonas pyocyanin1,2,3;P. flor.1,2,3*: *Pseudomonas florescence 1,2,3*).

In figure (3) showed that all non treated isolates were resistant to cefixime (5µg). The zone of inhibition of cefixime when treated with UV dose in exposed time (20) minutes were three isolates (*P. pyoc.1, P. pyoc.3 and P. flor.1*) susceptible to cefixime whereas the UV exposed time (40) minutes were three isolates (*P. pyoc.1, P. pyoc.2 and P. flor.3*) susceptible to cefixime while in (60) minutes was one isolate (*P. flor.3*) susceptible to cefixime. The exposure time of (20 and 40) minutes were better than of (60) minutes which had no effect on the sensitivity of bacterial species excpet (*P. flor.3*) while the expousre time (20 and 40) minutes were effect on the diffrent three bacterial species, which is the appropriate time of UV exposure compared with (60) minutess exposure time for this antibiotic. UV exposure effect of *Pseudomonas* species can develop resistance to cefixime either mutational processes that alter the expression and/or function of chromosomally encoded mechanisms[21].

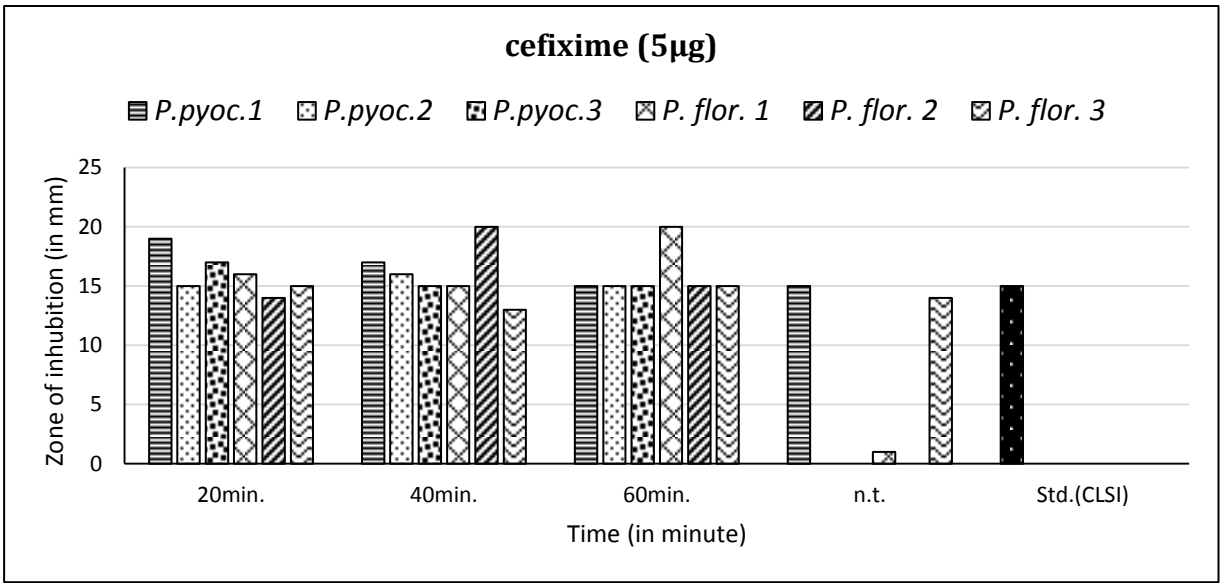

Figure (3): Inhibition zone of cefixime resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

The ciprofloxacin susceptibility of each species showed not identical results in all three UV exposure time compared to non treated isolates and Std.(CLSI). In figure (4) one isolate (*P. flor.2*) showed susceptible within tow time exposure (20 and 60) minutes whereas all isolates were resistance to ciprofloxacin. The UV exposure time (20 and 60) minutes which effect in *Pseudomonas florescence 2* with increased susceptibility to ciprofloxacin had previously recognised mutations in different combinations of genes. Mutations in more than one gene were associated with a higher level of resistance. associated with a high level of resistance in *Pseudomonas florescence 2.* [22]. ciprofloxacin resistance of *Pseudomonas* species isolates from countries with a restrictive usage of antibiotics is still rare and the problem is considerably smaller than that observed in certain hospital environments. These findings are relevant also for the third and fourth generation fluoroquinolones, as there is cross resistance between ciprofloxacin and these new compounds among Gram negative bacteria.[23].

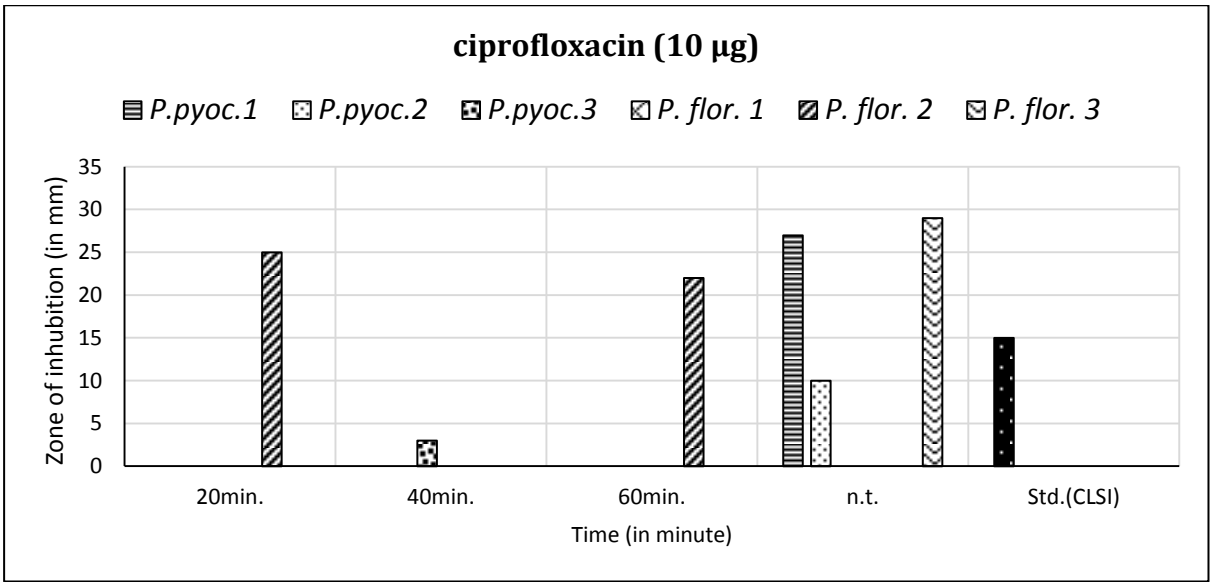

Figure (4): Inhibition zone of ciprofloxacin resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

All isolates were non treated with UV irradiation were resistance to ampicillin compared to Std.(CLSI) and the UV exposure time effect were significant in (40) minutes, while it was not effect of UV exposure time in (20 and 60) minutes except in one isolate (*P. pyoc.2*) was resistance to ampicillin. The UV exposure time (40) may be produce only low basal levels of ampicillin' and are susceptible to the antipseudomonal penicillins, ampicillin-inhibitor combinations, cephalosporins [24] and the greater impermeability of *Pseudomonas* species outer membrane may play an important role in allowing ampicillin overproduction to push cefepime MICs above the resistance breakpoint [25].

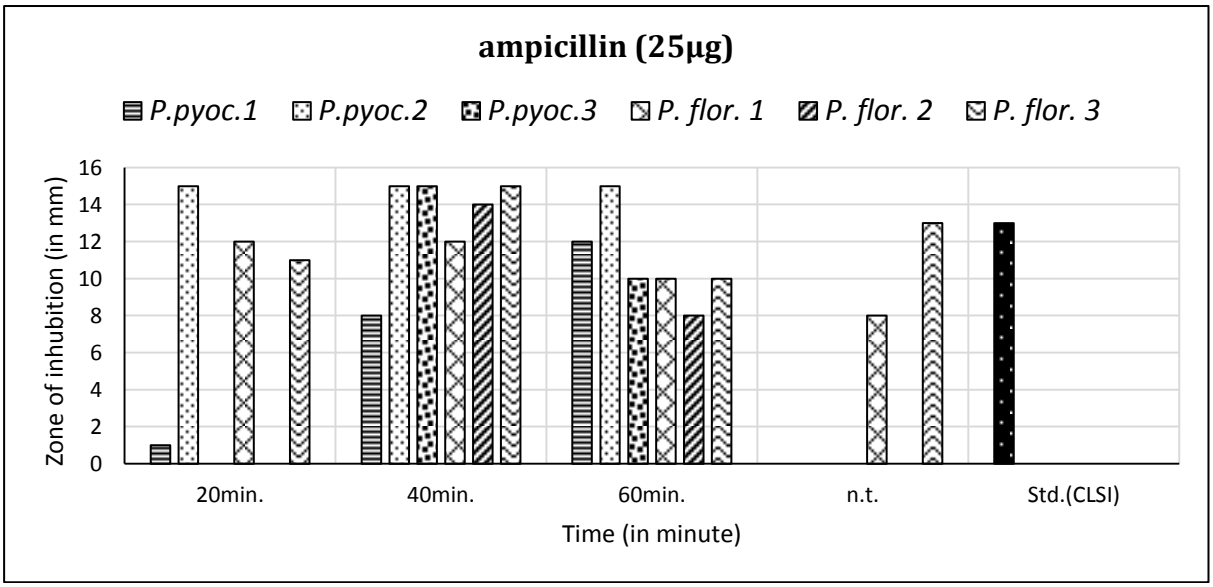

Figure (5): Inhibition zone of ampicillin resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

In figures (6), (7) and (8) Consistent with the observation of this study *Pseudomonas* species isolates presented in our work showed high sensitivity to

to amoxicillin , augmantine and gentamicin and that fluoroquinolones have lost their effectiveness against *Pseudomonas* species due to resistance, the UV expousure time were exhibit these antibiotics compared to n.t. and Std.(CLSI). The observations were not surprising as clinical and pharmaceutical environments tend to exert more selective pressure (leading to antibiotic resistance) on bacterial populations than non-clinical/non-pharmaceutical (e.g., municipal effluent) environments [26].

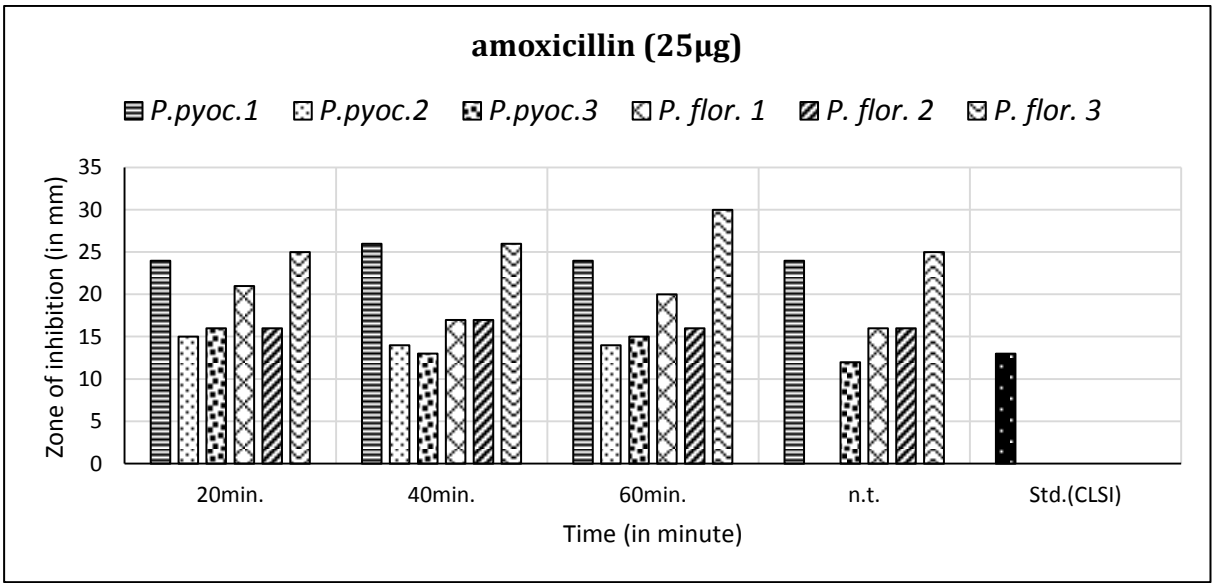

Figure (6): Inhibition zone of amoxicillin resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

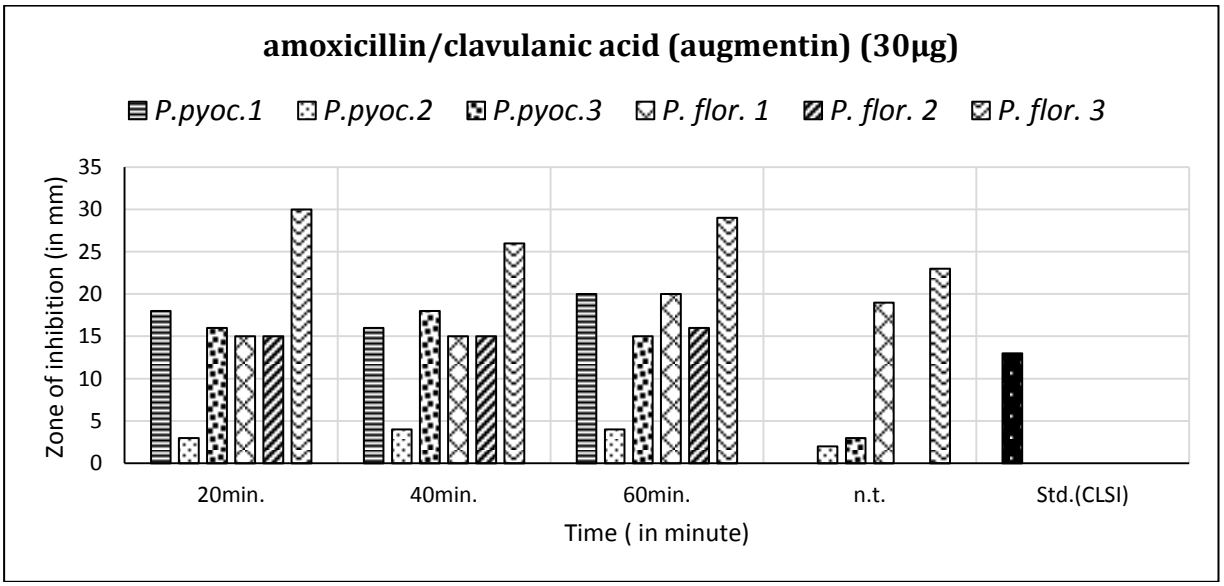

Figure (7): Inhibition zone of amoxicillin/clavulanic acid (augmentin) resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

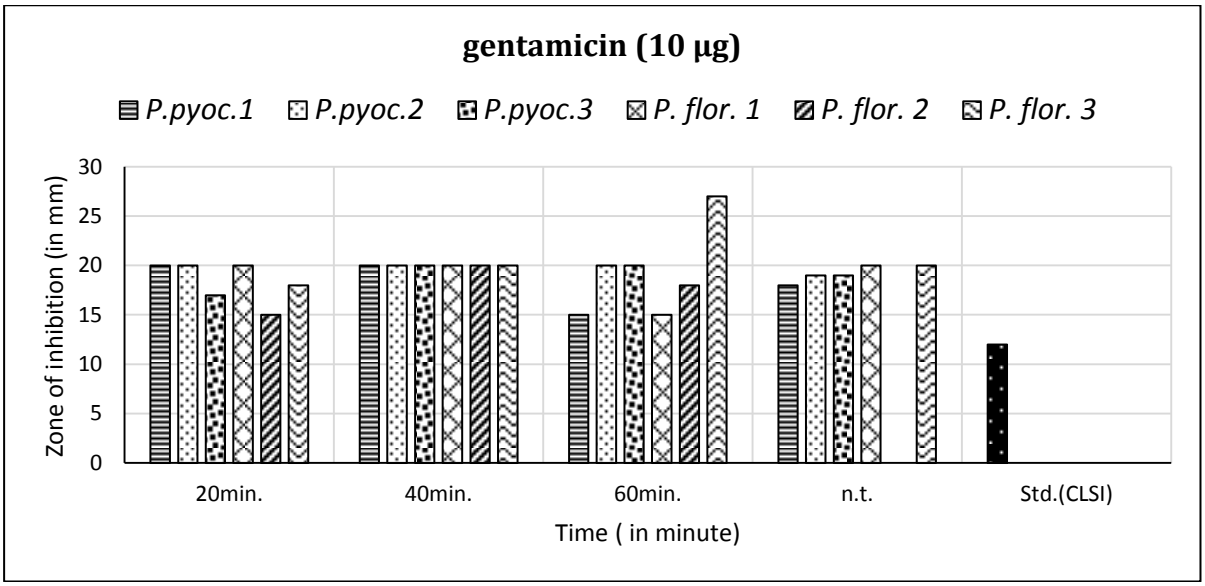

Figure (8): Inhibition zone of gentamicin resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

In figures (9) and (10) there were no effect of all exposure time were stiduded on the sensitivity of the bacterial species to antibiotics (tetracyclin and vancomycin). The isolates remained resistant to all antibiotics were treated compared with Std.(CLSI), the resistance of these species to this antibiotics may be caused to production of enzymes which capable of analyst the antibiotic molecules or modification and converted to inactive molecules, and R-plasmid encoding multiple antibiotics resistances undergo a remarkable change in character with the widespread occurrence of resistance transfer factors (RTF). RTF may transfer to drug-sensitive strains by conjugation in much the same way and with much the same type of kinetics as F transfer in *Pseudomonas* species. Furthermore, RTF can act as sex factors in promoting conjugation and transfer of chromosome [27]. The tetracyclin and vancomycin, were still active against *Pseudomonas* species. Resistance may nevertheless, emerge during long term treatment of chronic infections. Resistance to other antibiotic including antipseudomonal antibiotics may also occur in future [28]. Given this drug-resistant nature of *Pseudomonas* species, it is important from a public health viewpoint to know whether RTF can either occur in this species or be transferred to it from the Enterobacteria which located in sewage water.

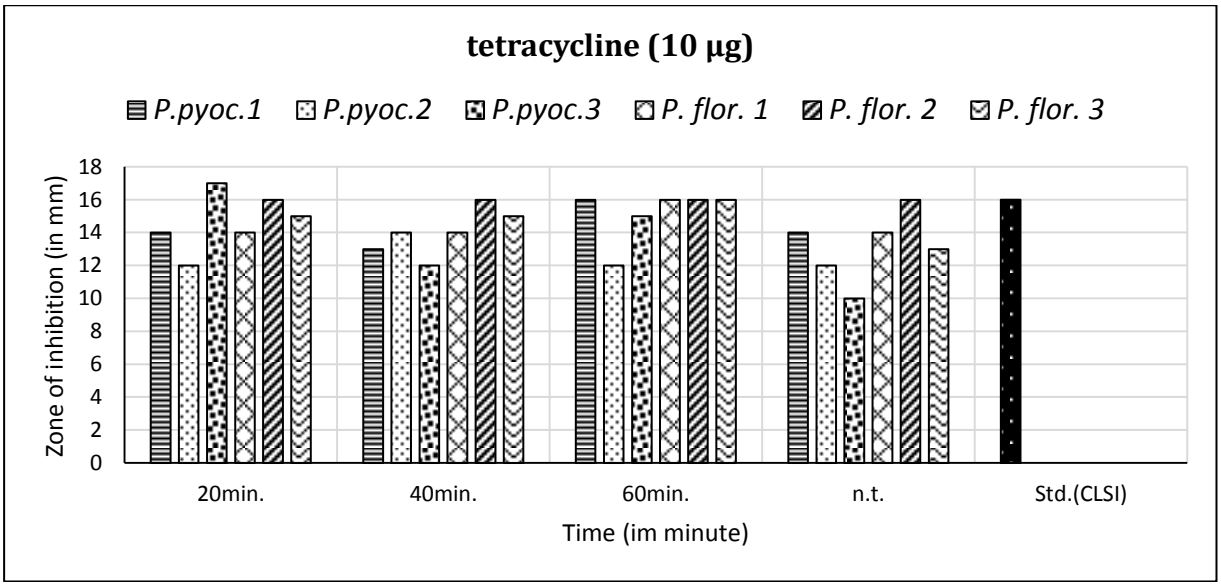

Figure (9): Inhibition zone of tetracycline resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

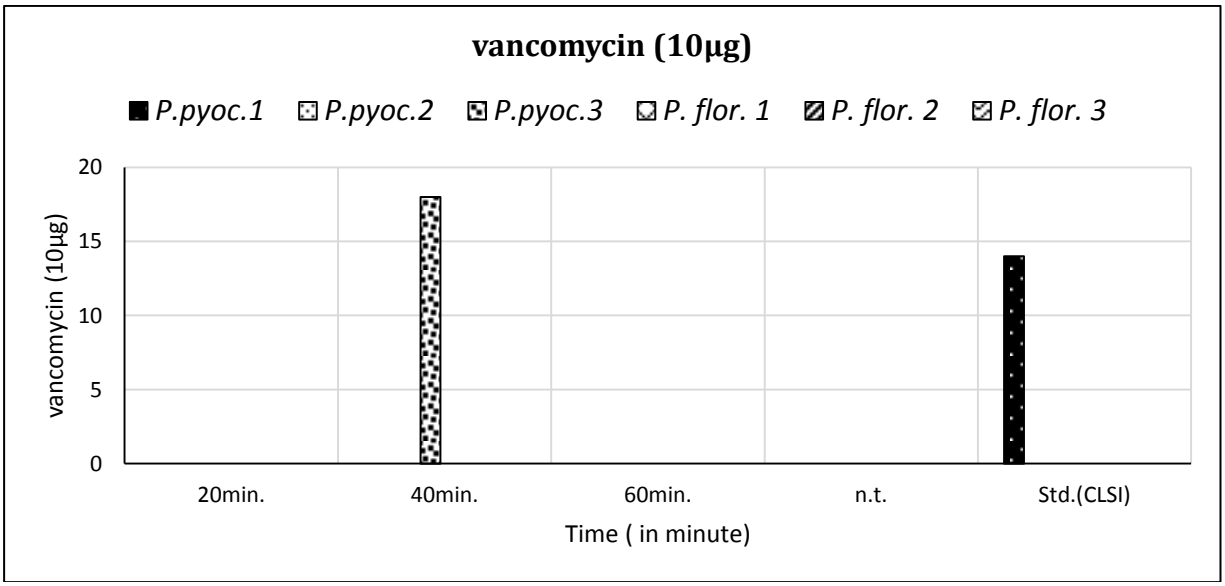

Figure (10): Inhibition zone of vancomycin resistance of *Pseudomonas* species treated with UV exposure in different time (20, 40, 60) minutes.

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