STUDY OF THE ANTIBIOTIC RESISTANCE OF PSEUDOMONAS AERUGINOSA ISOLATED FROM SOIL AND BURN WOUNDS INFECTION IN KARBALA PROVINCE

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ABSTRACT
The aim of this study is to isolate and diagnose P. aeruginosa from patients with inflammation of burns and soil samples with the study of antibiotic resistance to several antibiotics. Pseudomonas aeruginosawas isolated from clinical samples from burned wounds and soil samples from agricultural land. The isolated bacteria were distinguished based on standard cultured media, morphological and biochemical properties and by API 20 system. Disc diffusion and agar dilution method used for identified antibiotic susceptibility of the Pseudomonas aeruginosa. A total of 50 isolated bacteria (30 from a burned wound and 20 from soil), the bacteria was cultured and MIC and bacterial resistance were determined. Nine antibiotics were tacking in this study, the high resistance was recorded for P. aeruginosain samples of burn than in samples of soil and give less resistance for all antibiotics parameters, such as Ciprofloxacin, Streptomycin, Amikacin and Nalidixic acid not give any resistance for these bacteria, while the Tetracycline Trimethoprim and Amoxycillin give resistance but in less degree than in its recorded in samples from burn. Studying resistance to antibiotics allows to predict the future development and mentor the advancement of techniques to determine the quality of this resistance counteractive, thus necessitating periodic and detailed study of antimicrobial resistance in the endemic pathogens.

Key words:- P. aeruginosa, soil, burn, antibiotics resistance.

I. INTRODUCTION
Pseudomonas aeruginosa is important bacteria widely recognized reason for nosocomial diseases which influences principally immunocompromised patients in hospital, and specifically, is the main source of perilous contaminations in patients in units of burn [Floret N etal;2009, Leseva M etal;2013]. The medical significance of this bacteria is generally identified that it has strong obstruction to antibiotics medications, and notwithstanding its inborn protection from anti-infection agents, turns out to be rapidly safe against the medications within the medical therapy. [Tsutsui A etal;2011]. The predominance of medicine-safe detaches has been expanding worldwide and represents a major issue in emergency clinic cases, with huge ascent in cases horribleness and death [Cholley P etal;2010] Pseudomonas aeruginosa can identification in farming soils have been accounted for. Notwithstanding, regardless of whether these situations go about as a repository or a transient beneficiary of P. aeruginosa is still under discussion and information about components making its endurance and spread are rare [Green S. K etal; 1974, Marques A. M. etal;1979]. Due to its outstanding metabolic plasticity, P. aeruginosa can survive in diverse ecological niches including soil and aquatic habitats (Cabot et al. 2016), but it was usually observed in low abundance within these environments (Chatterjee et al. 2017). In contrast, P. aeruginosa was frequently isolated from hydrocarbon-contaminated sites (Kaszab et al. 2010) and can be dominant among hydrocarbon-degrading bacteria (Chaerun et al. 2004). The role of non-clinical environments as a reservoir or a transient recipient of P. aeruginosa is still under debate (Deredjian et al. 2014). Based on the absence of several virulence genes (Divya et al. 2018), or their non-haemolytic phenotype (Radhapriya et al. 2015), environmental P. aeruginosa isolates are sometimes considered as ‘non-pathogenic’ and are recommended for bioremediation (Ebadi et al. 2017), or plant protection purposes (Yasmin et al. 2017). Virulence models could provide tools for
the reliable assessment of the actual virulence of these strains such as wax moth (Galleria mellonella), a non-mammalian test organism, introduced as an in vivo model for the study of P. aeruginosa (Velikova et al. 2016).

The study of [Olga P. et al.; 2016] demonstrated that industrial wastewater effluents, composts, and hydrocarbon – contaminated sites should be considered as hot spots of high – risk clones of p. aeruginosa. it seems that Greek water bodies could serve as a potential reservoir of resistant P. aeruginosa isolates posing threats to human and animal health. The study of [Pachori P. et al.; 2019] provides a systemic review on major source of Pseudomonas aeruginosa in ICU. Further, study also emphasizes virulence gene/s associated with Pseudomonas aeruginosa genome for extended drug resistance. Study gives detailed overview of antibiotic drug resistance mechanism.

II. MATERIALS AND METHODS

Samples Tests
Microscopic organisms used for total 50 samples (30 from burn and 20 from soil) were disengaged from other ecological tests, for example, soil samples tests from horticultural locations at Karbala, and clinical samples tests from burn skin, going to the AL-Hussein Medical City in Karbala in February 2021. There are 30 samples were collected from burn with 60 % of total samples and 20 samples from soil with 40 % from total samples.

Identification of Bacteria and Antibiotics resistance
Identification of the Pseudomonas aeruginosa was by following: Morphological examination, Microscope Examination, Chemical Tests, API20E Identification system, and used recently Methods in diagnosis and isolation culture of the bacteria on gram staining, cetrimide agar, blood agar, MacConkey agar, growth on reaction in TSI case oxidase treatment catalase, and motion study in sulfide indole motility (SIM). P. aeruginosa were then kept win liquid culture at -70°C with 15% glycerol. The biochemical tests showed that 50 of the isolates were strains of this bacteria and then the resistance rates of the strains to many antibiotics were evaluated as indicator of resistance quality.

III. STATISTICAL ANALYSIS
Statistical analysis was performed using program SPSS version 20 for representation statistics by using bacterial data in tables. The aim was to recognize resistance range by significance measuring including frequencies, cross-tabulation of microbiological data. Chi square used in this study.

IV. RESULTS
There are 30 samples were collected from burn with 60 % of total samples and 20 samples from soil with 40 % from total samples. The Pseudomonas aeruginosa consider one of the most common bacteria infected the burn and resided the soil as in the figure (1).

Figure (1): Isolated bacteria from different sources (Burn and Soil)
Figure (2) Values of concentration of minimum inhibitory (MIC) to the bacteria isolated from soil to nine antibiotics. Ax: Amoxycillin; Ak: Amikacin; Ch: Chloramphenicol; Ci: Ciprofloxacin; G: Gentamicin; Nx: Nalidixic acid; S: Streptomycin; Te: Tetracycline; Tr: Trimethoprim.

**MIC of Antibiotic**

Figure (2) show the MICs of antibiotic *P. aeruginosa* W171 bacterial isolates, the MICs of AX, Ch and Nx determined in between 50 to 450, 100 150 and 450 µg/ml respectively. While the MICs ranged from 200 µg/ml to 250 µg/ml for Te, Tr, and G. from 0.5 µg/ml to 50 µg/ml for Ak S and Ci. This bacteria exposed high values of MIC to Ax (60 µg/ml), Ch (100 µg/ml), Te (250 µg/ml), Tr (200 µg/ml), G (200 µg/ml), Ak (10 µg/ml), and S (10 µg/ml). Therefore, it indicated resistance quality certainly.

**Figure (3) Antibiotic resistances values for the bacteria isolated from burn wounds and soil**

- burn: 19.8, 60, 18.8, 40.1, 85.2, 61.5, 88, 44.2, 36
- soil: 1, 0, 0, 0, 10.2, 32.1, 16, 0, 4
There are many differences in antibiotic resistance between samples that were taken from burn wounds and soil, where the resistance of P. aeruginosa in samples of soil burn give less resistance in all antibiotics parameters, such as Ciprofloxacin, Streptomycin, Amikacin, and Nalidixic acid. Chloramphenicol does not give any resistance for these bacteria. While the Tetracycline, Trimethoprim, and Amoxycillin give resistance but in less degree than in their recorded samples from burn.

V. DISCUSSION

To the best of our knowledge, this is the first time that an attempt has been made to determine P. aeruginosa resistance profiles circulating in Iraq from different sources. Additional information is provided for the presence of class 1 and class 2 integrons in some environments and their likely participation in the occurrence of resistance to several classes of antibiotics. Theoretically, wild isolates should be susceptible to all antibiotics presenting no acquired resistance mechanism while the intrinsic mechanisms are not expressed. However, in clinical isolates the intrinsic resistance mechanisms of P. aeruginosa are well characterized. The presence of resistant P. aeruginosa isolates in some environments is considered high and worrying while the combination with acquired mechanisms such as ESBLs may lead to complex resistant phenotypes. However, there is not much published information regarding soil P. aeruginosa isolates. In the present study, the majority of the resistant isolates may be presented non-enzymatic mechanisms with ATM-resistant isolates standing out. High resistance to ATM has been previously reported in environmental isolates deriving from soil or from hospital waste-water treatment.

In the present study, P. aeruginosa accounted for 60 percent of burn patient isolates from burn wounds and 40 percent from soil. Antibiotic sensitivity tests were done for detection of sensitivity and resistance of P. aeruginosa in all samples. This bacteria have high resistance for many antibiotics widely used in treatments of infections with these bacteria because these bacteria have many proteins channels called Porins inside the bacterial membrane, these channels responsible for inside influx for many components like antibiotics (such as beta lactamase). After culture the samples from burn wounds, the isolate show the Amikacin and Ciprofloxacin give lowest resistance for P. aeruginosa (18.8%, 19.8%) respectively and these results disagreed with Tsakris was reported the these bacteria give high resistance for these antibiotics. Amikacin antibiotic was give low resistance for bacteria from the other antibiotics used in these study may be due to these antibiotic have aminoxybutryl groups these prevent bacterial enzyme from binding to this antibiotics in different sites and binding to rRNA. The Ciprofloxacin antibiotic also show lowest resistance to this bacteria and these agreed with (Lomholt and Kilian 2003) were showed the resistance less than 5% from all samples, these results consider very low may be due to the Ciprofloxacin not activate excretion pumps systems including MexEF-OprN. This study reported high resistance to tetracycline and this agreed with (Lomholt and Kilian 2003) was reported about more than 60% resistance from all samples. The multidrug-resistant strains P.aeruginosa has improved change the resistance consciousness for many Antibiotic. It is therefore important to control nosocomial infections in burning units on a regular basis, and to perform culture and sensitivity tests to identify the most appropriate antibiotics for treatment. That mean the bacteria isolated from burn wounds considered more virulent bacteria because those bacteria penetrate the body's defenses and gain access to the bloodstream, thus demonstrating more antibiotic resistance than bacteria isolated from soil.

REFERENCES


