AL- Qadisiyah University

College of Biotechnology

Departement of Medical

Isolation and Diagnosis of bacteria *Pseudomonas aeruginosa*

Graduation research submitted to the Departement of Medical in the College of Biotechnology

By the students

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بسم الله الرحمن الرحيم

(يرفع الله الذين امنو منكم
والذين اوتوا العلم درجات والله
بما تعلمون خبير)

صدق الله العظيم

سورة المجادلة

رقم الاية:1

Dedication

To my Doctor Najlaa Abdullah Dawood.

To one of the cup's empty dishes to hold me a drop of love to those who wished us a moment of happiness to the one who took the bushes from Derby to guide me through the flagTo the great heart (my dear father)

To all who are in existence after His Messenger to my Sindhi and my strength and then to the spring of patience and hope and hopeTo the affectionate heart (my dear mother)

To those who follow me on their own, to those who taught me the science of life, to those who have shown me the beauty of life (my brothers)

To those who were refugees and refugees

To whom you have tasted the most beautiful moments

To whom I will miss .. I hope they miss meTo those who made them my brothers Ba .. And I loved them (Department of biotechnology department)

Huda, Hawraa, Hawraa

Abstract

In this study, 80 samples were collected from different sources in Diwaniyah Teaching Hospital and Burns Hospital. The random sampling process was conducted to identify the foci of pollution in *p.aeruginosa*, which was one of the objectives of this study and obtained diagnostic procedures. The samples were distributed to 45 clinical samples and 35 environmental samples. Results of agricultural tests and tests biochemistry 15 isolates of *p.aeruoginosa* bacteria The pharmacological sensitivity of 15 samples was tested against 10 types of antibiotics in the method of spreading the disk to *Kirby_bauer*. All isolates were almost resistant and there were 3 isolates. All antibiotics were resistant to severe resistance.

Contents

Introduction

P.aeruginosa was isolated for the first time from a pure farm in 1872 by the Schoroeter world of various environmental sources (Dowrkin *et al*., 2006) (Brooks *et al*., 2007), *Aeruginosa* bacteria are the most common types of *pseudomonas*.Bacteria are commonly used on commercial scale and traceableThese bacteria are the kingdom of Bacteria and the section of GracillicutesScotobacteri and *Pseudomonadalas*.

The family of *Pseudomonadaceae* by world classification Bergy(1994, Holt and), a Gram-negative bacterium Bacteria are generally spread in soil and water . Growth temperature Ideal for 73 m and can grow up to 23m.which have the ability to produce multiple dyes in the agricultural medium, including the dye of green pyocyanin (pheasant) , The pyoverdin pigment, the greenish fluorescein, the red poyrubin pigment, and the black pyomelanin pigment (Jawetz et al., 2010).

P.aeruginosa has a large genome consisting of 6.26 mpg For about 5567 genes compared to the Escherichia coli genome, which consists of 4.46 megabytes of WeigrassFledforabout4279Jane(Ratakia,2011). *Ps.aerugiosa* Secondary Opportunistic Pathogen rarely causes disease in healthy people but poses a real risk to patients in hospitals, especially cancer patients, burns, immunodeficiency and organ transplantation. It is one of the most important bacterial infections known in hospitals as Nosocomial infections, as these bacteria can grow On the floor of the hospital lobby, operation halls, surgical instruments, etc (Fonseca *et al*.;2007). It has the ability to stay in disinfectant and some types of sterilizer(Levinson,2004).

Ps.aeruginosa causes various medical conditions that are localized, especially after surgery and burns. The infection then spreads and causes fatal bacteremia.

Ps.aeruginosa infections are caused by their ability to possess effective adhesion fibers, low nutrient requirements and antibiotic resistance) and have the ability to invade topical tissue and destroy these tissues and have a tendency to invade the bloodstream And systemic diseases(Isturn,2008). Produce these bacteria many Factors of ferocity which help them on the invasion and settlement causing

damage histological invasion of blood stream and proliferation in the areas of the body is different and the most important these factors Hemolycin, Enzyme elastase and Urease enzyme that analyzes urea and other enzymes(Mask and hergenro,2008).

Ps.aeruginosa bacteria have the ability to resist a wide variety of antibiotics, making it one of the most dangerous and important causes of human diseases(Bukharia and Mowafi,2008). These bacteria can use a variety of resistance mechanisms. The most important of these mechanisms is reduced membrane permeability, Bacterial enzymes such as $\mathcal{B}$_*lactamases* and Cephalosproinases. The spread of these bacteria in various areas of the body and the constant exposure of antibiotics has led to the emergence of strains characterized by multiple resistance to drugs.

It is the most common cause of infections of burn injuries and of the outer ear (otitis externa) , and is the most frequent colonizer of medical devices (e.g., catheters). Pseudomonas can be spread by equipment that gets contaminated and is not properly cleaned or on the hands of healthcare workers. Pseudomonas can, in rare circumstances, cause community-acquired pneumonias, as well as ventilator-associated pneumonias, being one of the most common agents isolated in several studies. Pyocyanin is a virulence factor of the bacteria and has been known to cause death in C. elegans by oxidative stress. However, salicylic acid can inhibit pyocyanin production. One in ten hospital-acquired infections is from Pseudomonas. Cystic fibrosis patients are also predisposed to *P. aeruginosa* infection of the lungs. *P. aeruginosa* may also be a common cause of "hot-tub rash" (dermatitis), caused by lack of proper, periodic attention to water quality. Since these bacteria like moist environments, such as hot tubs and swimming pools, they can cause skin rash or swimmer's ear (Demiguel et al .;2005).

Pseudomonas is also a common cause of postoperative infection in radial keratotomysurgery patients. The organism is also associated with the skin lesion ecthyma gangrenosum. *P. aeruginosa* is frequently associated with osteomyelitis involving puncture wounds

of the foot, believed to result from direct inoculation with *P. aeruginosa* via the foam padding found in tennis shoes, with diabetic patients at a higher risk.

pathogenesis

Bacteria *p.aeruginosa* characterized by their ability to cause different types of infections in multiple locations of the body, especially after surgeries and injuries, burns and spread infection and cause cases of bacteremia (Demiguel martinez et al., 2005) .

As it penetrates bacteria *p.aeruginosa* severe burns causes weak resistance to skin tissue offered for burns and damage in addition to the presence of this bacteria in abundance in the surrounding environment in the patient's burns unit or nursing staff in hospitals (sheridan, 2005).

The severe bacterial infections *p.aeruginosal* be due to its ability to colonize different anatomical sites because of possession (effective mechanisms of adhesion and the requirements of low feed and antibiotic resistance) and have the ability to invade the host and destroy the events of systemic diseases, tissue (zeng, 2004) is a bacteria *p.aeruginosa* important cause In Chronic Respiratory Infection Associated with Cystic Fibrosis Which causes chest typhoid in adults and children, accompanied by disease secretion of mucus viscous can not be removed from the lungs, leading to indigestion in respiratory function (Hoiby et al., 2001) also caused bacteria *p.aeruginosa* many other such infections typhoid and typhoid eye and typhoid Urinary tract and skin infections (willenbrock et al., 2006). The incidence of these bacteria is increased in cases of cancer, immunodeficiency, and postoperative deficiencies (willenbrock andussery,2007).

Virulence Factors

P.aeruginosa is an opportunistic pathogens, especially in areas lacking naturaldefenses(Jawetzetal.,2008)

They have many factors, including cellular factors such as flagella, pilli, and fimbria, which help to move and adhere to host cells, as well as peripheral agents such as piacinin, buoferrin, and exotoxine A, which are lethal when injected into pure animals in exotoxine Its function is adhesion and prevention of phagocytosis in infected tissues (Jawetz *et al*., 2010). Other factors of virulence in *p.aeruginosa* bacteria are the mucous membrane (Todar, 2008), which is secreted by some *P.aeruginosa* strains and their colonies appear to be mucous (salyres and whitt, 2002) if they produce more in chronic infections Especially in the case of cystic fibrosis and has a role in reducing the permeability of the lethal concentrations of a number of antibiotics and sterilizers and preventing them from reaching the target sites in the bacterial cell, in addition to their role in increasing the resistance of bacteria tothechewing process (Jawetz et al., 2010) The invasion of host cells by bacteria requires the penetration or destruction of the outer cytoskeleton and occurs through physical methods or enzymatic means or both (musk and hergenro, 2008).

Enzyme factors play a key role in the breakdown or analysis of the structural units in the host's cell membrane, which is the lipid and proteins during the invasion of the host.

AIM*:*

1_Isolatio and diagnosis bacteria *P.aeruginosa* in diabetic patients in Diwaniyah Teaching Hospital and Burns Hospital.

2_The widespread use of antimicrobial agent leads to emergence of drug resistant organisms.Since the pattern of bacterial resistance is constantly changing over years,it is important to monitor the antibiotic susceptibility patterns of isolated organisms to ensure rational use of antibiotics for empirical and definitive treatment of body infections .

Materials and Methods

Materials

1// Equipment and apparatus

No	Equipment	Company
1.	Sensitive electronic balance	A & Co. (Japan)
2.	Refrigerator	Concord (Lebanon)
3.	Deep freezer	GFL (Germany)
4.	Autoclave	Hiclave (japan)
5.	Incubator	Memmert (Germany)
6.	Standard wire loop	Himedia (India)
7.	Hot plat	GallenKaamp (England)
8.	Milliporefilter paper	Difco (USA)
9.	Test tubes	Superestar (India)
10.	Sterilized cotton Swabs	SterellinLtd (England)
11.	Disposable Petri dishes	Al-Hani (USA)
12.	Conical flasks	BBL (USA)

13.	Hood	USA

2..Ready prepare material

NO	Material	Company
1.	Agar	Biolife (Italy)
2.	Glycerol (C3H803)	Fluka (switzerland)

3// Ready prepare media

NO	Media	Company
1.	MacConkey agar	Himedia (India)
2.	Blood agar	Himedia(India)
3.	Nutrient broth	Himedia(India)
4.	Muller Hinton agar	Oxoid (UK)

4//

Antibiotics

Antibiotics	Company
TRIME THOPRIM(TMP) 5mcg	CONDA pronadisa
NALIDIXIC ACID(NA) 30mcg	CONDA pronadisa
MEROPENEM(MEM) 10mcg	CONDA pronadisa
CHLORAMPHENICOL(C) 30mcg	CONDA pronadisa
GENTAMICIN(CN) 10mcg	CONDA pronadisa
RIFAMPIN(RA) 5mcg	CONDA pronadisa
CEFOTAXIME(CTX) 30mcg	CONDA pronadisa
AMOXICILLINI CLAVULANIC ACID(AMC) 30mcg	CONDA pronadisa
AZTREONAM(ATM) 30mcg	CONDA pronadisa
AMIKACIN(AK)30mcg	CONDA pronadisa

5// The kit ready made

Noun	Company
Api 20 E	BioMerieux(France)

Methods

/1/ Culture media preparation

a: Media preparation

attended the culture media according to the instructions of the manufacturer and adjust the pH to them as needed except for the synthetic agars, which included the blood agar.

b: Media sterilization

Sterilize all used culture media with autoclave at 121 ° C and 1 pressur for 15 minutes.

2/ Collection of samples

A total of 80 samples of 45 clinical samples were collected from various inflammatory cases, including urinary tract infections, burns, ear infections from patients, pedestrians, different ages for both sexes, and 35 environmental samples from the Diwaniyah Teaching Hospital and the Burning Hospital in Qadisiyah City from the period 8_9_2017 to 13_2_2018 .Samples (to avoid contamination of natural flora present in this area) were collected by swabs and recording information(sex & age). To investigate the *Ps.aeruginosa* Bacteria,take a swab of ear,burn,sputam,urine,operation room ,floor and emergency pans and plant it by cotton swabs and plan on the surface of the dishes of the blood agar base and MacConky agar and incubate the dishes for incubator(24_28h) at 37c .To study the sensitivity and resistance of bacteria causing more infections to some antibiotics used to treat more infection.

3/ Isolation of bacteria

For the purpose of isolating the p.aeruginosa bacteria,forgive blood agar and MacConkey agar by sampling swabs in a planning manner and incubated 37C 24_28 hours.

4/ Identification of Bacteria

Bacterial colonies were identified developing depending on:

a/// Phenotypic characteristics:

Observed Phenotypic characteristics for colonies developing forms and color and surface colonies and the presence of the smells featured her style decomposition blood on the blood agar and fermenting lactose on macconky agar(winn et al. 2006).

b/// Biochemical test

Oxidase test:

Transferring a part of a 24-hour colony with a sterile wooden stick to a filter paper saturated with oxidase and a violet color within 10 seconds indicates a positive test (Brown, 2007)

5//Preservation and Maintenance of Bacterial Isolates

A// Short_term Storage

The tubes containing the solid nutritious medium fed to the bacteria were conserved and stored at a temperature of 37 ° C for 24 hours and then kept at a temperature of 4 ° C. The preservation process was repeated to regenerate the isolates and avoid contamination(Collee et al., 1996).

B//Long_term Storage

The tubes containing the liquid nutrient-laden medium with 15% Under study and saved at 20c-(NCCLS,2003).

Antibiotic Susceptibility Testing

Tested sensitivity antibiotics for sample bacterial a way that drives depending on the way bauer and his group (1966) and (2012) CLSI. Included:

Activated bacteria *p.aeruginosa* stored in the refrigerator temperature 37c for a 2 hour after it has been moved colonies bacteria to tube containing 5ml of nutreint broth and put in lncubator temperature 37 for 24 hoursThen we dip the cotton swab in the nutreint broth and spread the bacteria on the center of the Muller_Henton steel in a manner of planning for more than one time and in different directions for the purpose of ensuring the spread of the bacteria to be tested for sensitivity in equal and left the dishes 15 minutes at room temperature to ensure the absorption of moisture, the tablets of antibiotics by 5 tablets in a measuring dish 100 ml and the distance between each tablet and another 20 ml from the center of the first disc to the center of the other disk, incubated the dishes at 37C for 24 hours for all antibiotics and then measured the diameters by using the ruler and compared with the standard values mentioned in CLSI (2012).

Results and Discussion*:*

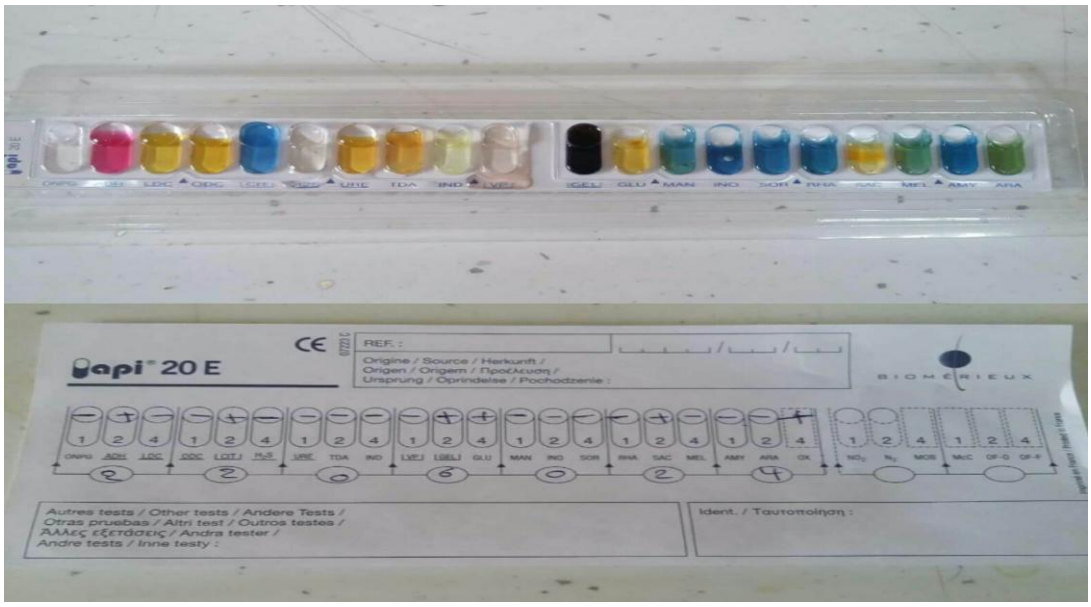
The current study included the collection of 80 samples of various clinical and environmental cases in Diwaniyah Teaching Hospital and Al-Burn Hospital. The random sampling was conducted to investigate the contamination of *P.aeruginosa* bacteria, resulting in diagnostic, preventive and therapeutic procedures. The samples were collected by ear from 10 ear swabs (12.5%), burns 25 (31.25%), and 5 (6.25%) and Urine 9 (11.25%) and floor and emergency pans 11 (11.75%) and operation room 20 (25%)

Table(1) types the samples and number of percentage

Sample	Type	The number	Percentage
Clinical	Ear	10	12.5%
Clinical	Burns	25	31.25%
Clinical	Sputam	5	6.25%
Clinical	Urine	9	11.25%
Environmental	Floor &emergency pans	11	13.75%
Environmental	Operation room	20	25%

Isolation and Identification

The main objective of the collection of samples was to isolate the bacteria *P.aeruginosa* and isolated the isolates depending on the phenotypic characteristics of the developing colonies as the appearance of the center of Agar color pale color of the inability to ferment the sugar lactose located in the center of the plant and has a smell similar to the smell of grape fermented while the colonies appeared dark color and most surrounded by A clear halo on the center of the blood agars, indicating its ability to decompose blood The results of the biochemical tests showed positive results for the oxidase test. All the isolates were characterized by their inability to produce hydrogen sulfide gas and they were not fermented for sucrose and lactose.The api ribbons were used to confirm the diagnosis *of P.aeruginosa*.



Figer(1) Api 20 E

The ability of bacteria to produce cytochrome oxidase can be determined by the sddition of the oxidase test reagent or test strip to colonies that have grown on plate medium.the appearance of violet indicates a positive test .

Incident and distribution of *P.aeruginosa*

Of the number of isolates *p.aeruginose* 15 isolated from the total of 80 samples with 18.7% and the results approximate to Ayman in India 2014 if the percentage change isolation 17% of the samples *p.aeruginosa* bacteria while our results do not agree with what recorded Salem and Yemen, who indicated the proportion of isolation of bacteria *P.aeruginosa* of different clinical cases were 76.2% and 51%, respectively.

The results of the present study Showed that the highest percentage of isolation of *P.aeruginosa* bacteria from burns amounted to 31.2%, and this result was compared to what recorded in the year 2013 with a rate of isolation of 29%, and these results more than recorded in 2014, if he pointed to the isolation rate of 23.6% of burns and the risk of isolation rate Paeruginosa is a high percentage of burns worldwide, due to the resistance of bacteria to antibiotics and antiseptics.

The results of the current study showed that *P.aeruginosa* bacteria is one of the main causes of urinary tract infections if the percentage of isolation of the blood is 11.2%. This ratio is close to that recorded by Abdel Wahab 2014 if the ratio of isolation was 10.4% of the blood. 10.5% of the blood in the United States. These results are not consistent with Faisal's results if the isolation rate is 6%. The second pathogen is *P.aerugionsa*, which is the second most frequent among the negative bacteria of chromatography (16.3%) in patients with urinary tract infection United States.

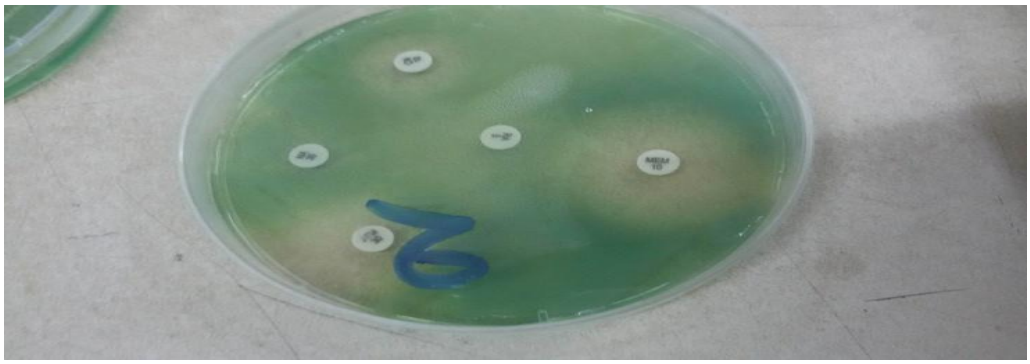
The current study recorded a ratio of isolates of *P. aeruginosa* from the respiratory system (sputam) was 6.2% and this ratio is close to that recorded by Ahmed 2013 if the rate of isolation of the sunscreen 4.4%, but in a study conducted in Iran, the rate of isolation was 9% This difference is attributed In the results of the current study with other studies to the fabrication of the season in which the collected samples and the difference of patients if the rate of injury in patients who are asleep and who already suffer from weakness in the immune systems of the body.

The results of the present study percentage of isolation of *P.aeruginousa* bacteria if the rate of isolation 12.5% and these results were close to what Ahmed obtained if the rate of isolation 13.9% in 2014 in hospitals Najaf, while our results do not agree what recorded Walid in hospitals in Nazareth This difference in the rates of isolation to the difference in the social and economic level of patients and to the geographical area and health culture.

Antibiotic Susceptibility Test

The antibiotic susceptibility test was measured in table (10). The antibiotic was chosen to be used in the treatment of some *P.aeruginosa* infections. Table shows the resistance and allergy between the bacteria to the antibiotic used and the results of the test About the antibiotic tablet and compare it with the standard tables according to CLSI 2012, in order to determine the resistance of bacteria to the antibiotics used in the hospitals of Diwaniya city and the seriousness of those resistance, which extends to a wide spectrum of different antibiotic.

The results of the effect of antibiotics samples bacteria *p.aerginousa*



Figer(2)antibiotic sensitivity pattern has been determined by the zone

Table(2)result of resistant and sensitive of antibiotics of *Ps.aeruginosa*

Antibiotic	Resistant	sensitive	Result
AK	8	7	R
AMC	14	1	R
C	9	2	R
TMP	15	-	R
CTX	15	-	R
NA	12	1	R
MEM	9	4	R
ATM	10	5	R
CN	9	5	R
RA	15	-	R

Table(3)The percentage of resistant and sensitive of antibiotics

Antibiotic	Resistance	Sensitive
AK	53.3%	46.6%
AMC	93.3%	6.6%
C	60%	13.3%
TMP	100%	-
CTX	100%	-
NA	80%	6.6%
MEM	60%	26.6%
ATM	66.6%	33.3%
CN	60%	33.3%
RA	100%	-

Recorded the present study that increased significantly in ratios resistant isolates *p.aeruginous* to antibody RA and CTX where resisted all isolates bacteria the proportion of resistant isolates to antibody C and MEM less than resistant isolates to antibody AMC and TMP . It can interpret the proportion of resistance highly expressed by isolates *p.aeruginous* to this antibodies vital in the present study to use the random to this antibiotics Besides evolution in resistance, which brought this bacteria because of the use of doses under the therapeutic contributed to the emergence of isolation mutation(magent and balanchard, 2005).

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