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Isolation and diagnosis of *Pseudomonas aeruginosa* from burn patients

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Abstract

The aim of this study was find out the zoonotic *Pseudomonas aeruginosa* of patient suffering from burns wound. The purpose of isolating these bacteria from burn patients is the high mortality rate in my country. (69) Samples were collected from teaching medical Al- Kendi hospital, during a period from October (2007) to June (2008). The samples cultured on blood agar media and incubated at (37 °) C for (24) h. These colonies were culture on the Blood agar; some of colony cultures on MacConky agar, after that subculture on Pseudomonas agar base. The patients divided into non mature group, their aged ranged from (3-11) years and mature group, their age ranged from (12-58) years, each group includes male, and females. The results showed (9) isolates of *Pseudomonas aeruginosa* were isolated from (69) burn wound samples (24) samples from kids (13) males and (11) females. (45) Samples collect from adults (20) sample from male and (25) samples from female. The percentage of bacterial isolation was (13%). Females showed higher percentage (7.2%) than males (5.7%) high percentage of bacterial isolates were rusticated at mature group (11.1%) compared with non-mature group (16.6%). The results of serotyping were P16 (33%). P15 (11.1%), P2 (11.1%), P9 (11.1%), P11 (11.1%) , P12 (11.1%). The serotype (P16) is prevailing among the strain classified. The present study suggested that *Pseudomonas aeruginosa* play important role in contamination of burn wound in human. Many burn patients die not because the burn but because the virulence of bacteria especially *Pseudomonas Aeruginosa*

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Introduction

Previously, it was believed that *Pseudomonas Aeruginosa* spores are harmless and have little significance in causing human and animal disease (Cherrington and Gildow, 1981). However, subsequent studies have shown that these opportunistic germs cause significant injuries in people with immune braking, such as cystic fibrosis in the lung, cancer, immunodeficiency, and skin burns, leading to a high rate of damage (Birch and Benner, 1920).

Pseudomonas aeruginosa are characterized by negative gram stain, *P. aeruginosa* is a facultative anaerobe and can achieve anaerobic growth with nitrate or nitrite like a terminal electron acceptor, *P.aeruginosa* motile with one polar flagellum (monotrichous) and some species have (1_3) polar flagellum, this bacteria non spore and non-capsule (Palleroni, 1984; Govan, 1996).

Pseudomonas aeruginosa are very important pathogenic bacteria that are widespread in nature and can live in different environmental conditions (Greenwood *et al*., 1998). They are found in soil, water, plants, skin, and it isolated from throat, healthy people's faeces, Pseudomonas aeruginosa found in the humid environment as sewerage, swimming pools and surrounding areas (Frazier and Wasthoff, 1985). *P. aeruginosa* consider main germs that cause injury to wounds and burns, cause local and topical lesions resulting in the failure of many internal organs of the host multivisceral failure leading to a high percentage of deaths up to (50%) as these bacteria cause severe tissue damage due to the production of many factors of virility and inflammation endocarditis, pneumonia, urinary tract injury, neuralgia, eye and external ear, It also has the ability to change its genetic structure constantly and the formation of mucous colonies, which increases the resistance of the bacteria to the process of phagocytosis and many antibiotics, where the problem of multiple resistance to antibiotics is one of the most important global problems and helped to complicate the problem of the presence of resistance with the plasmids associative mobility between bacterial species Which are naturally converging and present in the environment (Gori *et al*., 1996). Bacteria are bacilli, their diameter (0.5-0.7) microns, their lengths (1.5-3) microns and appear in swabs taken from the liquid and solid plant media in single or pair's formations, and may sometimes appear in short chains (Jawetz *et al*., 1987). It may be a letter (L) as referred to by the world Boneiw (1970) for the first time since it is isolated from the heart-craving inflammation in dogs. *Pseudomonas aeruginosa* grow on media of enterobacteriaceae family such as MacConky agar, Brilliant green agar, Trypticase soy agar, Xyloselysine deoxycholate, Blood agar, and Mueller Hinton agar, (Quinn *et al*., 2006).

Pseudomonas aeruginosa make mucous colonies on the blood agar accompanied by a beta-type hydrolysis, Colonies are characterized by growth bluish-green color due to the biosianin pigment produced by this bacterai (Ohman *et al*., 1980). The colonies are pale and non-fermented lactose and produce a Piacian pigment on MacConky agar (Quinn *et al*., 2006).They are (140) species of Pseudomonas genus (Atlas *et al*., 1995).

Pseudomonas has two types of siderophore, first type: pyeverdine, which is responsible for the Fluoresence pigment. This pigment consist from Dihydroxyquinolone chromophore which associated with a chain of peptides varying in length according to the production of bioferrins (Bayer *et a*., 1991).

The second type of Sidrophore is Pyochelin which is derived from Sialicylic acid and 2-cysteine residue (Budzikiewicz *et al*., 2001). The external receptors ferri-pyoverdin and ferri-pyochelin are the outer membrane proteins fpvA and fptA, respectively. Both Siderophores and their receptors are produced from the clinical isolates of *Pseudomonas aeruginosa* and play an important role in their virulence (Hancock and Worobec, 1998). (Meyer *et al.,* 1996) that mutant patterns that do not have this characteristic are weak and do not cause burns. *Pseudomonas aeruginosa* are very widespread in nature, as they are found in

soil and water and infect a wide range of field and wild animals as well as humans and plants, they have emerged as competitive germs that can survive and multiply in humid environments without the need for complex foodstuffs (Vasil *et al*., 1986). *P.aeruginosa* has been isolated from the skin of animals, mucous membranes and animal waste, as well as from purulent infections such as abscesses, mastitis and diarrhea from various wild and field animals, including cattle, pigs, chickens, ducks, rabbits, deer, bears and pigeons (Chen *et al*., 1987). (Sutter *et al*,.1996) noted that 5% of healthy people carry this germ in their saliva, these bacteria spread in the swimming pools and surrounding areas (Hoadely *et al*., 1975).Pseudomonas needs carbon and hydrogen as sources of energy (Holt *et al*.,1994). More than (50) organic compounds are used during the growth process (Vasil *et al*., 1986), as well as a need for a few nutrients as they grow in tap water and detergent solutions disinfection (Levinson and Jawetz, 1996). Pseudomonas also has a competitive advantage with other microbes living in their surroundings by inhibiting the growth of these bacteriostatic potentials to produce the piaciocin pigment that has the deadly bacteriocin action of isolates of the same species or other bacterial species (Timoney *et al*.,1988). *P.aeruginosa* has the viability and multiplicity of disinfectants and therapeutic fluids such as eye drops as well as anesthetic masks and the floor of operating theaters (Hawkey and Lewis, 1989).

Ayliff (1978) (6) blindness cases postoperative because the physiological solution used contaminated with *P.aeruginosa* bacterium and other similar cases were recorded in Thailand due to contamination of physiological solution and Hyaluronic acid with these bacteria (Swaddi *et al*., 1995).

Materials and methods

Bacterial germs and their sources

Pseudomonas aeruginosa strain received from the Zoonotic Disease Unit / College of Veterinary Medicine / University of Baghdad. (Holt *et al.,* 1994). Microbial isolates were standardized in the Central Health Laboratory, according to Book No. 215.

Reagents are used

Oxidase reagent, Kovac's reagent, Catalase reagent, Brothymol Blue Detector, Alpha naphthalimic acetate, Sulphanilic acid.

Samples culture

The samples cultured on blood agar media and incubated at (37 °) C for (24) h. Then the colonies were grown on the Blood agar; part of the colony was taken by a sterilized loop and culture on MacConky agar, fter that subculture on Pseudomonas agar base.

The examination included observation of colonies' colors and their production of dyes, as well as the shape and size of the colonies its aroma, its strength, its growth and the non-fermentation of lactose sugar on the center of the MacConky and form colonies, and after the growth of colonies on the media by making swabs of bacterial isolates, where a bacterial colony was taken by the bacterial carrier on a glass slide and a gram stain was made to study the bacterial interaction with the dye. The biochemical tests are: oxidase test, catalysis test, ureas test, triple sugar iron test, nitrate reduction test, gelatin test

Results of bacterial isolation

The isolates were identified according to phenotypic traits and biochemical methods. Nine samples were isolated from the pseudomonas by examining (69) burn cases of burn patients in Al Kindi Teaching Hospital for the period from October to June. The results showed (9) isolates of *Pseudomonas aeruginosa* were isolated from (69) burn wound samples (24) samples from child (13) males, (11) females and (45) samples taken from adults (20) sample from male and (25) samples from female.

The percentage of bacterial isolation was (13%). Females showed higher percentage (7.2%) than males (5.7%) high percentage of bacterial isolates were rusticated at mature group (11.1%) compared with non-mature group (16.6%). The results of serotyping were P16 (33%). P15 (11.1%), P2 (11.1%), P9 (11.1%), P11 (11.1%), P12 (11.1%). The serotype (P16) is prevailing among the strain classified.

Table 1. Shows the most important diagnostic differences of *Pseudomonas* species.

Species Characteristic	*Ps. aeruginosa*	*Ps. fluorescens*	*Ps. putida*	*Ps. cepacia*	*Ps. maltophilia*	*Ps. stutzeri*
Pyoverdin produced	+	+	+	*-	-	-
Oxidase	+	+	+	+	(-)	+
Growth at 5C°	-	+	(+)	-	-	+
Growth at 45C°	+	-	-	(+)	-	+
Gelatin	+	+	-	+	+	-
Urease	+	(+)	(+)	+	-	+
Oxidation of glucose	+	+	+	+	(-)	+
Oxidation of lactose	-	-	-	+	-	_
Oxidation of maltose	-	-	(-)	+	+	(+)
Arginine dihydrolyase	+	+	+	-	_	(+)
Maconkey agar	+	+	+	+	+	+
No. of flagella	1	>1	>1	>1	>1	1

+ = Positive reaction. - = Negative reaction

* = Some strains produce a yellowish non-florescent pigment

(+) = most strains positive (-) = most strains negative.

Table 2. Show laboratory devices.

Manufacture company	Used equipment
Memmert-Germany	Water bath
Many companies	Virnea
Towson-Japan	sonicator
Memmert-Germany	oven
Edelstahi-Rost	Incubator
Towson-Japan	Autoclave
Olympus-Japan	Microscope
Hearus-Germany	Centrifuge
Hearus-Germany	Cold centrifuge
Many companies	Water distillatory
Many companies	Benzene burner
ESHTAR- IRAQ	Refrigerator
ToWN-JAPAN	Spectrophotometer
PHILLIPS-HOLLAND meter	pH Meter
MEMMERT- GERMANY	Automatic Processors
MEMMERT- GERMANY	Microtome
	Petri dishes

Discuss

The results showed that the *Pseudomonas aruginosa* were very widespread in the contamination of burns patients in Al-Kindi hospital in Baghdad, where the percentage of isolation was (13.04%), which is much lower than that recorded in one of the burn centers in Tehran by (Rastegar *et al.*, 2007) in a study conducted in the educational hospitals in Baghdad to the rate of isolating the *Pseudomonas aruginosa* communities of wounds and burns was (10%) and the record Ergin and Mutlau (1999) the isolation rate of vesicular cultivars from wounds (1.48%) in Turkey,

and Savas (2005) recorded the isolation rate of these bacteria from the eye Wounds and burns (16.4%) in one of the teaching hospitals in Turkey. Al-Roubaeay (2002) recorded the isolation rate (45%) of wounds. Contamination of burns to pathogen is one of the most important problems faced by intensive care units.

Table 3. Show agar and broth use.

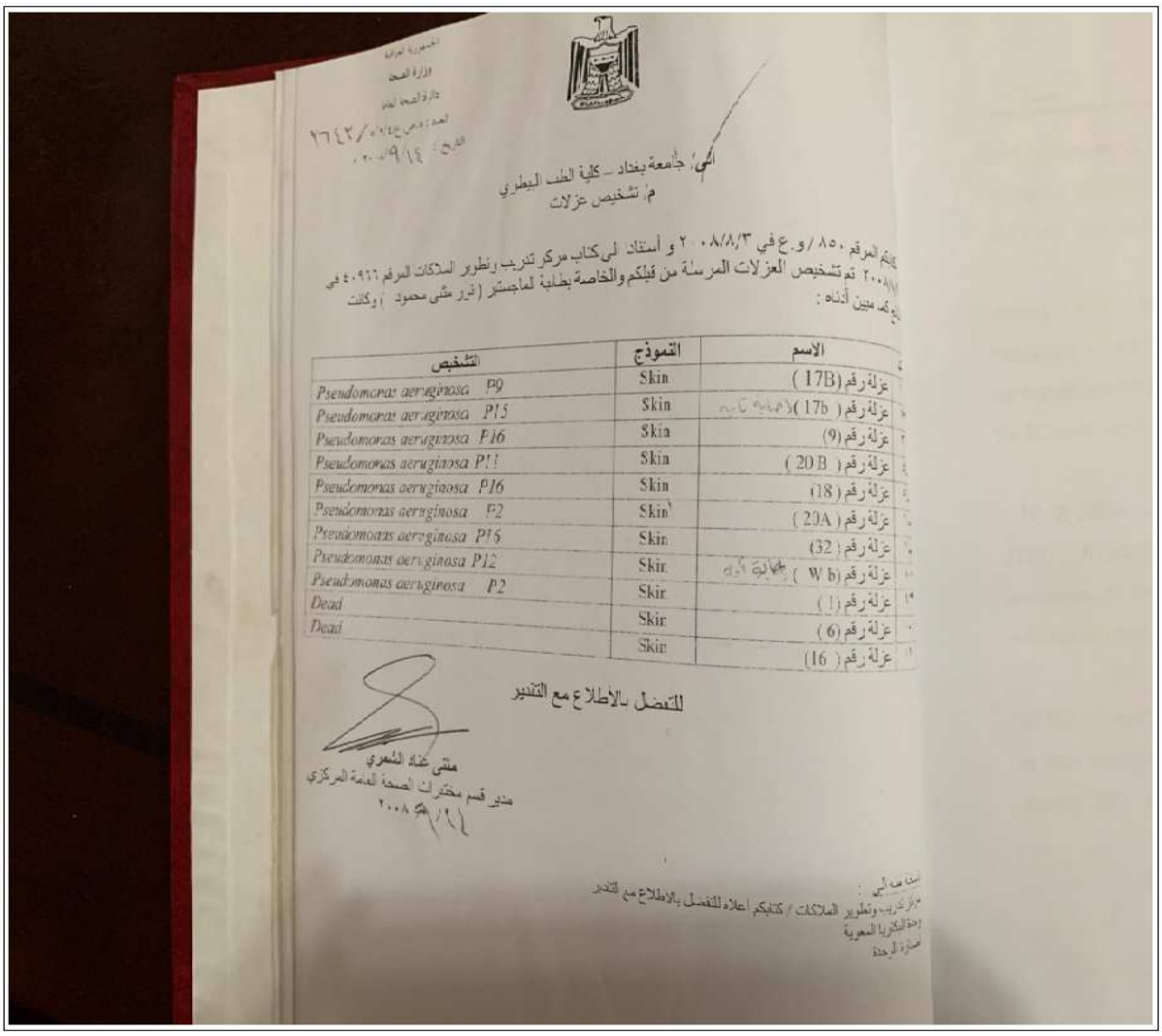
Brain and Heart infusion agar
Nutrient agar
Nutrient broth
MacConky agar
Blood agar
Urea agar
Pseudomonas agar base
Maintenance media agar
Gelatin agar

Table 4. Shows the results of laboratory testing of bacterial isolates.

Biochemical test	Test result
Pyoverdin produced	+
Oxidase	+ (dark blue)
Catalase	+ (bubbles)
Growth at 5c°	-
Growth at 42c°	+
Gelatin	+(medium liquefied after refrigerator)
Urease	1. (red color)
Oxidation of glucose	+
Oxidation of lactose	-
Oxidation of manitole	+
Oxidation of maltose	-
Maconkey agar	+
H_2s	-(no black color)
Indol	-(yellow circle)
Lysine decarboxylase	-
Nitrate reduction	+
Gram stain	Gve- (stained pink by using safranin)
Arginine dihydrolase	+
TSI	K / K No gas No H_2S

The reason for the different rates of infection with gram-negative bacteria, including *Pseudomonas aruginosa*, is the difference in number of samples and geographical location (Zaragoza *et al.*, 2002). Between (Clark *et al.*, 2003), that the bacteria are negative gram stain, including *Pseudomonas aruginosa* are common to the burns because of the possession of many factors of virility and genetic resistance to many of the factors of virility and genetic resistance of many antibiotics, and ranked first, followed by Staphylococcus aureus and intestinal germs (Revathi *et al.*, 1998). The susceptibility of these bacteria to live and reproduce under different conditions allows them to colonize and multiply in burned tissues leading to toxicity and multiple failures of internal organs and a high mortality rate of

up to 50% (Holder *et al.*, 1993). Burns provide suitable conditions for the invasion and propagation of pathogens within the incineration zone. Burns cause acute inflammatory response with localized changes that create the conditions for growth of opportunistic microbes, as well as systemic changes including increased vascular permeability, heart failure, increased hyperbolaemia, and weight loss, liver function and reduced immune response (Drost *et al.*, 1993).



الاسم	النموذج	التشخيص
عزلة رقم (17B)	Skin	Pseudomonas aeruginosa P9
عزلة رقم (17b)	Skin	Pseudomonas aeruginosa P15
عزلة رقم (9)	Skin	Pseudomonas aeruginosa P16
عزلة رقم (20B)	Skin	Pseudomonas aeruginosa P11
عزلة رقم (18)	Skin	Pseudomonas aeruginosa P16
عزلة رقم (20A)	Skin	Pseudomonas aeruginosa P2
عزلة رقم (32)	Skin	Pseudomonas aeruginosa P15
عزلة رقم (W b)	Skin	Pseudomonas aeruginosa P12
عزلة رقم (1)	Skin	Pseudomonas aeruginosa P2
عزلة رقم (6)	Skin	Dead
عزلة رقم (16)	Skin	Dead

Fig. 1.

In the current study, it was observed that the *Pseudomonas aruginosa* isolates were isolated from patients who had been hospitalized for (7-21) days, indicating the role of the hospital environment in the spread of psoriasis, (Mason *et al.*,1986) that the burns colonize the nurses after (5-7) days of occurrence and that these nurses originate from the natural fluorine of the digestive system and respiration of the injured, or from the vicinity of the hospital and the hands of health care workers or treatment solutions (Hydrotherapy), the direct or indirect transfer among patients and surgical instruments contaminated by the causes of the spread of dementia in hospitals (Hostacha and Mita, 1997). The percentage of cases of dementia is (10-20%) of total hospital-acquired injuries (Myrviik and Weiser, 1988). The current study showed that the percentage of female infection is higher than that of males. These results were not consistent with those reported by Al-Hadithi (2007). The percentage of males (11.82%) and females (3.7%) was due to lack of samples collected from male patients.

Conclusions

The *Pseudomonas aruginosa* are important contaminants for the wounds of the patients in burns burns units.

The serotype (P16) prevailing among the dorms classified.

The rate of infection of females is higher than that of males and that mature ages were more likely to be infected.

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