Morphological and Molecular Identification of Hospital *Pseudomonas* spp.

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الخلاصة

صممت الدراسة لعزل وتشخيص عزلات الزوائف من عدوى المستشفيات لدى الأطفال الحديثي الولادة ،حيث جمعت 288عينة من ثلاث مستشفيات تعليمية للنسائية والأطفال في مدينة الموصل،والتي اشتملت على عينات سريريه (134)للأطفال الحديثي الولادة الخدج والكاملي النضج دون السنة الأولى من العمر،بالإضافة إلى عينات بيئية (154)،للفترة من كانون الثاني 2011 وحتى تموز 2011 لغرض دراسة الصفات المظهرية من خلال طرق التشخيص التقليدية والجزيئية بتقنية التفاعل التضاعفي لسلسلة الـ DNA(PCR) لعزلات الزوائف.

تم عزل وتشخيص سلالات الزوائف *Pseudomonas* spp. باستخدام طريقة الزرع، إضافة إلى الاختبارات الكيموحيوية ونظام التشخيص Mini API 20NE كفحص توكيدي.من ناحية أخرى تم استخدام تقنية التفاعل التضاعفي(PCR) لسلسلة الـ DNA للكشف عن وجود الجين (*16SrRNA*) الخاص بتشخيص سلالات هذا الجنس.

أظهرت النتائج الحالية ،أن نسبة عزل الزوائف من العينات السريرية كانت 90/17(18.8%) بينما 111/30(27%)بالنسبة للعينات البيئية ،في حين بينت نتائج البلمرة أن هناك خصوصية عالية (100 %) في الكشف عن سلالات الزوائف تجاه الطرق التقليدية المختلفة كالزرع، الاختبارات الكيموحيوية ونظام التشخيص Mini API 20NE .

Abstract

The study was designed for isolation and identification of *Pseudomonas* spp. from neonatal nosocomial infections, from Three Teaching Hospitals of Maternity and Pediatrics in Al-Mosul city. The total collected samples were 288,which included clinical(134samples collected from preterm and full-term neonates who less one year of age) and environmental samples(154)from January 2011 till end July 2011, in order to evaluate the classical laboratory diagnostic

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procedures for diagnosis of *Pseudomonas* isolates and compared them with molecular technique on the basis Polymerase Chain Reaction (PCR).

Pseudomonas spp. has been isolated and identified by using cultural method, biochemical tests and Mini API 20NE system as confirmative test. On the other hand, PCR technique was utilized to detected *16SrRNA* gene as a house keeping gene, specific for diagnosis of *Pseudomonas* spp.

The present study showed that the isolation percent of *Pseudomonas* spp. from clinical samples was 17/30 (18.8%)and 30/111(27%) from environmental samples, whereas the PCR results showed that there were high specificity (100%) in detection of *Pseudomonas* spp. versus each of cultural, biochemical and Mini API 20NE system .

Introduction

Nosocomial infections(NI) also called hospital acquired infections are acquired during hospital care which is not present or incubating at admission, but appearing after discharge. Neonatal hospital infections are more frequent and more severe in newborn infants because of, the peculiar characteristics of this period of life allow for greater susceptibility to infections, the increasing of survival of preterm neonates and infants, following prolonged neonatal care units (NCUs) stay, use of invasive procedures and of wide spectrum antimicrobial as treatment and prophylaxis(1).

Pseudomonas is one of the most common pathogen involved in hospital infections causing opportunistic infections in humans, particularly among immunodeficiency patients(2).The hospital infections with this pathogen cause complication of medical care in neonatal care units, due to its ubiquitous nature with ability to survive in adverse conditions(3),while the emergence of multi-drug resistant bacterial strains of *Pseudomonas* spp. among nosocomial bacterial infections , is associated with highest mortality rate and is difficult to

eradicate from infected tissue, because those pathogens are virulent and have limited susceptibility to antimicrobial(4).

Genomic fingerprinting methods are now regarded as the most accurate methods for the typing of microorganisms for epidemiologic purpose(5). On the other hand, the molecular screening for detection of *16SrRNA* gene consider as a new way for diagnosis of bacteria (6).

Methodology

- **Samples collection**

A total of 288 samples were collected,134 from clinical and 154 from environmental samples(Table 1).

- **Samples processing**

All samples of this study were collected either by transport medium swabs or by added 1ml**.** of specimen into 5ml. of sterilized brain heart infusion broth**,** then they transported to the laboratory and incubated for 24 hours at 37˚C. Then a loop full of bacterial growth was streaked on the culture media (7).

- **Isolates identification**

- **Phenotypic screening** : The isolates were further identified by colonies morphology ,cell characteristics and biochemical tests (8). Also 21 isolates were confirmed by using API 20NE system according to the instructions of supplied company.

- **Molecular screening** :Polymerase Chain Reaction technique was used for detection of *16SrRNA* gene of *Pseudomonas* isolates, by the following steps:

A- DNA extraction : DNA extraction of 21 isolates of *Pseudomonas* spp. was carried out according to DNA extraction protocol of manufacturing company instructions(Geneaid, Genomic DNA Mini Kit, USA)(Figure 1).

B- Detection of 16SrRNA gene : The PCR amplification was performed using assay based on repetitive sequences of *16SrRNA* gene, the primer forward(5́-GGT CTG AGA GGA ATC AGT-3́) and reveres(5́-TTA GCT CCA CCT CGC GGC-3́). Also the laboratory protocol was carried out according to manufacturing company instructions (Bioneer / South Korea).

• **Statistical analysis :**

All results were performed by Chi-square test and Z-value at the level of significant when P-value(<0.01).The specificity, sensitivity and diagnostic accuracy of the results were also calculated by applying the following equations: (a / a + b) x 100 = sensitivity, (d / d + c) x 100 = specificity. diagnostic accuracy = (a+d / a+b+c+d)x100 . Also calculated positive predictive value (PPV) =(a / a + c)x 100 and negative predictive value (NPV) =(d / b + d)100. (a = the total number of positive cases, b = false positive those bearing positive reading from negative samples, d = total number of true negatives, c = those with negative reading from positive cases(9).

Results and discussion

• **Frequencies of *Pseudomonas* isolates**

This study showed that the percent of *Pseudomonas* isolation from hospitalized samples was 47/288(16.3%),the clinical isolation was 17/134(12.68%) and 30/154(19.48%) as environmental samples)(Table 2).

In Iraq, there are several studies that conduct on isolation and identification the causatives (such as *Pseudomonas aeruginosa*)of neonatal nosocomial infections, which based on routine laboratory techniques such as :(10);(11);(12);(13);(14) and(15).

• **Relationship between PCR (*16SrRNA* gene) and other tests used for detection *Pseudomonas* isolates.**

The results showed that out of 21 tested isolates were positive for culturing method, biochemical tests and Mini API 20NE system as *Pseudomonas* spp., the results of molecular screening for detecting of *16SrRNA* gene revealed that all tested (21)isolates were positive for amplification product approximately 990-1Kb.(Figure 2).On the other hand, 10 cases of non *Pseudomonas* spp.(control group) gave negative results for culture, Mini API 20E and PCR method(Table 3).

• **Comparison between the PCR method and other tests used for detection of *Pseudomonas* isolates**

Table (4) shows the sensitivity, specificity, diagnostic accuracy, PPV. and NPV. of the PCR test for *16SrRNA* gene were (90.4% ,100%,93.5%,100%,83.3%)respectively, while for cultural, biochemical tests and Mini API 20NE there were(100% ,100%,100%,100%,100%) respectively.

Locally there is no available data based on molecular diagnosis for rapid identification of *Pseudomonas* spp. as an important contributor in neonatal nosocomial infections .

Many studies were carried out overall the world to diagnosis of *Pseudomonas* spp., as example the primer set that utilized for detection of *16SrRNA* gene in the present study was designed by(16)who developed it according to the convention of Oligonucleotide Probe Database(OPD). In another study was carried out by(17), the previous primer set(*16SrRNA* gene)was used for detection of 50 *Pseudomonas* isolates with amplification product was approximately (990 bp.). On the other hands,(18) was used the same primer set for diagnosis of *Pseudomonas* spp. by molecular screening for detection of *16SrRNA* gene with PCR product at the predicted size1kb.

Table (1): distribution of hospitalized collected samples .

Clinical samples	No.	Environmental samples	No.
Throat swab and tracheal aspirate	21	Distilled water	7
Oral mucous membrane	8	Tap water	12
Gastrointestinal tract infections	24	Kitchen	20
Blood stream infections	13	Evaporator	9
Umbilical stumps	11	Normal saline	12
Urinary tract infections	14	Washing water of breast pumps	10
Vagina	8	Antiseptics &disinfectants	14
Nasal	9	Incubator	13
Conjunctiva	7	Air-flow	8
Axillaries folds	7	Fluid suckers instruments	21
Skin lesion	12	Hand of medical staffs	28
Total	134	Total	154

Table(2):Percentage of *Pseudomonas* Isolates from pediatric hospitals (NCUs. and related units).

Isolation results	No.	%
Positive results	47	16.3
Negative results	241	83.7
Total	288	100
Z-Value =11.37 , P. Value =8.031, significance.		

Table(3) : Relationships between results of PCR technique (16SrRNA gene)and other used tests for detection Pseudomonas isolates.

Tests		PCR				Total
		Positive		Negative		
		No.	%	No.	%	
Culture, biochemical, and Mini API 20NE	+ve 21 21	21	100	0	0	31
	-ve 10 10	0	0	10	100	

Table (4):Comparison between the results of sensitivity, diagnostic accuracy, PPV and NPV for both of phenotypic and genotypic diagnosis of *Pseudomonas* isolates.

Gene	Tests	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)	PPV (%)	NPV (%)
16Sr-RNA	PCR method	100	100	100	100	100
	Cultural, biochemical and Mini API 20NE	100	100	100	100	100

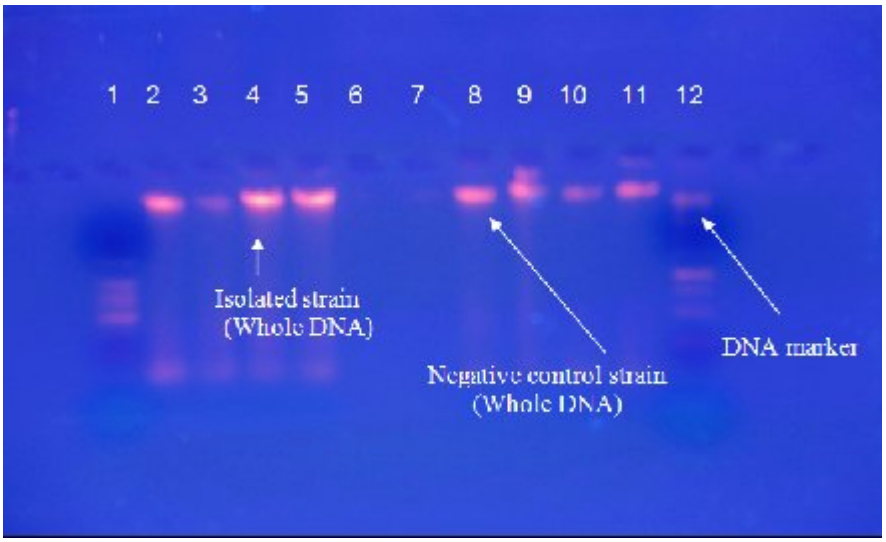
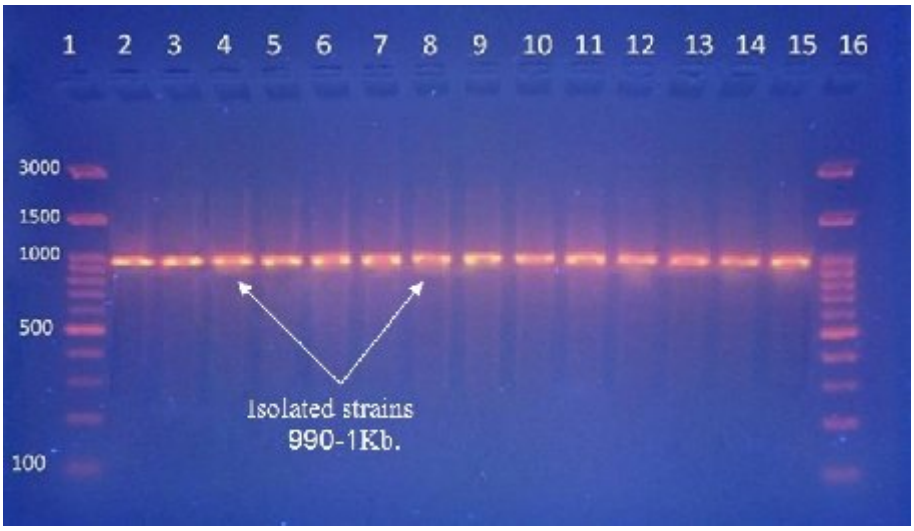


Figure (1): Ethedium bromide-stained agrose gel of DNA extracted from *Pseudomonas* isolates. Lane(1)and (12) DNA molecular size marker (100bp.). Lanes (2-5) *Pseudomonas* DNA extraction and lanes (8-11) negative control DNA extraction strains .



Figure(3-9):Ethidium bromide-stained agarose gel of amplification products *16SrRNA* gene from *Pseudomonas* isolated strains. Lanes (1-16) DNA molecular marker size(100-bp. ladder). Lanes(2-15)*Pseudomonas* isolates show positive results for amplification of *16SrRNA* gene with amplicon size (990-1Kb).

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