RESEARCH ARTICLE

Bacteriological Study of *Pseudomonas aeruginosa* Isolated from Tonsillitis Patients

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ABSTRACT

The present study included (50) clinical samples were collected from patients suffering from tonsillitis signs during the period from) November 2018 to January 2019). All specimens were cultured for macroscopic and microscopic study.

Results show that out of 50 patients, the male was 60%, and 40% were female. Several morphological, physiological, and biochemical tests showed that *P. aeruginosa* constituted 18 isolates (36%) of these isolates. *P. aeruginosa* isolates were 18 isolates diagnosed by the morphological, cultural, and biochemical characters; the identification was confirmed by automated VITEK-2 compact system and molecular method for the presence of *OprL*.

The results showed that only 12 (66.6%) isolates diagnosed as *P. aeruginosa* by automated VITEK-2 compact system, and that was carrying *OprL*, which are diagnosed as *P. aeruginosa* by PCR.

According to the different diagnostic above, VITEK and PCR method were more sensitivity for *P. aeruginosa* detection among tonsillitis patients.

Keyword: Tonsillitis patient, PCR, Bacterial, Isolation, VITEK.

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INTRODUCTION

Tonsillitis is an inflammation of the tonsils and is a common type of upper respiratory tract infections (URTIs) which is most commonly caused by a bacterial, viral, and fungal infection.[1] Tonsillitis can be defined as the state of inflamed condition of palatine tonsils, pharyngeal tonsils, and lingual tonsils.[2]

Studies recorded that the tonsillitis can appear with other infections like fever, malaise sore throat, neck swollen lymph nodes, and red or swollen tonsils with whitish debris in sometimes.. If tonsillitis is not treated promptly or adequately it could lead to several complications can be appeared when patients aren't treated tonsillitis infection these complications included severe odynophagia, trismus, neck stiffness or muffled (hot potato voice) or nasal voice.[3] The treatment of tonsillitis by antibiotic lead to improvement providing appropriate antibiotics.[4] *P. aeruginosa*, consider as important infectious causes tonsillitis, it's an obligate aerobic gram-negative a non-fermentative, have measured (0.5–0.8) µm by (1.5–3.0) µm. Strains are motile and have a single polar flagellum. The Typical biochemical characteristics are positive for the oxidase test, the growth period is 42°C, the capability to arginine and gelatine hydrolysis, and reduction of nitrate. Also it can produce two soluble pigments types included pyoverdin and pyocyanin, which is a blue pigment produced in f low-iron content media used in iron metabolism. The genome is large, containing 6.26 Mbp encoding to 5567 genes.[5] Therefore it has a considerable additional genetic capacity, which gives the ability to develop antibiotics resistance. Usually The prevalence of colonization is low in healthy individuals, higher incidence appeared in hospitalization, among patients treated with broad-spectrum antibiotics[6] several disruptions of the physical barriers (skin or mucous membrane) must be happened for infection, also by-passing invasive devices, and/or an underlying dysfunction of the immune defense mechanisms.[7] Also, the mucoid phenotype it can be causing damage of the lung tissue and decreased pulmonary function.[8]

METHODOLOGY

Samples collection

Clinical samples (50) were collected from patients suffering from tonsillitis signs during the period from November 2018 to January 2019. From patients attending to AL-Hakem General

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Hospital and AL-Sadder Medical City, according to ethical approval of the environment and health ministry of Iraq, All samples were cultured on the MacConkey agar plates and incubated at 37°C under the aerobic condition for 18–24 hour.

Detection *OprL gene*

Deoxyribo nucleic acid extraction

Total DNA was extracted from isolates by boiling method according to[9] with modifications. A single colony was scraped using sterile toothpick from the surface of agar plates and suspended in 40 µl Tris-EDTA buffer. The suspension was heated for15 min at 100 ºC, followed by 5 min on ice rapidly. The suspension containing DNA was stored at -20 ºC till it used.

Primers set and polymerase chain reaction (PCR) conditions

Primer sets were synthesized by Bioneer company (Korea), as showed in Table 1, the PCR conditions, as in Table 2.

RESULTS

Bacterial Isolation

A total of 50 clinical specimens were collected from patients suffering from tonsillitis signs during the period from) November 2018 to January 2019). These specimens were collected from patients attending to AL-Sadder Medical City and AL-Hakem General Hospital during the studied period. All samples were cultured on the MacConkey agar plates and incubated at 37°C under the aerobic conditions for 18–24 hours.

In 50 patients, 30 (60%) were male and 20 (40%) were female as in Figure 1. The prevalence of tonsillitis was more in male patients compared with female patients, probably because the number of patients admitted was more than female patients.

Pseudomonas aeruginosa Isolation and Identification

The initial identification of bacterial specimens depended on some criteria, which included cultural, morphology, and biochemical tests. The final identification was performed with the automated VITEK-2 compact system using GN-ID cards, which contained 64 biochemical tests and one negative control. Confirmation of *Pseudomonas aeruginos* was conducted using PCRsystem. Several morphological, physiological, and biochemical tests were made to identify bacterial isolates.

Results showed that *Pseudomonas aeruginosa* constitute 18 isolates (36%) of these isolates, The other bacterial isolates were *Klebsiella pneumoniae, Enterobacter aerogenes* and *Streptococcus spp.* Bacterial isolates were identified according to their cultural, microscopical, and biochemical characteristics that were in agreement with.[12,17]

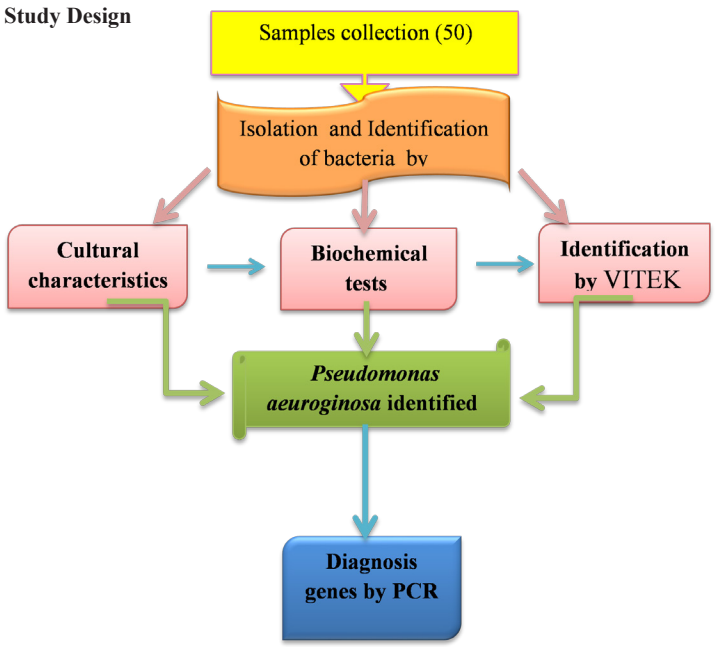

Figure 1: Study the most important topics.

Table 1: Sequences and Product size of each primer.

Primer type	*Primer (5'-3')*		*Product size(bp)*	*References*
OprL	F	ATGGAAATGCTGAAATTCGGC	504	(10)
	R	CTTCTTCAGCTCGACGCGACG		

Table 2: PCR conditions of *OprL gene detection*

	Temperature (°C) / Time					
	Initial	*Cycling conditions*			*Final*	
Name of Gene	*Denaturation*	*Denaturation*	*Annealing*	*Extension*	*extension*	*Cycles number*
OprL	94/5 min	94/1 min	55/1 min	72/1 min	72/10 min	30

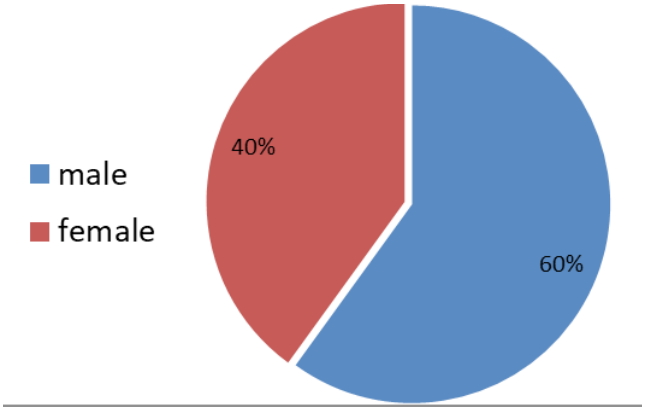

Figure 1: Distribution of infected patients according to sex

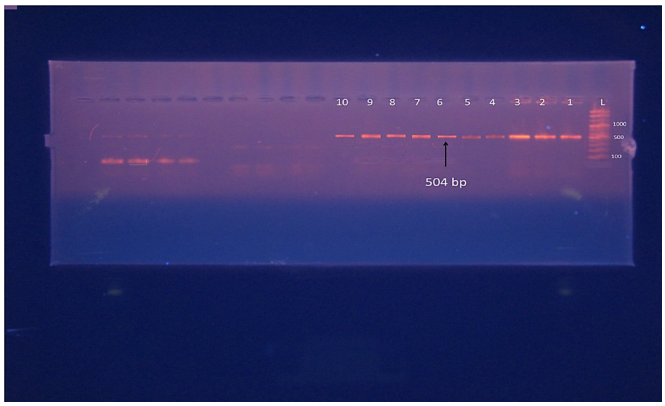

Figure 3: Ethidium brmide-stained agarose gel electrophoresis of PCR amplification products of *P.aeruginosa* isolates that amplified with *OprL* gene primers with product 504bp for 1 hr. at 80volt/cm.

Colony morphology

The primary identification of bacterial isolates was done after incubated aerobically on MacConkey agar plates at 37 C° for 24–48 hours. *P.aeruginosa* colonies appeared on MacConkey agar as smooth round colonies and pale yellow color (non-fermented), occasionally produced pigments that diffuse in the agar[13], Figure 2.

Biochemical test

Pseudomonas aeruginosa was produce pyocyanin and giving strongly oxidase-positive, and give indole (Indole test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole, vogues procures (is a test used to detect acetoin in a bacterial broth culture), methyl red (test detects the production of sufficient acid during the fermentation of glucose .

Pseudomonas aeruginosa isolates have given in biochemical tests; a positive result for oxidase, also it is able to utilize citrate as a sole source for carbon, in Kligler iron agar have given alkaline slant and did not change the bottom, H_2S negative without gas production due to fact that it is strictly aerobic.[14]

Identification of *P.aeruginosa* by VITEK-2 compact system

The identification was performed with the automated VITEK-2 compact system using GN-ID cards, which contained 47 biochemical tests and one negative control well. The results

Figure 2: *Pseudomonas aeruginosa* colonies on nutrient agar media

Table 3: The morphological, cultural and biochemical characters of *P. aeruginosa*

Test	*Result*
Cell shape	Bacilli
Oxidase	+
MacConkey agar	Lactose nonferment
Indole production	-
Methyl red	-
Voges –Pproskauer	-
Simmons Citrate	+
Kliglar iron agar (KIA)	Alkaline slant /No change bottom, No gas, No H_2S

demonstrated that only 12 (66.6%) isolates were confirmed as *P.aeruginosa* with ID message confidence level best (Probability percentage from 92–99).

OprL gene detection

A collection of 12 (66.6%) isolates of *P.aeruginosa* are covered from clinical specimens was molecularly screened for the presence of (*OprL*) genes as a diagnosis gene. The results demonstrated that all ten isolates were carrying *the OprL* gene and confirmed as *P.aeruginosa*. These results correlate with the results obtain by Al-Ahmadi,[15] who diagnosed *P.aeruginosa* by using *OprL* gene. As in Figure 3.

Whereas using molecular technique takes a long time to perform and require extensive hands-on work by the technologist, both for setup and ongoing evaluation. Various methods have been developed to rapidly and accurately identify *P. aeruginosa* species as a medically important bacterium. According to these, PCR has the potential to identify microbial species rapidly and precisely by amplification of gene sequences unique to a particular organism.16 According to the results obtained by this research, confirm by VITEK and PCR method were more sensitivity for *P. aeruginosa* detection among tonsillitis patients.

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