AlQalam
Alq J Med App Sci
https://journal.utripoli.edu.ly/index.php/Alqalam/index eISSN 2707-7179

Review article

The Use of *ecfX* Gene as a Specific Identification Target of *Pseudomonas aeruginosa* Isolated from Infected Wounds

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ARTICLE INFO

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***Received**: 29-10-2024*
***Accepted**: 10-12-2024*
***Published**: 20-12-2024*

***Keywords**. Pseudomonas Aeruginosa, Phenotypic Characteristics, Conventional PCR, Real-Time PCR, EcfX Gene.*

ABSTRACT

Pseudomonas aeruginosa is a significant pathogen in medical settings, accounting for 10% to 20% of infections acquired. This oxidase-positive, Gram-negative bacteria is well-known for its ability to cause respiratory problems, wound infections, and pneumonia related to ventilator use especially in individuals with cystic fibrosis. Prompt and precise diagnosis of P. aeruginosa is essential in of the growing antibiotic resistance, particularly of the limited data on resistance patterns in Libyan healthcare settings. This investigation examines the frequency of P. aeruginosa infections in wounds at Misurata Medical Center and tests the effectiveness of RT-PCR, which targets the ecfX gene, in detecting the pathogen. In this study obtained 165 clinical samples were obtained from patients suffering from wound infections, and using both traditional and molecular methods, we were able to identify P. aeruginosa. The study showed that, in comparison to conventional biochemical approaches, RT-PCR targeting the ecfX gene provides a dependable technique for the quick and accurate detection of P. aeruginosa in clinical samples.

***Cite this article.** Teka I, Elfaitori A, Hajer Almuaget. The Use of ecfX Gene as a Specific Identification Target of Pseudomonas aeruginosa Isolated from Infected Wounds. Alq J Med App Sci. 2024;7(4):1566-1570.* https://doi.org/10.54361/ajmas.247490

INTRODUCTION

One important opportunistic pathogen that causes a variety of diseases in hospital environments is *Pseudomonas aeruginosa*. Treatment and management are complicated by its capacity to form biofilms, innate resistance mechanisms, and acquired resistance to several antibiotics [1,2]. The issue has been made worse by the emergence of antibiotic-resistant strains, especially in nosocomial infections where prompt and precise identification is essential for successful treatment [3].

Conventional approaches to *P. aeruginosa* identification, including as culture-based procedures and biochemical tests, may have drawbacks. According to Kidd *et al*. (2009) [4], these techniques can be laborious and might not always be able to distinguish between closely related bacterial species.

For the purpose of identifying bacteria, molecular methods specifically, PCR-based assays have become more efficient and accurate [5]. Because of its high specificity and sensitivity, the *P. aeruginosa*-specific ecfX gene has been suggested as a target for PCR-based detection [7]. In addition to comparing the efficacy of RT-PCR targeting the *ecf*X gene with traditional identification techniques, this study attempts to assess the frequency of *P. aeruginosa* in wound infections at Misurata Medical Center.

METHODS

Bacterial collection and isolation

Between January and June 2020, 165 clinical samples were collected from patients at the Misurata Medical Center in Libya who had wound infections. The study encompassed patients ranging in age from 2 months to 98 years. The laboratories where samples were processed were the Microbiology Department of the Medical Misurata Center, Animal Health Center Laboratories, Biological Research Center (BRC), NCDC Reference Laboratories (Misurata – Tripoli), and Misurata Medical Technology College laboratories.

Samples were cultured on MacConkey agar, Blood agar, and Nutrient agar and incubated aerobically at 37°C for 24 hours. Single colonies were tested for biochemical characteristics using the Oxidase test and API 20NE system [6].

Isolation of Genomic DNA

Colonies were grown overnight in LB broth at 37°C, and DNA extraction was performed following the manufacturer's protocol usin the QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany).

Real-Time PCR Assays

Quantitative PCR (qPCR) was conducted using the Bio-Rad CFX Connect Real-Time PCR Detection System. The assay targeted a 146bp fragment of the *ecf*X gene specific to *P. aeruginosa*. The RT-PCR mix included 10µl GoTaq Probe qPCR MasterMix (Biospeedy - Turkey), 5µl of the *P. aeruginosa* oligo mix containing FAM-labeled *ecf*X gene primers and HEX-labeled human RNase-P gene as an internal control, and 5µl of DNA template in a total volume of 20µl.

The RT-PCR program consisted of an initial denaturation step at 95°C for 3 minutes, followed by 40 cycles of 95°C for 10 seconds and 55°C for 40 seconds. Detection was performed using two channels: FAM for the *ecf*X gene and HEX for the internal control (Figure 1).

RESULTS

Phenotypic and Biochemical Characterization

Phenotypic identification of *P. aeruginosa* was based on Gram staining, pigment production, and biochemical tests (Oxidase test and API 20NE). Of the 165 clinical samples, 32 isolates were confirmed as *P. aeruginosa* based on their cultural and biochemical characteristics (Table 1)

Table 1. Biochemical Test Results (+=Positive ,- =Negative)

Oxidase test	Api-20E	Sample No	Oxidase test	Api-20E
+	+	17	+	+
+	+	18	+	+
+	+	19	+	+
+	+	20	+	+
+	-	21	+	+
+	+	22	+	+
+	+	23	+	+
+	+	24	+	-
+	+	25	+	+
-	-	26	+	+
+	+	27	+	+
-	-	28	+	+
+	+	29	+	+
+	+	30	+	-
-	+	31	+	+
-	+	32	+	+

Real-Time PCR Results

Real-Time PCR successfully detected the *ecf*X gene in all 32 *P. aeruginosa* isolates, confirming the specificity of the assay. The RT-PCR results showed a high level of sensitivity and specificity for detecting *P. aerugionsa*.

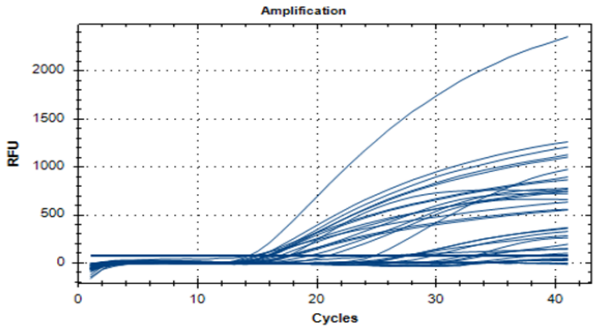

Figure 1. The (FAM) signal in RT-PCR correspond to Amplification of ecfX Gene from P. aeruginosa isolates using Real time PCR technique

RT-***PCR results verification***

To confirm the RT-PCR results, *ecf*X gene was targeted in all DNA sample using conventional PCR on the basis of molecular size electrophoresis fragmentation, through prepared agarose gel by adding 1% (w/v) agarose (Invitrogen) to 1× TAE buffer and boiling the solution in a microwave to allow the agarose to dissolve. Once the mixture had cooled to 40-50 °C, SYBR safe (Invitrogen, 1:10,000) was added. DNA samples were mixed with 6× DNA loading buffer (Promega), and loaded into the wells alongside DNA 100 bp ladder (Grisp). Electrophoresis was performed at 70V in 1× TAE buffer. The bands were visualized under UV illumination in a Gel Doc system (Bio-Rad). The band corresponds to a desired DNA fragment (size 146bp) was considered positive, and if absent then considered negative otherwise.

Table 2. ecfX Oligonucleotides

Oligonucleotides	Sequence (5'- 3')'	Reference
ecfX – F	TTCCATGGCGAGTTGCT	**Cattoir et al,2010**
ecfX – R	CGGGCGATCTGGAAAAGAA	**Cattoir et al,2010**
ecfX prob	FAM-GCTGAAATGGCCGGGC-BHQ	**Cattoir et al,2010**

Conventional PCR also confirmed the presence of the *ecf*X gene in the isolates, with a 146-bp band corresponding to the expected size. Agarose gel electrophoresis results corroborated the RT-PCR findings.

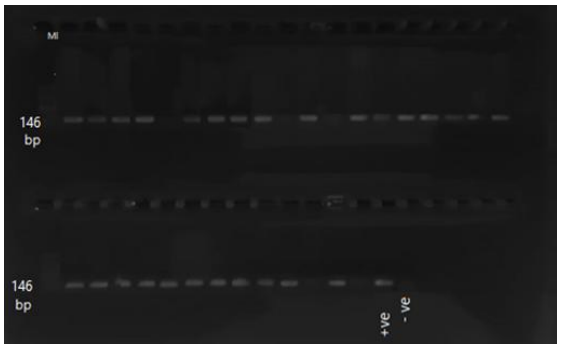

Figure 2. Agarose Gel Electrophoresis of PCR Products of amplified ecfX gene (146 bp) of P. aeruginosa isolates using PCR. Lane1 ML denoted size marker of 100bp, lanes +ve and -ve denoted negative and positive control respectively, lanes 2 – 32 denoted the studied samples

Statistical Analysis

using SPSS software (26.0 version, Chicago, IL, USA) revealed that RT-PCR had a higher sensitivity (94.5%) compared to traditional methods like the Oxidase test (84.6%) and API-20NE system (75%) (Table 3).

Table 3. Sensitivity rates of different Tests

Test	Sensitivity Rate
RT-PCR (*ecf*X gene)	9494.5%
Oxidase Test	84.6%
API-20NE	75.0%

Seeing all the above, The RT-PCR method demonstrated superior accuracy in detecting *P. aeruginosa* compared to traditional biochemical methods.

DISCUSION

The emergence of *Pseudomonas aeruginosa* strains resistant to antibiotics highlights the significance of prompt and precise diagnostic techniques. While helpful, traditional phenotypic and biochemical testing have drawbacks in terms of turnaround time and specificity [7].

A major benefit of the *ecf*X gene-targeting RT-PCR technique is its great sensitivity and specificity. The study discovered that *P. aeruginosa* in wound infections may be detected with great efficacy by RT-PCR using the *ecf*X gene. This was in line with earlier research [8,10]. that showed the *ecf*X gene to be a trustworthy marker for *P. aeruginosa* identification. When compared to conventional techniques, RT-PCR's high sensitivity indicates its potential to increase diagnostic precision and lower misidentification rates. Moreover, the RT-PCR method offers faster results, which is crucial for timely treatment and management of infections. This aligns with findings from other studies highlighting the benefits of molecular techniques in clinical diagnostics [5,8,9].

The results of this study demonstrate how inaccurately traditional phenotypic and biochemical techniques can detect *P. aeruginosa*. Despite being widely used, the Oxidase test and API-20NE system are prone to errors in identification and results delays. In contrast, *P. aeruginosa* could be found quickly, precisely, and selectively using RT-PCR that targets the *ecf*X gene [8,10,11].

Comparing molecular technologies to traditional procedures, previous research has demonstrated improved sensitivity and specificity [8,10]. Corroborating findings from previous studies, the *ecf*X gene, a marker unique to *P. aeruginosa*, is a useful target for PCR-based detection [7,11].

CONCLUSION

Accurate identification of *Pseudomonas aeruginosa* is essential for efficient infection management and control. This work shows that, when compared to conventional biochemical testing, RT-PCR targeting the *ecf*X gene is a more effective technique for the quick and accurate detection of *P. aeruginosa*. For the diagnosis of *P. aeruginosa*, RT-PCR using the *ecf*X gene ought to be the method of choice because to its exceptional sensitivity and dependability. In clinical settings, routine *P. aeruginosa* diagnoses should be conducted using RT-PCR, as it has a high sensitivity and specificity towards the *ecf*X gene. For prompt and efficient patient care, it provides a noteworthy benefit over conventional techniques in terms of speed and precision.

Acknowledgments

The authors wish to thank the personnel and labs at Misurata Medical Center, Animal Health Center Laboratories, Biological Research Center (BRC), Misurata Medical Technology College, and NCDC reference laboratories for their assistance with sample collecting and laboratory analysis. Sincere gratitude is extended to the technical personnel and researchers who worked on this project.

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إستخدام جين *ecf*X كهدف تعريفي محدد لبكتيريا *Pseudomonas aeruginosa* المعزولة من عدوي الجروح

إبراهيم تيكة، آمال الفيتورى، هاجر المؤقت

كلية التقنية الطبية مصراته والمركز الليبي للتقنيات الحيوية طرابلس، ليبيا

المستخلص

Pseudomonas aeruginosa هو مسبب مرضي مهم في البيئات الطبية، حيث يمثل 10% إلى 20% من حالات العدوى المكتسبة. هذه البكتيريا إيجابية الأوكسيديز وسلبية الجرام معروفة بقدرتها على التسبب في مشاكل الجهاز التنفسي والتهابات الجروح والالتهاب الرئوي المرتبط باستخدام أجهزة التنفس الصناعي خاصة في الأفراد المصابين بالتليف الكيسي. التشخيص السريع والدقيق لـ *Pseudomonas aeruginosa* أمر ضروري في ظل مقاومة المضادات الحيوية المتزايدة، وخاصة في ظل البيانات المحدودة حول أنماط المقاومة في البيئات الصحية الليبية. هدفت هذه الدراسة الي تقييم معدل الإصابة ببكتيريا *Pseudomonas aeruginosa* في الجروح في مركز مصراتة الطبي وإمكانية إستخدام تقنية RT-PCR الذي يستهدف جين *ecf*X في الكشف المباشر عن هذه البكتيريا. في هذه الدراسة تم الحصول على 165 عينة سريرية من مرضى يعانون من التهابات الجروح، وباستخدام الطرق التقليدية والجزيئية، تمكنا من تحديد *Pseudomonas aeruginosa*. وأظهرت الدراسة أنه بالمقارنة مع الطرق الكيميائية الحيوية التقليدية، فإن تقنية تفاعل البوليميراز المتسلسل العكسي (RT-PCR) التي تستهدف جين *ecf*X توفر تقنية يمكن الاعتماد عليها للكشف السريع والدقيق عن البكتيريا السيدوموناس آريجينوزا (*P. aeruginosa*) في العينات السريرية.

الكلمات المفتاحية: *Pseudomonas Aeruginosa*، الخصائص الظاهرية، تفاعل البوليميراز المتسلسل التقليدي، تفاعل البوليميراز المتسلسل في الوقت الحقيقي، جين *ecf*x.