Molecular detection of phzD, phzA and phzG Gene among Pseudomonas aeruginosa Isolated from Burn Infections

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Abstract

Pseudomonas aeruginosa's ability to produce phenazine poses a severe healthcare issue with a range of effects on patients and the healthcare system. The study's goal was to find out how phenazine affects bacterial burn infections. Because of this, 223 swabs from burn infections were collected and grown on suitable conditions; the initial identification was based on morphological and culture features, biochemical tests, and then identification with the vitek-2 system. Phz D, Phz A, and Phz G genes that code for phenazine synthesis were studied genetically using the PCR technique. All 34 (97%) P. aeruginosa isolates tested positive for the phz D gene, 34 (97%) isolates tested positive for the phz A gene, and 23 (66%) isolates tested positive for the phz G gene.

Introduction

One of the most common types of trauma, burn injuries primarily cause morbidity and mortality in low- and middle-income nations. Burns can result from I coming into touch with high-temperature materials (Mofazzal Jahromi et al.,2018).

P. aeruginosa is a human pathogen that is obligately aerobic, gram negative, and oxidase positive and is linked to several nosocomial infections that can be fatal. Numerous virulence factors that actively harm host tissues are produced by this bacteria (Baker et al., 2013). Numerous varieties of soluble pigments are made by bacteria.

such as the most prevalent kind, pyocyanin and pyoverdin. In low-iron content media, the blue pigment is produced in large quantities and is involved in the metabolism of iron. The bacterium can also create pyomelanin (brown), pyorubin (red), and pyoverdin (yellow/green) colours (Kothari et al.,2022).

The broad class of heterocyclic nitrogen-containing compounds known as phenazines has different chemical and physical characteristics depending on the kind and location of the functional groups. Over 6,000 compounds with phenazine as a core moiety have been synthesized, and more than 100 distinct phenazine structural variants have been found in nature (Zhou et al.,2022).

The roles of the enzymes PhzA, PhzB, and PhzG in the condensation and rearrangement events that lead to PCA are virtually fully understood.

The single phenazine chemical produced by P. fluorescens is PCA, which is the end product of the main phenazine biosynthetic operon,

phzABCDEFG. However, when it comes to Pseudomonas strains like P. aeruginosa, P. chlororaphis, and others, PCA is changed to various phenazine derivatives (Price et al.,2007). PhzH, a putative transamidase that allegedly transforms a portion of PCA into phenazine-1-carboxamide PCN), is present in P. chlororaphis PCL1391 and P. aeruginosa PAO1, allowing these strains to produce both PCA and PCN ((Chin-A-Woeng et al. 2001). P. aeruginosa phenazines can also induce the production of two neutrophil chemotaxins, IL-8 and leukotriene B4, by alveolar macrophages. These chemotaxins draw neutrophils into the airways, where they trigger an inflammatory response and neutrophil-mediated tissue destruction (Caldwell et al.,2009).

We have investigated the potential application of phzD , phzA and phzG Gene as markers for the capacity to produce bioactive phenazines in the detection of new isolates from burns infection.

Materials and Method

Two hundred and three (223) swabs from patients with burn infections were collected throughout the study's May 2022–July 2022 period. The specimens were then cultured on appropriate media and incubated for (18–24) hours at 37 C.

A commercial extraction technique was used to extract genomic DNA (Favorgen, Taiwan). Pseudomonas aeruginosa Isolated from Burn Infections was tested using a PCR assay to find the phz A, phz D, and phz G gene sequences (Table 2).

Table (1) Oligonucleotide primers of pigment genes

gene	primer	PCR products	reference
Phz A	F:TCAGCGGTACAGGGAAACAC R:GAAGTGGTTCGGATCCTCGG	283 bp	This study
Phz D	F: AAGTACATGAAT ACCAAAGGCCAGG R;AATTCGCGGTTGC GCTTGATCTTGC	150 bp	(daCruz Nizer *et al*.,2021)
Phz G	F; GAGAGCAGCCAGCAGATCAT R: AGTTCGAACAGGCAGTAGCC	212 bp	This study

Table 2: PCR program that apply in the thermo-cycler:

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cycles
phzA	72˚C for 5min	72˚C for 1min	58˚C for 30 sec	94˚C for 30 sec	94˚C for 1min	30
phzg	72˚C for 5min	72˚C for 1min	58˚C for 30 sec	94˚C for 30 sec	94˚C for 1min	30
pvdD	94˚Cfor 5min	94˚Cfor 30sec	55.5˚C for 30sec	72˚C for 30sec	72˚C for 1min	30

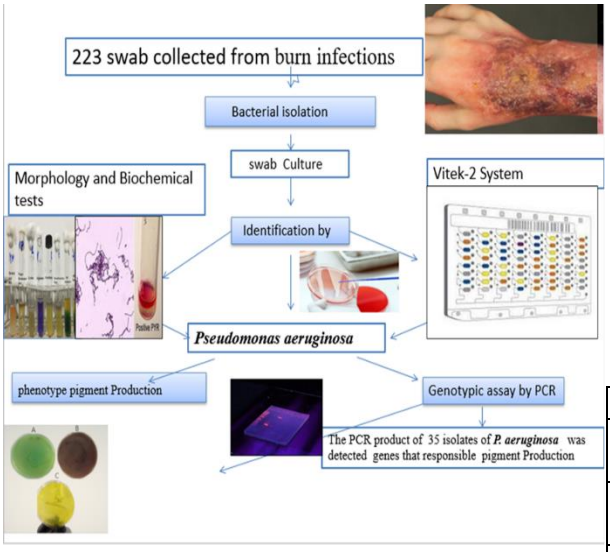


Figure (1)scheme of current study

Results and Discussion

phenotype pigment Production of P. Aeruginosa

From (35) P. Aeruginosa, 25(71%) isolates produce pigment and 10(29%) no produce pigment

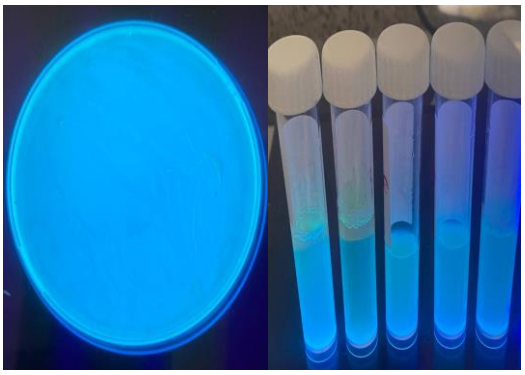


Figure (2) Shown fluorescent pigment.

Detection of Genes Encoding for Pigment by Using PCR Technique

This technique have been used to detect genes responsible for pigment production. The DNA of all (35) isolates was extracted by using lysis buffer used as a template for gene amplification

1-4 phz D gene

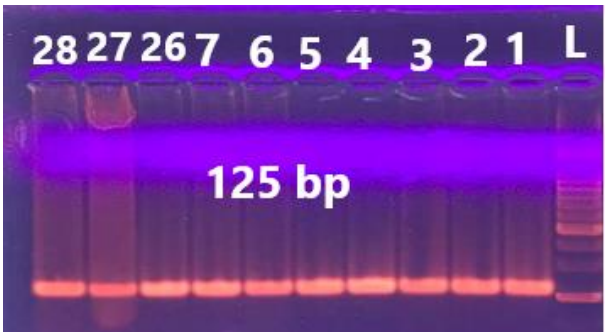
According to the amplification results of the PCR investigation for phz D, 9 (90%) of the non-pigmented isolates and 25 (100%) of the pigmentation isolates both showed positive results for the phz D figure(3) and table (3)

Table (3) Occurrence of phz D gene in P. aeruginosa isolates

phz D gene	positive	Negative	total
***phz* D pigmentation isolates**	**25**	**0**	**25**
***phz* D non pigmentation isolates**	**9**	**1**	**10**
total	**34**	**1**	**35**

In table (3), there is 25isolates of P. aeruginosa isolates produce pigmentation(25 positive for phz D gene ,0 negtive for phz D but produce pigmentation) and there is 9 isolates of P. aeruginosa isolates.

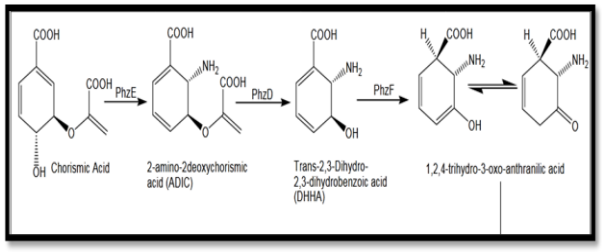
Figure (3): PCR amplification for phz D gene at molecular size=125 bp in P. aeruginosa isolates: L-100bp DNA ladder, lanes positive isolates of P. aeruginosa isolates in all isolate.



According to the previous results, the prevalent rate of phz D gene was 29(73%) as shown in table (3). The results in this study are different from the result of Fitzpatrick et al (2009) considered as a local study where the study illustrated that prevalent rate of appearance phz D gene is (70.51%).

After PhzD incubation of ADIC produced trans-2,3-dihydro-3-hydroxyanthranilic acid (DHHA), and PhzA-G incubation of DHHA resulted in complete conversion to PCABoth these chemicals, they were therefore validated as intermediates of phenazine synthesis (Hu et al .,2017). The conversion of ADIC into DHHA is catalyzed by the enzyme isochorismatase PhzD. The structural analysis of PhzD by Parson et al. crystal. recently reinforced this result (2003).

Figure (4) The phenazine biosynthetic pathway's putative intermediates



2- phz A gene

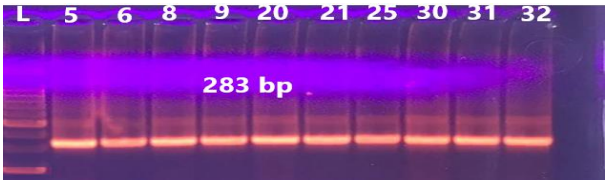
The amplification of the P. aeruginosa phz A gene (283 bp) by PCR revealed that 24 (96%) isolates from pigmentation and 8 (80%) from non-pigmentation isolates had positive results for the phz A gene. figure(4) and table (4)

Table (4) Occurrence of phz A gene in P. aeruginosa isolates

***phz* A gene**	**positive**	**Negative**	**total**
***phz* A pigmentation isolates**	**24**	**1**	**25**
***phz* A non pigmentation isolates**	**8**	**2**	**10**
total	**34**	**1**	**35**

In table (4), there is 30 isolates of P. aeruginosa isolates produce pigmentation(20 positive for phz A gene ,0 negtive for phz A but produce pigmentation) and there is 9 isolates of P. aeruginosa isolates.

Figure (5): PCR amplification for phzA gene at molecular size=283 bp in P. aeruginosa isolates: L-100bp



According to the previous results, the prevalent rate of phz A gene was 30 (100%) as shown in table (4). The results in this study are different from the result of Fitzpatrick et al (2009) considered as a local study where the study illustrated that prevalent rate of appearance phz A gene is (60.94%).

Trans-2,3-dihydro-3-hydroxyanthranilic acid (DHHA), which is completely converted to PCA, is produced by incubating ADIC with the enzyme PhzD. Thus, the identity of both of these substances as phenazine biosynthetic intermediates was established (Ke et al .,2022). The genes phzA and B were shown to be important for quantitative synthesis but not necessary for PCA biosynthesis. share more than 70% similarity (Mavrodi et al.,2001). However, the phenazine biosynthesis operons of every Pseudomonas species contain both of these enzymes. The PhzA-B enzymes were believed to function after DHHA synthesis, But because these enzymes showed no similarities to any known-function enzymes in the NCBI database, no additional structural or biochemical data was available. It was found that PhzC, D, E, and G had bacterial counterparts. For PCA synthesis, the remaining enzymes PhzC, D, E, F, and G are essential. (Dietrich et al .,2006).

Figure (6) The envisioned components of the process for producing phenazine (Dietrich et al .,2006).

3- phz G gene

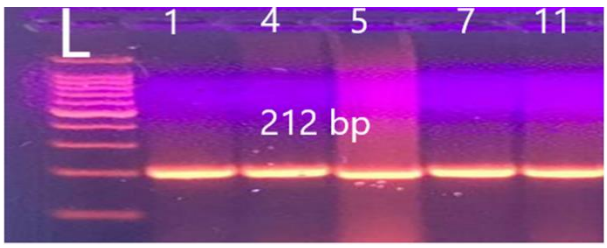
The amplification results of PCR study for phz G appear that 19(76%) isolates gave positive results phz G gene from pigmentation isolates and 3(30%) from non pigmentation isolates figure(6) and table (5).

Table (5) Occurrence of phz G gene in P. aeruginosa isolates

phz G gene	positive	Negtive	total
phz G pigmentation isolates	19	6	25
phz G non pigmentation isolates	3	7	10
total	23	12	35

In table (5), there is 30 isolates of P. aeruginosa isolates produce pigmentation(18 positive for phz G gene ,2 negtive for phz G but produce pigmentation) and there is 10 isolates of P. aeruginosa isolates non pgmentation (3 positive for phz G gene ,7 negtive for phz G gene).

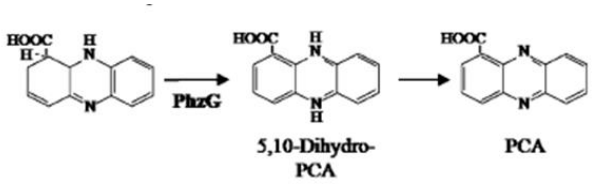
Figure (7): PCR amplification for phz G gene at molecular size=212 bp in P. aeruginosa isolates: L-100bp



The prevalence rate of the phz G gene was 70 (100%) based on the prior findings, as indicated in table (5). The findings of Fitzpatrick et al. (2009), which were deemed to be local results, showed that the prevalence rate of the appearance of the phz G gene is (71.62%).

PhzG, is similar to bacterial It was thought to act in steps past the formation of 2,3-dihydro-3-oxo anthranilic acid, the function of the enzymes PhzA, PhzB and PhzG are almost enlightened and their role in the condensation and rearrangement reactions to form PCA is demonstrated(Gohain et al.,2009).

Figure (8) phenazine biosynthesis's intermediaries (Pierson et al.,2010).



Conclusions

1. The present results have shown a great emergence of P.aeruginosa isolates from patients with burn infection, P. aeruginosa isolates are the most prominent causing agents of burn infections.

2. The high percentage of P.aeruginosa isolates were have the phz C , phz E and phz F Gene that responsible produce the phenazine.

Recommendations

1. Studies the relation between pigment production and Biofilm formation of Pseudomonas aeruginosa and pigment role in antibiotic resistance.

2. Studies other type of Microbial pigments.

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