Original Article

# Molecular Technology for the Detection of *Pyoviridine* Gene in *Pseudomonas aeruginosa* Isolated From Burn Cases

**Eman Nassir Hussan AL-Jesmany[1], Oruba Khalid Abbas[2], Basima QASIM Hasan AlSaadi[3]**

[1]Department of Babylon Health, Ministry of Health, Baghdad, Iraq, [2]Department of Microbiology, College of Medicine, AL Mustansiriyah University, Baghdad, Iraq, [3]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

## Abstract

**Objectives:** Using molecular technology for the purpose of confirmation of the diagnosis of *Pseudomonas aeruginosa* which depends on the detection of proteins like *Pyoverdine* gene of *P. aeruginosa* as diagnostic test. **Materials and Methods:** This work was done on 110 patients who had sustained burn injury from hospitals Baghdad, Al-Yarmouk and the Medical City Teaching Hospital during the period from October 2020 to the end of March 2021. The collected samples were cultured on different media (blood agar, MacConkey agar, nutrient agar, and Cetrimide agar) for isolation of *P. aeruginosa* bacteria as well as automated biochemical tests such as Api20E and VITEK 2 systems. The results showed that 76 (69%) samples had bacterial growth of *P. aeruginosa*. Antibiotic susceptibility testing was evaluated using the VITEK 2 compact. The *Pseudomonas* was detected using species-specific gene 16SrRNA gene using polymerase chain reaction (PCR) method and also detected by (PCR) using newly designed primers with a molecular size (389 bp) for *pyoviridine* gene. **Results:** This work showed that the predominant growth of burn wound infections was *P. aeruginosa* 76 samples (69%). Antibiotic susceptibility testing results showed the same sensitivity pattern of *P. aeruginosa* isolates to ceftriaxone and cephalothin (67.1%), gentamicin, piperacillin, ceftazidime, cefepime, and cefotaxime (65.7%). Resistance to imipenem, tobramycin, ticarcillin, and meropenem were (57.8%), (51.3%), (56.5%) and (55.2%) respectively, and had the highest sensitivity to amikacin (34.2%). Moreover, the highest resistance was to ciprofloxacin (69.7), and norfloxacin (71%). The results of using the 16SrRNA gene for the detection *Pseudomonas* give positive results (100%). *Pseudomonas* spp was detected by (PCR) for *pyoviridine* gene, revealed that 50% isolates give positive results. PCR product pyoviridine of the isolates (3) with the highest resistance to fluoroquinolones (ciprofloxacin and norfloxacin) was sent to nitrogen-based sequencing, and the sequencing results revealed the mutation presence. **Conclusion:** This study shows the current resistant pattern of *P. aeruginosa* against different classes of antibiotics and the involvement of several virulence genes in resistance mechanisms by using PCR which ultimately helps to select appropriate antibiotics useful for the treatment of many burned complicated by *P. aeruginosa*.

**Keywords:** Burn patients, multi-drug resistance, *Pseudomonas aeruginosa*, *pyoviridine* gene

## Introduction

*Pseudomonas aeruginosa* is a Gram-negative rod bacterium, which has a remarkable ability to adapt and thrive in a variety of environments: Water, soil, occupational places, such as metalworking, fluids.[1] *P. seudomonas* causes the most common bacterial infection coupled with hospital infections in burns and pneumonia that are related to ventilation. It is responsible for otitis media, inflammation of the follicle, inflammation of the cornea, Burns wounds, diabetic foot infections, urinary tract infections, bacteremia, and inflammation in cystic fibrosis patients.[2] *P. aeruginosa* can produce multiple dyes in the agricultural medium, including the dye of green pyocyanin, pyoverdine pigment, the red poyrubin pigment, and the black pyomelanin pigment.[3] many virulence factors are produced that include secreted factors such as cytotoxic pigment pyocyanin, siderophores, alkaline protease, elastase, exotoxin A, lipopolysaccharide, pili, flagella, and biofilm formation (BF).[4] Pyoverdine, a siderophore produced by *Pseudomonas* spp., is also known to contribute to its virulence.[5]

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_14_21

**Address for correspondence:** Mrs. Eman Nassir Hussan AL-Jesmany, Department of Babylon Health, Ministry of Health, Baghdad, Iraq.
E-mail: eman.aljesmany@gmail.com

**Submitted:** 30-Jul-2021 **Accepted:** 06-Sep-2021 **Published:** 30-Jun-2022



**How to cite this article:** AL-Jesmany EN, Abbas OK, Hasan AlSaadi BQ. Molecular technology for the detection of *Pyoviridine* gene in *Pseudomonas aeruginosa* isolated from burn cases. Mustansiriya Med J 2022;21:23-8.

Pyoverdine can also hijack iron from other host sources, including mitochondria, which apply to considerable damage on the host.[6] Iron-bound pyoverdine (known as ferripyoverdine) functions as a signaling molecule that triggers the release of the alternate sigma factor PvdS from sequestration by the intermembrane FpvA/FpvR complex.

Once released, PvdS promotes the expression of at least two secreted toxins (exotoxin A and the protease PrpL) and also its own biosynthetic machinery. The iron provided by pyoverdine is required for BF.[7]

Burn infections are caused by both Gram-positive and Gram-negative microorganisms, currently, the common pathogens isolated from burn patients are *P. aeruginosa*, *Staphylococcus aureus*, β-hemolytic *streptococci*, *Escherichia coli, Klebsiella* species, and various coliform bacilli.[8]

## Materials and Methods

A total of 110clinical specimens were collected at the period between the beginning of October 2020 to the end of march 2021from patients suffering from burns infections. Samples were collected from different age groups, ranging from 5 to 65 years, from different parts of the body including lower extremities, upper extremities, and trunk, in different proportions. The isolates were identified based on the colony morphology on different culture media, MacConkey agar, and blood agar and incubated at 37°C for 24 h after the incubation period, nonlactose fermented colonies were transferred on cetrimide agar medium, which is a special selective medium for *P. aeruginosa* and incubated at 37°C for 24 h. Bacterial isolates were diagnosed via Oxidase Test, Catalase Test. and confirmed by using IMViC biochemical tests, Api 20 E and Vitek compact 2 system. Furthermore, *Pseudomonas* was detected by using species-specific gene 16SrRNA gene using polymerase chain reaction (PCR) method and species-specific primers. Kirby–Bauer method was followed for antibiotics susceptibility for all isolated specimens as *P. aeruginosa*. The enrolled isolates were subjected to DNA extraction using Presto Mini g DNA bacteria Kits extraction genomic DNA, Purification depending on the instruction of manufacturing company (iNtron) (Kore).

Genomic DNA was prepared from overnight cultures grown, 1000 microliter of broth was centrifuged, 100 µl buffer lysis + 100 µl protease were added and incubated for 1 h at 56°C, then 220 µl ethanol was added; 600 µl were transferred to spin column, centrifuged at 8000 rpm/1 min. The rest of the sample was transferred to spin column and centrifuge. Then, 500 µl of wash 1 buffer was added centrifuged. Transfer to new wash tube and 500 µl wash 2 was added. Spin column transferred to new wash tube, centrifuged at 13,000/3 min, then transfer to elution tubes and add 50 µl preheated elution Buffer (60°C). Incubate 3 min at room temperature; centrifuge 14000 rpm/1 min.[9] Primer Preparation Lyophilized forward and reverse primers for the *Pyoverdine* gene and 16S rRNA [Table 1]. Were suspended in nuclease-free water to give a final concentration of (100 pmol/µl) as stock solution; to prepare 10 pmol/µl concentration as work primer re-suspended 10 µl in 90 µl of nuclease-free water.

The molecular detection of *16S rRNA* gene, this step was carried out by adding 12.5 µl from OneTaq (NEB) master mix, 5 µl of DNA sample, 1.5 µl 10 pmol/µl from each primer, and 4.5 µl of free-nuclease water. The reaction was done under the optimal PCR conditions for gene. The annealing temperature in amplifying *Pyoverdine* gene was 58°C. PCR products were separated by gel electrophoresis on 2% agarose gel containing 0.5 µg/ml ethidium bromide. with 80 Volt/cm for 80 min [Table 2].[10]

## Results

The total 110 clinical samples gain from study patiens were submitted to microbiological culture technique, 76 (69%) were *P. aeruginosa*, while 34 (31%) belong to other genera of bacteria (*Klebsiellapneumonia* = 9 (8.2%), *Escherichia coli* = 10 (9.0%), *Acinetobacter baumannii* = 3 (2.8%), *Proteus mirabilis* = 8 (7.3%), and 4 (3.7%) patient have mix growth.

The results on culture media (blood agar, MacConkey agar, and cetrimide agar) reveal that the colony on the blood agar appear as white to gray color, sticky textures bacteria and examined their ability to hemolyze blood and showed type beta hemolysis,[11] while on MacConkey agar appeared as small pale colonies due to lactose non-fermenting[12] and capable of growing on cetrimide agar as blue-greenish colonies (at 37°C for 24 h). The outcome of biochemical tests gave the same results for all 76 specimen isolation as shown in Table 3, Identification of the isolates using Api 20E system and Vitek compact 2 system.[13]

Antimicrobial susceptibility of the 76 selected *P. aeruginosa* isolates from burns wound infection to antibiotics. Antimicrobial sensitivity tests were conducted for *P. aeruginosa* using (14)

**Table 1: The sequence forward and reverse primers of *pyoverdine* gene and 16S ribosomal RNA**

| Genes | Sequence (5'-3') | Size (Pb) | References |
|---|---|---|---|
| PVC | F: CTATGAGAGCCATTATTCCG | 389 | In this study |
| | R: GTAGATCTGCTTGTACAGGTA | | |
| 16S rRNA | F: GGGGGATCTTCGGACCTCA | 956 | |
| | R: TCCTTAGAGTGCCCACCCG | | |

PVC: *Pyoverdine*, rRNA: Ribosomal RNA

**Table 2: The polymerase chain reaction conditions for amplifying *Pyoverdine* gene**

| Steps | Temperature (°C) | Time | Cycles number |
|---|---|---|---|
| Initial denaturation | 94 | 5 min | 1 |
| Denaturation | 94 | 30 s | 35x |
| Annealing | 58 | 45s | 35x |
| Extension | 72 | 45 s | 35x |
| Final extension | 72 | 7 min | 1 |

types of antibiotics used in the hospitals According to the results obtained in Table 4.

PCR has the potential to identify microbial species rapidly and precisely by amplification of gene sequences unique to a particular organism.[14] All isolates gave positive results for *16S rRNA* gene that used the same primer with molecular size (956 bp) then the isolates screened for the presence of *Pyoverdine* gene. The result of gel electrophoresis for amplification PCR for *pyoviridine* gene product showed that the presence of bands of the samples. That means the primers of this genes bind specifically to complementary sequences within the DNA template. The molecular weight of the band was (389 bp) compared with DNA ladder 1500 bp as shown in Figure 1. The prevalence of the *pyoviridine* gene in the target isolates was (50%).

PCR products for three selected isolates (1, 2, and 3) for *Pyoviridine* gene were choice for sequencing that depending on their resistance for fluoroquinolones (ciprofloxacin, norfloxacin). These sequences were analyzed for the detection of differences in the nucleotides (mutations) and changes in amino acids.

The results of the alignment of the *Pyoverdine* sequences of the local isolates (1, 2, and 3) with the reference strain *P. aeruginosa* as shown in Table 5.

**Table 3: Morphological and biochemical features for the identification of *Pseudomonas aeruginosa* isolates**

| Tests | Results |
|---|---|
| Gram stain | Gram -ve rods |
| Catalase | + ve |
| Oxidase | + ve |
| Growth at 42 c° | + ve |
| B-hemolysis | - /+ |
| H2S production | - ve |
| Indol test | - ve |
| Kligler's iron agar | K/K |
| Methyl-red | - ve |
| Voges-Proskauer | - ve |
| Pigments production | + ve |
| Simmon's citrate | + ve |
| Urease | + ve |
| Motility | + ve |

## Discussion

Severe burns are very devastating forms of trauma which require immediate and specialized medical care. This pathogen is responsible for morbidity and mortality in

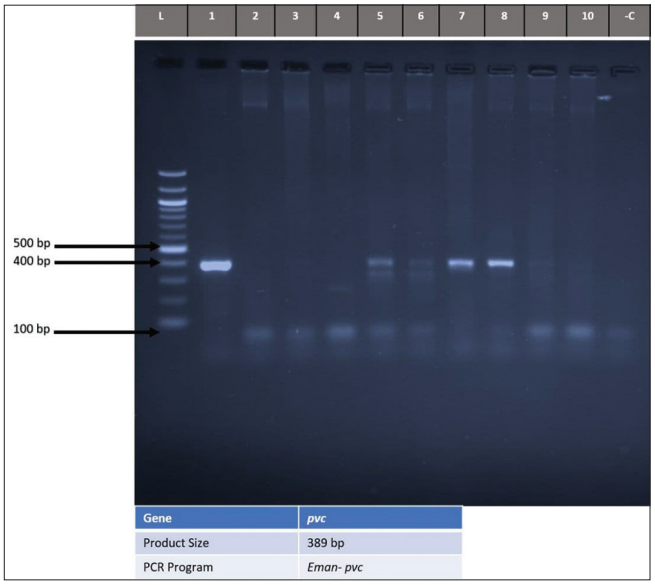


**Figure 1:** Agarose gel electrophoresis (2% agarose, 100 vol/80 min) of traditional polymerase chain reaction amplification products of *Pseudomonas aeruginosa pyoviridine* gene (389bp) 1–10: Show positive results: Ladder 100 bp

**Table 4: Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* by Bauer - Kirby method**

| Antibiotics | Resistance, *n* (%) | I, *n* (%) | Sensitive, *n* (%) | Total, *n* (%) | *P* |
|---|---|---|---|---|---|
| CIP | 53 (69.7) | 3 (3.9) | 20 (26.3) | 76 (100) | 0.0001** |
| NOR | 54 (71) | 2 (2.6) | 20 (26.3) | 76 (100) | 0.0001** |
| MER | 42 (55.2) | 1 (1.3) | 33 (43.4) | 76 (100) | 0.0001** |
| IMI | 44 (57.8) | 2 (2.6) | 30 (39.4) | 76 (100) | 0.0001** |
| TOB | 39 (51.3) | 0 | 37 (48.65) | 76 (100) | 0.0001** |
| GN | 50 (65.7) | 4 (5.2) | 22 (28.9) | 76 (100) | 0.0001** |
| AK | 26 (34.2) | 3 (3.9) | 47 (61.8) | 76 (100) | 0.0001** |
| Ti | 43 (56.5) | 1 (1.3) | 32 (42.1) | 76 (100) | 0.0001** |
| PRL | 50 (65.7) | 3 (3.9) | 23 (30.2) | 76 (100) | 0.0001** |
| CTX | 50 (65.7) | 4 (5.2) | 22 (28.9) | 76 (100) | 0.0001** |
| CRO | 51 (67.1) | 0 | 25 (32.8) | 76 (100) | 0.0001** |
| CAZ | 50 (65.7) | 7 (9.2) | 19 (25) | 76 (100) | 0.0001** |
| FEP | 50 (65.7) | 7 (9.2) | 19 (25) | 76 (100) | 0.0001** |
| KF | 51 (67.1) | 1 (1.3) | 24 (2.6) | 76 (100) | 0.0001** |
| *P* | 0.0001** | 0.0392* | 0.0001** | - | - |

*$P \leq 0.05$, **$P \leq 0.01$. CIP: Ciprofloxacin, MER: Meropenem, NOR: Norfloxacin, IMI: Imipenem, TOB: Tobramycin, GN: Gentamicin, AK: Amikacin, Ti: Ticarcillin, PRL: Piperacillin, CTX: Cefotaxime, CRO: Ceftriaxone, CAZ: Ceftazidime, FEP: Cefepime, KF: Cephalothin

immunocompromised patients[15] Within this study These findings may be due to develops the resistance by different mechanisms, including multidrug-resistance (MDR), efflux pumps, BF.[16] The prescience of virulence factors lets the pathogen to create efficient invasion, colonization, and persistence inside the host organism.[17]

The current study showed a high prevalence of *P. aeruginosa* infections among burned patients which were in agreement with the result of other investigators in Iraq/Mosul,[18,19] and in many economically developing countries such as Zimbabwe,[20] South Korea[21] and India.[22]

Although *P. aeruginosa* is not a classic pathogen of burn wound infections in economically developed countries, a few burn centers in Canada and the USA, France, and Italy have reported *P. aeruginosa* as an important microorganism in the burn unit.[23] while the results are slightly more than.[24,25] and it was very far from the percentage reported by.[26]

Incidence of "*P. aeruginosa*" according to the gender of the patient showed a higher rate of "*P. aeruginosa*" among females compared to males which is compatible with study in Iraq/ *Sulaimaniyah*[27] Otherwise, these results differ with the study in Nigeria[28] and Egypt[29] where males were the most affected. Isolation of *Pseudomonas* according to the site of infection was a result similar to a study in Sulaimaniyah, Iraq Which found that the majority of the specimens were taken from the lower limbs (43%) followed by the trunk (23%) and the upper limbs (17%).[27]

The growth of *Pseudomonas* on different media, blood agar, MacConkey agar, and cetrimide agar showed that only 76 isolates were capable of growing on cetrimide agar, which is a special selective medium for *Pseudomonas.* Depending on the shape of the bacterial isolates on a selective medium and the results of biochemical tests, as well as the results of Api 20E which showed that only seventy-six out of one hundred ten bacterial isolates were *P. aeruginosa*, while 31% belonged to other bacteria.[18]

Othman used API 20 E as final confirmation was made using the analytical profile index (API 20E system).[27] Zbinden reported that the accurate identification of *Pseudomonus aeruginosa* by VITEK 2system.[30]

The results obtained in this study revealed that *P. aeruginosa* isolates have shown high resistanc against Ciprofloxacin and norfloxacin. These results are close to the result belonging to AL-Buaiji[19] and higher than resistance against ciprofloxacin, 20.6% belonging to Al-Doory[31] and higher than the result of norfloxacin, 38.8% belonging to.[32] while the resistance of Meropenem was 55.2%, imipenem was 57.8% which is similar to Al-Doory.[31] Pseudomonads may develop resistance to carbapenems through combined mechanisms such as target in accessibility and overexpression of efflux systems.[33] Gentamicin group has shown high resistance to this group of antibiotics 65.7%. This result is compatible with[19,31] results and disagreement with the results obtained by[34] who showed extremely low resistance rate to imipenem and meropenem, 8% both and 4% to imipenem in Pakistan[35] Gentamicin has shown high resistance antibiotic 65.7% This result compatible with[19,31] results that were 60% and 65.7% respectively. and incompatible to the results of 47.6% resistance to *P. aeruginosa.*[36] Tobramycin resistanc rate was 51.3% which is similar to,[32] while less than the results of resistance to Tobramycin is 88.2%.[37] resistance to Amikacin was (34.2%), this result coincides with the finding of amikacin resistance which reported 34.9%,[36] and incompatible with and funding of Amikacin resistance were seen in (67%).[38]

The aminoglycosides inhibit protein synthesis in the bacterial cell by binding to 30S subunit of the ribosome and the Aminoglycoside-resistance in *Pseudomonas* sp. is primarily due to changes in the target enzymes and inactivation of the antibiotics[39] have mentioned.

Piperacillin resistance rate is 65.7% which is close to findings that reported 57.9%.[19] The result of this study crossed with the results finding, 35.8% resistance[31] while Ticarcillin resistance rate is 56.5% which is similar to the study which found that resistance to Ticarcillin was 61%.[40] While the difference in result is apparent compared with results found that all *P. aeruginosa* isolated from burn were resistant to ticarcillin 100%.[32]

The levels of resistance to cephalosporins including ceftazidime, cefotaxime, cefepime, cephalothin, and ceftriaxone. This study, *P. aeruginosa* isolates has revealed high resistance (65.7%) for both ceftazidime and cefepime. This result coincides with the finding of (65.7, 68.4) for ceftazidime and cefepime, respectively.[19] Results obtained in this study revealed that the levels of resistance to cefotaxime and ceftriaxone are (65.7, 67.1). This result coincides with the finding[41] which found 50.75 resistance to cefotaxime and74.95 to ceftriaxone, while the result incompatible with

**Table 5: Mutation in *Pvc* gene in isolates numbers 1, 2, and 3**

**Source: *Pseudomonas aeruginosa***

| Number of sample | Type of substitution | Location | Nucleotide | Nucleotide change | Amino acid change | Predicted effect | Sequence ID with compare | Gene | Identities (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | - | - | - | - | - | - | ID: CP034908.2 | PvcD | 100 |
| 2 | Transition | 2476605 | C\T | CGC\TGC | Arginine\cysteine | Missense | ID: CP034908.2 | PvcD | 99 |
| | Transition | 2476740 | G\A | GAC\AAC | Aspartic acid\asparagine | Missense | | | |
| 3 | Transition | 2476621 | G\A | TGC\TAC | Cysteine\tyrosine | Missense | ID: CP034908.2 | PvcD | 99 |

high resistance 100% to both of them.[38] The MDR, defined as resistance to three classes of antimicrobials, increased amongst these organisms making it difficult to choose appropriate suitable antimicrobial therapy.[42]

Recently various methods have been developed to rapidly and accurately identify *Pseudomonas* 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.[14] The results of this study showed that the number of the *P. aeruginosa* isolated from the burn by using PCR detection, all the *P. aeruginosa* isolates (100%) were positive which based on the 16S rRNA gene. The results of *16S rRNA* gene matched with the study in Kurdistan.[37]

A total of 15 pyoverdine genes have now been identified that are essential for pyoverdine synthesis in *P. aeruginosa* PAO1 and it is likely that most, if not all, of the genes that are essential for pyoverdine synthesis in this strain are now known. The result of gel electrophoresis for amplification PCR product found that the presence of bands, the results indicated 50% of the isolates were carrying the *pyoviridine* genes and confirmed as *P. aeruginosa*.

In this study, it will be addressed the pvc genes lie at about 66-70 min on the genetic map,[43] about 240 kb away from pvdS. A separate cluster of four genes (pvcABCD) has been reported to be required for synthesis of the pyoverdine chromophore.

pvc mutants can make pyoverdine in some growth media so that these genes are not essential for pyoverdine synthesis.[44] The existence of pyoverdine-negative isolates of *P. aeruginosa*has prompted the need for accurate and enhanced genotyping procedures based on the determination of the gene sequence of ferripyoverdine receptor fpvA of *P. aeruginosa.*[45]

No matching found with pvc gene detection and sequencing study except[46] found that the proximity of the ptxR gene to the pvcABCD operon it seemed possible that PtxR plays a role in the expression of pvcABCD and, thus, pyoverdine[46] reported that the ptxR deletion in strain PAO1 completely abrogated pvc expression, with no pvc mRNA detected under iron-limited or iron-replete conditions.

The isolates that had double mutation in *Pyoverdine* isolation number (2) had higher ciprofloxacin and norfloxacin resistance than those with a single mutation in *Pyoverdine* isolation number (3).

Resistance to fluoroquinolone antibiotics is multifactorial and can be via one or a combination of target-site gene mutations, increased production of MDR efflux pumps, modifying enzymes, and/or target-protection proteins.[47]

Characterized mechanisms of fluoroquinolone resistance among *P. aeruginosa* isolates have been restricted to chromosomal genes, including target mutations and active efflux Similar to the case for other gram-negative bacteria, DNA gyrase is the primary target for the fluoroquinolones in *P. aeruginosa.*[48]

In Gram-negative bacteria, GyrA is the preferred target of fluoroquinolone, and resistance mutations thus tend to occur in this enzyme first with additional mutations in topoisomerase IV seen in some highly resistant isolates.[49]

The current study concluded that *P. aeruginosa* isolate is high despite modern techniques and sterilization and disinfection solution and has abundant ability to form biofilms, produce virulence enzymes, and antibiotic resistance. For these isolates that were identified by phenotypic testing used detection of PCR and DNA sequencing analyzes designed to make sure identification Burn wound infection isolates, as well as identification of mutations, if any.

## Conclusion

The highest prevalence of *P. aeruginosa* was located in burn, the highest frequency resistance was against fluoroquinolones (Ciprofloxacin and Norfloxacin) and the lowest resistance was to Amikacin. Half selected local isolates of *P. aeruginosa* carried Pyoverdine genes can uptake iron from host sources, which apply to considerable damage on the host. Using PCR is highly sensitive, specific and rapid method which vastly improved the detection of *P. aeruginosa.*

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Karadzic I, Masui A, Zivkovic LI, Fujiwara N. Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid. J Biosci Bioeng 2006;102:82-9.
2. Liew SM, Rajasekaram G, Puthucheary SA, Chua KH. Antimicrobial susceptibility and virulence genes of clinical and environmental isolates of *Pseudomonas aeruginosa*. PeerJ 2019;7:e6217.
3. Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA. Medical Microbiology. Jawetz, Melnick and Adelbergs, 25th ed. McGraw-Hill Companies, 2010,213-219.
4. El-Mahdy R, El-Kannishy G. Virulence factors of carbapenem-resistant *Pseudomonas aeruginosa* in hospital-acquired infections in Mansoura, Egypt. Infect Drug Resist 2019;12:3455-61.
5. Visca P, Imperi F, Lamont IL. Pyoverdine siderophores: From biogenesis to biosignificance. Trends Microbiol 2007;15:22-30.
6. Kang D, Kirienko NV. Interdependence between iron acquisition and biofilm formation in *Pseudomonas aeruginosa*. J Microbiol 2018;56:449-57.
7. van Tilburg Bernardes E, Charron-Mazenod L, Reading DJ, Reckseidler-Zenteno SL, Lewenza S. Exopolysaccharide-repressing small molecules with antibiofilm and antivirulence activity against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2017;61:e1997-16.
8. Nudegusio L, Algimantas T, Rytis R. Analysis of burn patients and the isolated pathogens. Lithuanian Surg 2004;2:190-3.
9. Nikbin VS, Aslani MM, Sharafi Z, Hashemipour M, Shahcheraghi F, Ebrahimipour GH. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious

origins. Iran J Microbiol 2012;4:118-23.
10. Sambrook J, Russell D. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2001. p. 999.
11. Korgaonkar A, Trivedi U, Rumbaugh KP, Whiteley M. Community surveillance enhances *Pseudomonas aeruginosa* virulence during polymicrobial infection. Proc Natl Acad Sci U S A 2013;110:1059-64.
12. Forbes BA, Sahm DF, Weissfeld A, editors. Bailey and Scott's Diagnostic Microbiology. 11th ed. St. St Louis: Mosby Inc.,; 2002.
13. Guckan R, Kilinc C, Demir AD, Capraz A, Yanik K. Antimicrobial susceptibility of *Acinetobacter baumannii* complex isolated from different clinical samples in a tertiary care hospital. J Antibiot Res 2015;1:103.
14. Nikbin VS, Abdi-Ali A, Feizabadi MM, Gharavi S. Pulsed field gel electrophoresis and plasmid profile of *Pseudomonas aeruginosa* at two hospitals in Tehran, Iran. Indian J Med Res 2007;126:146-51.
15. Rocha AJ, Barsottini MR, Rocha RR, Laurindo MV, Moraes FL, Rocha SL. *Pseudomonas Aeruginosa*: Virulence Factors and Antibiotic Resistance Genes. Brazilian Archives of Biology and Technology 2019, v. 62, e19180503. ISSN 1678-4324.
16. Ali MD, Abdulrahman ZF. Molecular identification, susceptibility pattern, and detection of some virulence genes in *Pseudomonas aeruginosa* isolated from burn patients. Plant Arch 2020;20:2573-80.
17. Que YA, Hazan R, Strobel B, Maura D, He J, Kesarwani M, *et al*. A quorum sensing small volatile molecule promotes antibiotic tolerance in bacteria. PLoS One 2013;8:e80140.
18. Al-Habib HM, Al-Gerir AZ, Hamdoon AM. Profile of *Pseudomonas aeruginosa* in burn infection and their antibiogram study. Ann Coll Med 2011;37:2.
19. Al Buaiji AK. Molecular study parC and gyrA genes of multidrug resistant *Pseudomonas aeruginosa* isolated from clinical specimens, M.S.C thesis Institute of Genetic Engineering and Biotechnology For Post Graduate Studies/University of Baghdad; 2019.
20. Igumbor E, Gwanzura L, Chirara M, Obi C, Muza D. Antibiotic sensitivity and plasmid profiles of *Pseudomonas aeruginosa*. Cent Afr J Med 2000;46:296-300.
21. Song W, Lee KM, Kang HJ, Shin DH, Kim DK. Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. Burns 2001;27:136-9.
22. Kaushik R, Kumar S, Sharma R, Lal P. Bacteriology of burn wounds – The first three years in a new burn unit at the Medical College Chandigarh. Burns 2001;27:595-7.
23. Rastegar Lari AR, Alaghehbandan R, Akhlaghi L. Burn wound infections and antimicrobial resistance in Tehran, Iran: An increasing problem. Ann Burns Fire Disasters 2005;18:68-73.
24. Al Mamory HH. Characterization of *Pseudomonas aeruginosa* isolated from patients and hospital environment in Hilla City. (M. sc. thesis). University of Bablon; 2011.
25. AL Kaisse AA. Molecular detection of OXA 4, OXA 10 and VEB 1 genes in *Pseudomonas aeruginosa* isolated from burn's wound patients. (MS. thesis), genetic engineering and biotechnology Institute, Baghdad university; 2013.
26. Al-Daraghi WA, Al-Badrwi MS. Molecular detection for nosocomial *Pseudomonas aeruginosa* and its relationship with multidrug resistance, isolated from hospitals environment. Med Leg Update 2020;20:631-6.
27. Othman N, Babakir-Mina M, Noori CK, Rashid PY. *Pseudomonas aeruginosa* infection in burn patients in Sulaimaniyah, Iraq: Risk factors and antibiotic resistance rates. J Infect Dev Ctries 2014;8:1498-502.
28. Okon K, Agukwe P, Oladosu W, Balogun S, Uba A. Antibiotic resistance pattern of "*Pseudomonas aeruginosa*" isolated from clinical specimens in a tertiary hospital in northeastern Nigeria. J Microbiol 2009;8:5-7.
29. Mohamed H. One year prevalence of critically ill burn wound bacterial infections in surgical ICU in Egypt: Retrospective study. Egypt J Anaesth 2016;32:431-4.
30. Zbinden A, Böttger EC, Bosshard PP, Zbinden R. Evaluation of the colorimetric VITEK 2 card for identification of gram-negative nonfermentative rods: Comparison to 16S rRNA gene sequencing. J Clin Microbiol 2007;45:2270-3.
31. Al Doory IA. A diagnostic study of *Pseudomonas aeruginosa* isolated from contaminated burns and wounds using cultural and molecular methods. M. Sc. Thesis. College of Science for Women, University of Baghdad, Iraq; 2012.
32. Al Zaidi JR. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolated from clinical and hospital environmental samples in Nasiriyah, Iraq. Afr J Microbiol Res 2016;10:844-9.
33. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? Clin Infect Dis 2002;34:634-40.
34. Mohammed AS. Phenotypic and genotypic detectionof metallo β lactamase producing *Pseudomonas aeruginosa* from local isolates. Ph. D. Desseration. College of Medicine, University of Babylon, Iraq; 2012.
35. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. Burns 2009;35:1020-5.
36. AL-Mayyahi AW, AL-hashimy A, ALawadi K. Detection of (exoT, exoY, exo S and exoU) genes in *Pseudomonas aeruginosa* isolate from different clinical sources. Iraqi J Biotechnol 2018;17:1-8.
37. Merza NS, Hanoon RA, Khalid HM, Qader MK, Jubrael JM. Molecular differentiation and determination of multi-drug resistant isolates of *Pseudomonas* species collected from burn patients in Kurdistan Region, Iraq. Zanco J Med Sci 2018;22:394-400.
38. Hasan SA, Najati AM, Abass KS. Isolation and identification of multi-drug resistant "*Pseudomonas aeruginosa*" from burn wound infection in Kirkuk City. Eurasia J Biosci 2019;13:1045-50.
39. Matsuo Y, Eda S, Gotoh N, Yoshihara E, Nakae T. MexZ-mediated regulation of mexXY multidrug efflux pump expression in *Pseudomonas aeruginosa* by binding on the mexZ-mexX intergenic DNA. FEMS Microbiol Lett 2004;238:23-8.
40. AL-Shamaa NF, Abu- Risha RA, AL-Faham MA. Virulence genes profile of *Pseudomonas aeruginosa* local isolates from burns and wounds. Iraqi J Biotechnol 2016;15:31-9.
41. Hongqi L, Mengchen J, Lei X, Li Y. Analysis of infection distribution of *Pseudomonas aeruginosa* on burned patients and its drug resistances. J Clin Case Rep 2016;6:8.
42. Yayan J, Ghebremedhin B, Rasche K. Antibiotic resistance of *Pseudomonas aeruginosa* in pneumonia at a single university hospital center in Germany over a 10-year period. PLoS One 2015;10:e0139836.
43. Stintzi A, Cornelis P, Hohnadel D, Meyer JM, Dean C, Poole K, *et al.* Novel pyoverdine biosynthesis gene(s) of *Pseudomonas aeruginosa* PAO. Microbiology (Reading) 1996;142:1181-90.
44. Lamont IL, Martin LW. Identification and characterization of novel pyoverdine synthesis genes in *Pseudomonas aeruginosa*. Microbiology (Reading) 2003;149:833-42.
45. De Vos D, De Chial M, Cochez C, Jansen S, Tümmler B, Meyer JM, *et al.* Study of pyoverdine type and production by *Pseudomonas aeruginosa* isolated from cystic fibrosis patients: Prevalence of type II pyoverdine isolates and accumulation of pyoverdine-negative mutations. Arch Microbiol 2001;175:384-8.
46. Stintzi A, Johnson Z, Stonehouse M, Ochsner U, Meyer JM, Vasil ML, *et al.* The pvc gene cluster of *Pseudomonas aeruginosa*: Role in synthesis of the pyoverdine chromophore and regulation by PtxR and PvdS. J Bacteriol 1999;181:4118-24.
47. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol 2014;22:438-45.
48. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22:582-610.
49. Jacoby GA. Mechanisms of resistance to quinolones. Clin Infect Dis 2005;41 Supp 2:S120-6.