Case Report

narra j

Case Report

Detection of *Pseudomonas aeruginosa* pus wound isolate using a polymerase chain reaction targeting 16S rRNA and *gyrB* genes: A case from Indonesia

Indra P. Jamaluddin[1], Susan H. Musa[2], Stalis N. Ethica[3], Arif NM. Ansori[1,4,5], Valensa Yosephi[1], Peter Y. Atmaja[6], Ahmad AA. Murtadlo[5,7], Sukma Sahadewa[8], Fara D. Durry[9], Maksim Rebezov[10,11], Marina Derkho[12], Sin W. Naw[13], Rahadian Zainul[14,15]* and Kadek Rachmawati[16]

[1]Master of Immunology Study Program, Universitas Airlangga, Surabaya, Indonesia; [2]Associate Degree Study Program of Health Analyst, Faculty of Nursing and Health Science, Universitas Muhammadiyah Semarang, Semarang, Indonesia; [3]Master of Clinical Laboratory Science, Faculty of Nursing and Health Science, Universitas Muhammadiyah Semarang, Semarang, Indonesia; [4]Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India; [5]Virtual Research Center for Bioinformatics and Biotechnology, Surabaya, Indonesia; [6]Soeradji Tirtonegoro Hospital, Klaten, Indonesia; [7]Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia; [8]Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia; [9]Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jawa Timur, Surabaya, Indonesia; [10]Department of Scientific Research, V. M. Gorbatov Federal Research Center for Food Systems, Moscow, Russian Federation; [11]Faculty of Biotechnology and Food Engineering, Ural State Agrarian University, Yekaterinburg, Russian Federation; [12]Department of Natural Sciences, Institute of Veterinary Medicine, South Ural State Agrarian University, Troitsk, Russian Federation; [13]Department of Chemistry, Myitkyina University, Myitkyina, Myanmar; [14]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Padang, Indonesia; [15]Center for Advanced Material Processing, Artificial Intelligence, and Biophysic Informatics (CAMPBIOTICS), Universitas Negeri Padang, Padang, Indonesia; [16]Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: rahadianzmsiphd@fmipa.unp.ac.id

Abstract

Infectious wounds on the skin surface are easily colonized by bacteria from pyogenic group that manifest as inflammation, such as *Pseudomonas aeruginosa*. *P. aeruginosa* is a Gram-negative bacterium and an opportunistic pathogen known for causing invasive state in critically ill and immunocompromised patients. The aim of this study was to detect the 16S rRNA and *gyrB* genes in *P. aeruginosa* using polymerase chain reaction (PCR) method. The sample in this study was pus isolate from a 5-year-old boy with leg wounds. The bacteria were isolated on brain heart infusion broth (BHIB) media and identified with molecular identification. Sequencing and BLAST analysis were carried out to determine the similarity of gene identity by comparing sample sequence with other isolate sequences on the Gene Bank. The results of molecular identification showed amplification DNA band of around 934 base pairs (bp) for 16S rRNA and 225 bp for *gyrB* gene. The BLAST program demonstrated that the sample had 99.89% similarity with *P. aeruginosa* strain XC4 (accession code ON795960.1) for the 16S rRNA gene. Meanwhile, the *gyrB* gene exhibited 99.10% similarity with the *P. aeruginosa* strain PSA-1.2 (accession code KP172300.1).

Keywords: *Pseudomonas aeruginosa*, 16S rRNA, *gyrB*, PCR, sequencing

Copyright: © 2024 by the authors. This is an open access article distributed under the terms and conditions of the CC BY-NC 4.0.

Introduction

Wounds represent damage to the normal anatomical structure and function of the skin due to pathological processes originating both internally and externally that affect specific organs [1]. Bacterial infection of a skin wound is a primary cause of delayed wound healing with high bacterial levels impending wound healing process [2]. A study has predicted that antimicrobial-

Received: March 29, 2024 - Accepted: June 20, 2024 - Published online: July 4, 2024

resistant pathogens infection may result in 10 million annual deaths by 2050 [3]. Bacterial infection is typically accompanied by inflammation, which when it is persistent, can lead to cell death, tissue necrosis and further injuring surrounding soft tissues [4]. Additionally, excessive inflammation response is a significant factor in delayed wound healing and may precipitate severe complications [5]. Pus is a protein-rich fluid and byproduct of inflammatory process that is formed by leukocytes, extracellular fluid, and cellular debris. Prolonged pus formation indicates the continued presence of bacteria in the wound area [6]. In contrast to other forms of traumatic injury, the microenvironment of wounds is more complex, leading to a greater susceptibility to bacterial colonization and significant exudate production, both of which contribute to delayed wound healing [7].

Pseudomonas aeruginosa is a Gram-negative bacterium and one of the leading causes of opportunistic infections (e.g., wound infection, eyes, and respiratory system) that can spread systematically through the bloodstream, causing bacteremia, multiple organ failure, and death [8,9]. The 16S rRNA is an essential genes for determining *P. aeruginosa* bacteria through its genetic characteristic based on 16S DNA ribosomes [10]. Antibiotic resistance by *P. aeruginosa* can be influenced by several factors, including the presence of gene mutations in the DNA gyrase (*gyrB*) subunit. Many studies have utilized *gyrB* gene as a target for detecting *P. aeruginosa* due to its specificity that allows for accurate detection of the microorganism [11-13]. The *gyrB* is a gene that encodes the ATPase domain of DNA gyrase, an essential enzyme for DNA replication that contribute to the virulence of bacteria and have been employed as molecular tools for bacterial species identification and phylogenetic analysis [14].

It has been reported that the *gyrB* gene is a reliable polymerase chain reaction (PCR) target in the detection of *P. aeruginosa* detection [11]. PCR is a molecular technique to identify infectious diseases and has numerous advantages [15]. These advantages include producing accurate, fast, and specific product amplification, requiring a small number of samples, and overcoming the shortcomings of conventional diagnostics [16]. The aim of this study was to detect *P. aeruginosa* from a pus wound isolate from a child using PCR based on 16S rRNA and *gyrB* genes.

Case

The bacteria were isolated from a wound on the injured leg of a 5-year-old boy from Kedungmundu, Semarang, Indonesia, who suffered an iron nail puncture in leg that was not treated properly. After several days, the signs of infection appeared, including reddish swelling, pus production, and pain at the wound site. Bacterial isolation was performed using nutrient and MacConkay agar by employing the scratch method to obtain separate colonies and incubate them in the incubator at 37°C for 24 hours. Gram staining was then conducted following standard protocol [17]. The characteristics of isolated bacteria colonies in nutrient and MacConkey agar are shown in **Figure 1A-1B** and **Table 1**. On nutrient agar, the colonies were circular, greenish, and medium-sized, with entire edges, a raised elevation shape, and smooth consistency. On MacConkey agar, the colonies were circular, greenish, and medium-sized, with undulated or uneven edges, a convex or convex elevation shape, smooth consistency, and non-lactose fermenting. The bacteria staining resulted in red color with a solitary arrangement (**Figure 1C**).

Figure 1. Characteristics of the isolated bacteria on nutrient agar media (A), MacConkey agar (MCA) media (B), and Gram staining (C).

Table 1. Colony and cell morphology of pus wound bacterial isolate

Isolate characteristic	Result	
	Nutrient agar	MacConkey agar
Colony shape	Circular	Circular
Colony elevation	Raised	Convex
Colony size	Medium	Small-medium
Colony edges	Entire	Undulate
Colony color	Green	Green
Gram staining	Gram negative	Gram negative
Cell shape	Bacilli (rods)	Bacilli (rods)

DNA extraction and quantification were subsequently conducted using the MCA culture results. The bacteria were inoculated on brain heart infusion broth (BHIB) media and incubated at 37°C for 48 hours. DNA extraction was conducted using Geneaid Presto Mini gDNA Bacteria Kit (Genetika Science, Tangerang, Indonesia). The purity of the extracted genomic DNA was assessed using a NanoDrop spectrophotometer (Maestrogen Pro, Las Vegas, USA), which measures DNA purity against contaminants by analyzing the A260/280 absorbance ratio of the DNA sample.

The PCR amplification was carried out with a mixture consisted of 12.5 µL master mix, forward and reverse primer each 2 µL, DNA template 2 µL, and nuclease-free water 6.5 µL. The PCR program included pre-denaturation 95°C for 4 min, denaturation 95°C for 30 s, annealing 58°C (16S rRNA) and 62°C (*gyrB*) for 30 s, extension 72°C for 1 min with 35 cycles, and final extension 72°C 5 min as recommended previously [18]. Published primers were used to amplify the 16S rRNA [10] and *gyrB* gene [19]. PCR products were electrophoresed on a 2% agarose gel by mixing 8 µL of the PCR product with 2 µL of loading dye and visualized with a UV transilluminator. Gel electrophoresis yielded two bands ~934 and ~225 base pairs for 16S rRNA and *gyrB*, respectively.

The subsequent sequence was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, CA, USA) and an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA). Sequence analysis was conducted using BioEdit software DNA Base Assembler 7.2.5 version (UNT Art and Sciences, Denton, TX, USA), and the generated sequences were matched with data in the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI), National Institute for Health, USA to identify the type of bacterial species. Our data indicated that the sequences were more than 99% similar to *P. aeruginosa* (**Table 2**).

Table 2. BLAST gene 16S rRNA and *gyrB* results

Genes	Description	Scientific name	Percentage identity (%)	Length (bp)	Accession number of the comparator
16S rRNA	*Pseudomonas aeruginosa* strain XC4 16S ribosomal RNA gene, partial sequence.	*Pseudomonas aeruginosa*	99.89	1411	ON795960.1
gyrB	*Pseudomonas aeruginosa* strain PSA-1.2 gyrase B (*gyrB*) gene, partial cds.	*Pseudomonas aeruginosa*	99.10	539	KP172300.1

A phylogenetic tree analysis was carried out using the Molecular Evolutionary Genetics Analysis (MEGA) Version 11 (Mega XI Software, USA) to determine the relationship between bacterial species. The phylogenetic of *P. aeruginosa* isolate BP-1 (Boy's pus 1) was analyzed based on the similarity of the 16S rRNA sequence using the *Neighbor-Joining* method with the *Tamura-Nei* algorithm and a bootstrap of 1000 repetitions. The phylogenic analysis of the isolated bacteria based on 16S rRNA gene is presented in **Figure 2**. The BP-1 sample had a great relationship with

Jamaluddin et al. Narra J 2024; 4 (2): e774 - http://doi.org/10.52225/narra.v4i2.774

Pseudomonas sp. from Gene Bank using *Acinetobacter lactucae*. A similarity of 99.89% was identified for BP-1 bacterial isolate, confirming it as *P. aeruginosa*.

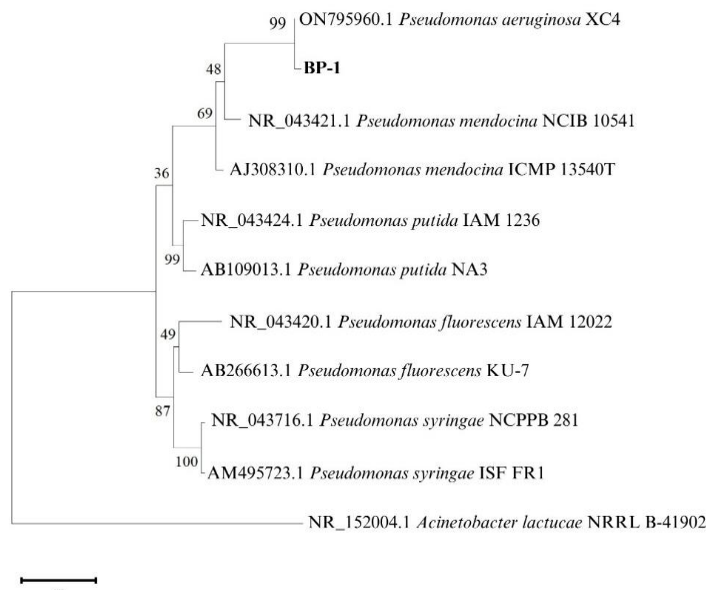

Figure 2. Results of phylogenic analysis of the 16S rRNA gene of the BP-1 sample isolated from the patient.

Discussion

We reported a case of a 5-year-old boy with a leg infection due to improper management. A sample was collected from infected wound with pus production. Our nutrient agar culture indicated that bacterial colonies produced pyoverdine pigment, which gives the media a greenish color. This phenomenon aligns with previous reports that *P. aeruginosa* produced various pigments, such as pyocyanin (greenish blue) and pyoverdine (yellowish-green fluorescent pigment) [20]. In addition, the colonies were unable to ferment the lactose (non-lactose fermenter) and Gram staining confirmed the presence of Gram-negative bacteria consistent with previous report for *P. aeruginosa* [21,22]. PCR amplification and electrophoresis yielded the DNA band sizes of ~934 bp for 16S rRNA and ~225 bp for *gyrB* gene, consistent with previous studies [10,19]. The amplified DNA had sufficient concentration to indicate that the sample underwent DNA amplification using primers for each gene. The suitability of the primers and the efficiency of the PCR process contributed mainly to the success of PCR technique.

Our sequencing and BLAST indicated that the sample had a 99.89% resemblance to *P. aeruginosa* strain XC4 (access code ON795960.1) for the 16s rRNA gene and a 99.10% similarity to *P. aeruginosa* strain PSA-1.2 (accession code KP172300.1) for *gyrB* gene sequence (**Table 2**). The phylogenetic analysis results indicated that the 16S rRNA gene of the sample was similar to the *P. aeruginosa* strain XC4 gene (access code ON795960.1) (**Figure 2**). Based on these results, the pus isolate was confirmed as *P. aeruginosa* according to the base sequence of the 16S rRNA and the *gyrB* genes. The 16S rRNA gene is one of the important genes for determining bacteria through its genetic factors due to its conversed (sustained) region, making it suitable for the use of PCR and sequencing analysis for taxonomy, phylogeny, and species diversity studies [23]. The 16S rRNA gene is located at the 5' terminus and codes for the formation of ribosomal RNA in a small ribosomal subunit separated by a spacer region called intergenic spacer region (ISR), which will form transfer RNA (tRNA) and plays a role in the process of protein synthesis [10]. The *gyrB* gene is a specific gene for *P. aeruginosa* and many studies have used it as a target for detecting *P. aeruginosa* due to its specificity, allowing for accurate microorganisms detection [11-13]. *gyrB* is a gene that encodes the ATPase domain of DNA gyrase, an enzyme essential for DNA replication [11]. These genes contribute to bacterial virulence and have been used as molecular tools for bacterial species identification and phylogenetic analysis [14,24,25,26]. The use of 16S rRNA and

gyrB genes could facilitate the diagnosis, treatment, and prevention of infection cases because of *P. aeruginosa* in Indonesia.

This case has limitations, as the patient was not monitored for antibiotic treatment until the wound healed, and only molecular identification of the pus sample using the 16S rRNA and *gyrB* gene was performed. Future research could focus on monitoring wound treatment until the healing phase and investigating other types of virulence genes.

Conclusion

Molecular identification based on the 16S rRNA and the *gyrB* gene indicated that the isolate from the wound infection was *P. aeruginosa,* with 99.8% and 99.1% similarity for the 16S rRNA and *gyrB* gene, respectively. The isolate was identified as *Pseudomonas aeruginosa* strain BP-1 (Boy's pus 1). The use of 16S rRNA and *gyrB*-based PCR could be used to detect *P. aeruginosa* and holds potential for development to identify other bacteria in advanced health centers in Indonesia.

Ethics approval

The parent of the patient provided consent to use the case as a case report.

Acknowledgments

None.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Jamaluddin IP, Musa SH, Ethica SN, *et al.* Detection of *Pseudomonas aeruginosa* pus wound isolate using a polymerase chain reaction targeting 16S rRNA and *gyrB* genes: A case from Indonesia. Narra J 2024; 4 (2): e774 - http://doi.org/10.52225/narra.v4i2.774

References

1. Wang PH, Huang BS, Horng HC, *et al.* Wound healing. J Chinese Med Assoc 2018;81(2):94-101.
2. Li S, Renick P, Senkowsky J, *et al.* Diagnostics for wound infections. Adv Wound Care 2021;10(6):317-327.
3. Walsh TR, Gales AC, Laxminarayan R, *et al.* Antimicrobial resistance: Addressing a global threat to humanity. PLOS Med 2023;20(7):e1004264.
4. Arciola CR, Campoccia D, Montanaro L. Implant infections: Adhesion, biofilm formation and immune evasion. Nat Rev Microbiol 2018;16(7):397-409.
5. Hou X, Zhang X, Zhao W, *et al.* Vitamin lipid nanoparticles enable adoptive macrophage transfer for the treatment of multidrug-resistant bacterial sepsis. Nat Nanotechnol 2020;15(1):41-46.
6. Fatimawali, Kepel B, Tallei TE. Potential of organic mercury-resistant bacteria isolated from mercury contaminated sites for organic mercury remediation. Pakistan J Biol Sci 2019;22(1):45-50.
7. Zhou L, Liu N, Feng L, *et al.* Multifunctional electrospun asymmetric wettable membrane containing black phosphorus/Rg1 for enhancing infected wound healing. Bioeng Transl Med 2022;7(2):e10274.
8. Wood SJ, Kuzel TM, Shafikhani SH. *Pseudomonas aeruginosa*: Infections, animal modeling, and therapeutics. Cells 2023;12(1):199.

9. Sathe N, Beech P, Croft L, *et al. Pseudomonas aeruginosa*: Infections and novel approaches to treatment "Knowing the enemy" the threat of *Pseudomonas aeruginosa* and exploring novel approaches to treatment. Infect Med 2023;2(3):178-194.
10. Abdullah RM, Mehdi AF. Identification of *Pseudomonas aeruginosa* from clinical specimen by using 16S rDNA gene. J Biotechnol Res Cent 2016;10(1):45-49.
11. Aghamollaei H, Moghaddam MM, Kooshki H, *et al.* Detection of *Pseudomonas aeruginosa* by a triplex polymerase chain reaction assay based on lasI/R and *gyrB* genes. J Infect and Public Health 2015;8(4):314-322.
12. Varela-Rodríguez EN, Arvizu-Gómez JL, Martínez-Rizo AB, *et al.* Molecular identification of *Pseudomonas aeruginosa* and antibiotic resistance testing. GSC Biol Pharm Sci 2023;24(2):308-316.
13. Feng X, Zhang Z, Li X, *et al.* Mutations in *gyrB* play an important role in ciprofloxacin-resistant *Pseudomonas aeruginosa*. Infect Drug Resist 2019;12:261-272.
14. Foysal M, Biswas G, Raihan M. PCR based detection of *gyrB2* gene from *Pseudomonas* sp. affected human clinical isolates. Int Curr Pharm J 2012;1(9):235-238.
15. Liu HY, Hopping GC, Vaidyanathan U, *et al.* Polymerase chain reaction and its application in the diagnosis of infectious keratitis. Med Hypothesis Discov Innov Ophthalmol 2019;8(3):152-155.
16. Schoonbroodt S, Ichanté JL, Boffé S, *et al.* Real-time PCR has advantages over culture-based methods in identifying major airway bacterial pathogens in chronic obstructive pulmonary disease: Results from three clinical studies in Europe and North America. Front Microbiol 2023;13:1098133.
17. Wulandari D, Purwaningsih D. Identification and characterization of Amylolytic bacteria in tubers of Colocasia esculenta L. morphological, biochemical, and molecular. J Bioteknol Biosains Indones 2019;6(2):247-258.
18. Adenipekun EO, Akinleye EF, Tewogbade OA, *et al.* Detection of virulence genes and multidrug resistance in *Pseudomonas aeruginosa* clinical isolates from a public hospital in Lagos, Nigeria. Sci Afr 2023;22:e01950.
19. Mulamattathil SG, Bezuidenhout C, Mbewe M, *et al.* Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles. J Pathog 2014;2014:1-11.
20. Ekawati ER, Nur HSY, Herawati D. Identification of germs in pus from skin infected wounds. Jurnal SainHealth 2018;2(1):31-35.
21. Kayser F. Haemophilus and Pasteurella. In: Fritz H, Kayser F, Kurt A, Eckert J, Kayser D, editors. Medical Microbiology. 10th ed. Stuttgart: Georg Thieme Verlag; 2005.
22. Thairu Y, Nasir IA, Usman Y. Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub Saharan Afr J Med 2014;1(4):168-174.
23. Hussein AM, Muhialdin AJ, Karim FR, *et al.* 16 s rRNA sequencing for *Pseudomonas aeruginosa* isolated form clinical samples. Materials Today: Proceedings 2022;65:2899-2903.
24. Al-Shimmary SMH, Mohamed NS, Al-Qaysi SAS, *et al.* Phylogeny analysis of *gyrB* gene and 16S rRNA genes of *Pseudomonas aeruginosa* isolated from Iraqi patients. Res J Pharm Technol 2021;14(5):2517-2521.
25. Saad JM, Farhan MB. Isolation and identification of *Pseudomonas aeruginosa* causing otitis media and investigation of some genes of virulence factors of it in Ramadi city. Turk J Phys Rehabil 2021;32(3):11097-11103.
26. Febriyanti I, Djuminar A, Merdekawati F, *et al.* Comparison of purity and concentration values of *Mycobacterium tuberculosis* DNA extraction result from the boiling and spin column method. Indones J Med Lab Sci Technol 2023;5(2):133-145.