CASE REPORT
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Gene similarity examination *Pseudomonas aeruginosa* in Intensive Care Unit (ICU) at Prof. Dr. dr. I.G.N.G Ngoerah hospital using the ERIC-PCR method: case reports

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ABSTRACT

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen that has the potential to cause severe degrees of healthcare-associated infections, especially in immunocompromised patients who have AIDS, cancer, burns, and respiratory tract infections. Antibiotic resistance genes by genetic elements are increased so that it becomes a worldwide concern and also causes the evolution of resistant strains (MDR) ERIC-PCR technique is a PCR-based method in which the location and number of different ERIC sequences in bacteria are used as genetic markers of bacterial diversity.

Case Presentation: We presented three patients with *Pseudomonas aeruginosa* infection treated in the ICU (Intensive Care Unit) room of Prof. dr. I. G. N. G Ngoerah Hospital. While being treated in the ICU, the three patients' beds were located side by side in one room. Three patients, in this case, reports were treated in the same room where the beds were side by side and experienced a worsening of their condition. After examination, it was found that the bacteria that caused the infection was *Pseudomonas aeruginosa*. However, after conducting molecular analysis using the ERIC-PCR method, there was no gene similarity between the three isolates examined. This might have happened because the patient had been treated in a different room before being admitted to the ICU.

Conclusion: We presented a discrepancy between *Pseudomonas aeruginosa* infection in three patients treated in the same ICU room. However, the ERIC-PCR results found no gene similarity between the three isolates examined.

Keywords: ERIC-PCR, ICU, nosocomial infection, *Pseudomonas aeruginosa*.
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INTRODUCTION

Many opportunistic pathogens cause disease causing high mortality and morbidity. One of the pathogens often used as an opportunistic agent is *Pseudomonas aeruginosa. Pseudomonas aeruginosa* is an opportunistic pathogen that can cause severe healthcare-associated infections, especially in immunocompromised patients with AIDS, cancer, burns, and respiratory tract infections.[1] Burn and the respiratory tract are the most suitable sites for multiplication. The difficulty of administering therapy, especially in *Pseudomonas aeruginosa, is a major concern worldwide* because it has different antibiotic resistance mechanisms.[2]

Antibiotic resistance genes by genetic elements increase, becoming a worldwide concern and causing resistant strains' evolution (MDR).[3] Integron is very important in developing bacterial resistance, especially gram-negative bacteria. Integrons are genetic elements associated with transposons, chromosomes and plasmids.[4] Integrons consist of three important elements, of which three essential regions between the two ends are conserved (5′CS and 3′CS), including *you*, a gene that encodes the integrase enzyme, *acts*, a site recognized by integrase for integrase cassette integration, and *APC* promoter leading to the transcription of the encoded gene.[5,6]

Nosocomial infections occur worldwide, affecting both developed and resource-poor countries. Acquired healthcare-associated infections are the leading cause of death in hospitalized patients, and their prevalence is increasing.[7] *Pseudomonas aeruginosa* is a ubiquitous bacterium considered the fourth most frequently isolated nosocomial pathogen among all nosocomial infections. Widely distributed in the natural environment, it causes serious and widespread nosocomial infections such as urinary tract infections, burns, respiratory tract infections, meningitis, chronic and external otitis media, pseudomonas endocarditis, and sepsis.[7,8] Mortality associated with this pathogen is high, especially in immunocompromised patients, and there is excessive mortality and morbidity associated with ineffective empiric treatment, leading to complications during treatment. The strain type is very important to determine the epidemiology of nosocomial infections and helps

the development of pathogen control methods.[9,10] *Pseudomonas aeruginosa* has been classified based on phenotypic characteristics such as serotype, biotype, bacteriophage type, myosin type, and antimicrobial susceptibility test. For the antibacterial profile, P. aeruginosa. Due to the property of phenotypic plasticity, molecular techniques (inherently more stable than phenotypic traits) have gained popularity for identifying strains and the epidemiological study of many organisms. Molecular techniques such as pulsed-field gel electrophoresis (PFGE) and DNA fingerprinting have recently been used for P. aeruginosa typing.[11]

Pseudomonas aeruginosa, an aerobic and oxidizing gram-negative bacterium, is one of the main causes of nosocomial infections, especially in the intensive care unit (ICU). This bacterium generally does not cause disease but causes opportunistic infections in people with compromised immune systems, such as intensive care patients. It is a ubiquitous common organism, especially in environments such as soil, standing water, sediment, food, and hospital settings. Pathogenic mechanism *Pseudomonas aeruginosa is* caused by the production of several cytotoxic and extracellular factors. aeruginosa includes exotoxin S (exoS), exotoxin U (exoU), exoenzyme A (exoA), secretory protein III, elastase, alkaline protease, and protease IV, each of which has toxic effects on mammals. Cells.[11] This bacterium accounts for approximately 13.5% of Gram-negative infections in the ICU and is the leading cause of ventilator-associated pneumonia in the ICU. The release of these bacteria in the intensive care setting is difficult to control because they are resistant to many antibiotics through several mechanisms.[12] The ERIC-PCR technique is a PCR-based method in which the location and number of different ERIC sequences in bacteria are used as genetic markers of bacterial diversity. Therefore, this study aims to investigate the Gene Similarity *Pseudomonas aeruginosa* obtained by the patient after being treated at the same time and place and whether there was the transmission of the Pseudomonas aeruginosa gene in the intensive care Unit (ICU) Prof. Dr.I.G.N.G Ngoerah Hospital with the ERIC-PCR technique.

CASE PRESENTATION

In a case report study with 3 patients with 3 clinical trial samples, Pseudomonas aeruginosa isolates were identified and confirmed by conventional microbiological and biochemical tests.[13] DNA extraction and PCR were extracted from isolates*Pseudomonas aeruginosa* using a commercial DNA extraction kit (Qiagen, Hilden, Germany). Genomic DNA was extracted from P. aeruginosa isolates using a commercial DNA extraction kit (Qiagen, Hilden, Germany). Virulence genes, including exoA, ExoS and exoU, were detected using specific primers.[13] A total of 3 patient samples were treated in the ICU (Intensive Care Unit) room of Prof.dr.I.G.N.G Ngoerah Hospital. While being treated in the ICU, the three beds of this patient were located side by side; during treatment, the patient experienced a worsening of his condition. Therefore, the treating doctor did a culture examination. Examine culture by obtaining the results of the same bacterial culture, namely bacteria *Pseudomonas aeruginosa*. Thus, an ERIC-PCR examination was carried out to determine the similarity of genes in the bacteria *Pseudomonas aeruginosa.* This technique was performed in thermal circulation (Bio-Rad, Inc. USA) using the primers ERIC (F): 5′-ATG TAA GCT CCT GGG GAT TCAC-3′ and ERIC (R): 5′-AAG TAA GTG ACT GGG GTG AGC G3′ (Pishgam Biotech Co., Iran) according to the following procedure: initial denaturation (94°C for 5 minutes) followed by 0 cycles of denaturation (91°C for 1 minute), annealing (25°C for 2 minutes), elongation (72°C for 2 min), and a final elongation cycle at 72°C for 5 min. The PCR products were loaded onto a 2% agarose gel (Sigma-Aldrich) at 70 V for 1 hour, and the band patterns were visible under ultraviolet light.[13]

The ERIC sample was analyzed using an online data analysis service (inslico. ehu.es). The ERIC profile was compared using the Dice method and synthesized with the UPGMA program.

Case 1

In case 1, a woman with the initials IWN, 56 years old, the patient was treated at Prof. Dr. dr. I.G.N.G Ngoerah General Hospital diagnosed with Adenocarcinoma of the right lung empyema. The patient was treated in Ratna's room when he first came to the hospital. The patient entered with complaints of shortness of breath and was given ceftriaxone therapy by the treating doctor. On chest x-ray examination of the lungs, there was a picture of pneumonia and bilateral pleural effusion. In the treatment in the ratna room, there was a clinical deterioration in the patient Shiengga on July 2, 2021. The patient was transferred to the ICU (Intensive Care Unit) room, a thoracotomy was performed, and a ventilator was installed to help the patient's breathing. During treatment in the ICU, the patient was examined for a Sputum exit culture site on July 6, 2017, in Prof. Dr. dr Microbiology's laboratory. I.G.N.G Ngoerah. On July 9, 2017, there were isolated results of pseudomonas bacteria *Multidrug Resistant* Organism against all the antibiotics tested, including Piperacillin/Tazobactam = Resistant, Cefazolin = Resistant, Cefuroxime = Resistant, Ceftazidime = Resistant, Cefepime = Resistant, Cefixime = Resistant, Aztreonam = Intermediate, Meropenem = Resistant, Amikacin = Resistant, Gentamicin = Resistant, Ciprofloxacin = Resistant, Levofloxacin = Resistant, Tigecycline = Resistant).

Case 2

In case 2, a man with the initials FFM, 46 years old, the patient was treated at Prof I.G.N.G Ngoerah General Hospital with a suspected diagnosis of GBS on April 20, 2021. When he first arrived, the patient was admitted to the Ratna room, complaining of weakness in both legs. On April 24, 2021, the patient experienced a worsening condition and was transferred to the ICU (Intensive Care Unit) room. The patient's condition at that time was DOC with a ventilator as his breathing assistance. On May 25, the patient underwent a craniotomy due to a worsening of the patient's condition. The doctor treating the patient conducted a tube sputum culture examination on June 3, 2021, which was sent to the Clinical Microbiology Laboratory of Prof I.G.N.G Ngoerah Hospital. On June 5, 2021, the results of the culture examination came out with the outcomes identified as bacteria *Pseudomonas aeruginosa*,

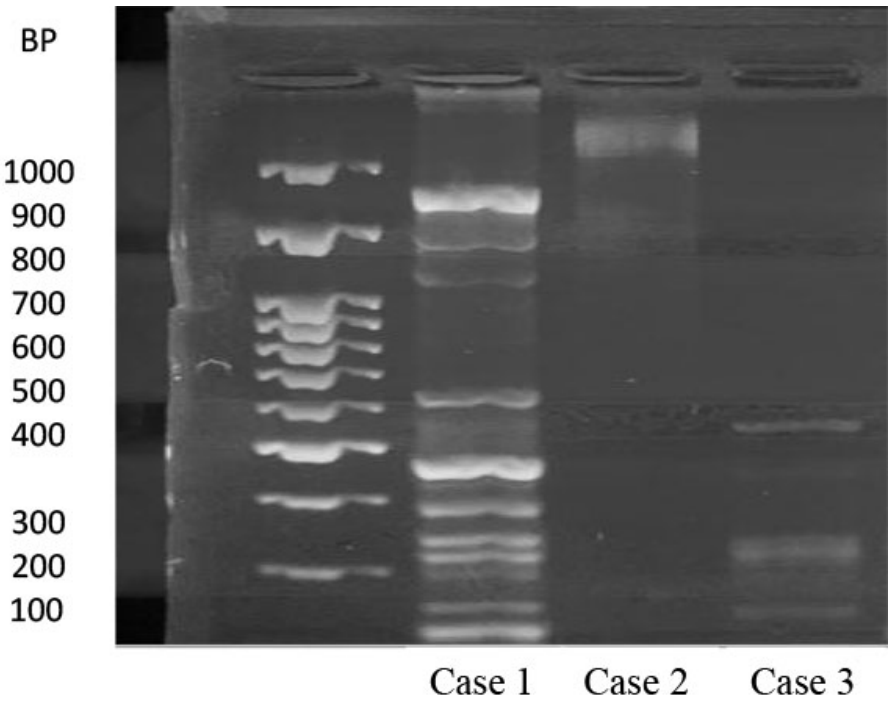

Figure 1. Molecular analysis of the analysis using the ERIC-PCR method on the three samples.

which is *Multidrug Resistant* Organism against all the antibiotics tested, including Piperacillin/Tazobactam = Resistant, Cefazolin = Resistant, Cefuroxime = Resistant, Ceftazidime = Resistant, Cefepime = Resistant, Aztreonam = Intermediate, Meropenem = Resistant, Amikacin = Resistant, Gentamicin = Resistant, Ciprofloxacin = Resistant, Levofloxacin = Resistant, Tigecycline = Resistant).

Case 3

In case 3, a 50-year-old man with the initials IKGA, the patient came to the Emergency Room at Prof I.G.N.G Ngoerah Hospital with reduced consciousness on May 24, 2021; the patient was diagnosed with Covid-19. The patient was treated in the Mawar room on May 25, 2021, and on May 28 the patient was transferred to the Awar room. On June 9, 2021, the patient experienced a worsening of his condition where the patient experienced Sepsis and DOC due to metabolic encephalopathy. The treating doctor conducted a tube sputum culture examination on July 15, 2021, at the Clinical Microbiology laboratory. On June 18, 2021, the results of the culture examination came out with bacteria identified *as Pseudomonas aeruginosa*, which is *Multidrug Resistant* Organism against all the antibiotics tested, including Piperacillin/Tazobactam = Resistant, Cefazolin = Resistant, Cefuroxime = Resistant, Ceftazidime = Resistant, Cefepime = Resistant, Aztreonam = Intermediate, Meropenem = Sensitive, Amikacin = Sensitive, Gentamicin = Sensitive, Ciprofloxacin = Resistant, Levofloxacin = Resistant, Tigecycline = Resistant).

In the three cases above, the three patients were treated in the same room with adjoining beds and experienced a worsening condition. Therefore, the doctor treating the patient carried out a culture examination to find out the agent causing the infection in the patient. After examination, it was found that the bacteria that caused the infection was: *Pseudomonas aeruginosa* in these three patients. However, after conducting molecular analysis using the ERIC-PCR method, there was no gene similarity between the three isolates examined, as seen in Figure 1.

DISCUSSION

Pseudomonas aeruginosa, an aerobic and oxidizing gram-negative bacterium, is one of the main causes of nosocomial infections, especially in the intensive care unit (ICU). These bacteria generally do not cause disease but do cause opportunistic infections in people with weakened immune systems, such as intensive care patients.[12,14] In examining these three samples, it was found that there were differences in the genes in each sample which were examined in the clinical microbiology laboratory at Prof. Dr. I. G. N. G Ngoerah Hospital. In the case of 1 woman with IWN aged 56 years, the patient was treated at Prof. Dr. dr. I.G.N.G Ngoerah General Hospital diagnosed with Adenocarcinoma of the right lung empyema. The patient was treated in Ratna's room when he first came to the hospital. The patient entered with complaints of shortness of breath and was given ceftriaxone therapy by the treating doctor. On chest x-ray examination of the lungs, there was a picture of pneumonia and bilateral pleural effusion. In the treatment at the Ratna Room, there was a clinical deterioration in the patient. On July 2, 2021, the patient was transferred to the ICU (Intensive Care Unit) room, a thoracotomy was performed, and a ventilator was installed to help the patient breathe. In

Case 2, a man with the initials FFM, 46 years old, the patient was treated at Prof I.G.N.G Ngoerah General Hospital with a suspected diagnosis of GBS on April 20, 2021. When he arrived, the patient was admitted to the Ratna room, complaining of limb weakness. On April 24, 2021, the patient experienced a worsening condition and was transferred to the ICU (Intensive Care Unit) room. The patient's condition at that time was DOC with a ventilator as his breathing assistance. On May 25, the patient underwent a craniotomy due to worsening the patient's condition. In case 3, a 50-year-old man with the initials IKGA, the patient came to the Emergency Room at Prof I.G.N.G Ngoerah Hospital with reduced consciousness on May 24, 2021; the patient was diagnosed with Covid-19. The patient was treated in the Mawar room on May 25, 2021; on May 28, the patient was transferred to the Awar room. On June 9, 2021, the patient experienced a worsening condition where the patient experienced Sepsis and DOC

ec metabolic encephalopathy. The treating doctor conducted a tube sputum culture examination on July 15, 2021, at the Clinical Microbiology laboratory.

After examination, it was found that the bacteria that caused the infection was: *Pseudomonas aeruginosa* in these three patients. However, after conducting molecular analysis using the ERIC-PCR method, there was no gene similarity between the three isolates examined. This might have happened because the patient had been treated in a different room before being admitted to the ICU. It is known that Pseudomonas aeruginosa is an environmental bacterium and one of the nosocomial pathogens.[15,16]

CONCLUSION

We presented three cases of *Pseudomonas aeruginosa* infection in patients treated in ICU with beds located side by side in one room. However, after conducting molecular analysis using the ERIC-PCR method, there was no gene similarity between the three isolates examined. This might have happened because the patient had been treated in a different room before being admitted to the ICU.

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

ETHICAL CONSIDERATION

This case report was obtained from the patient's family and conducted based on the ethical conduct of research from the Ethics Committee of the Medical Faculty, Udayana University and Prof. Dr. dr. I.G.N.G Ngoerah Hospital.

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AUTHOR CONTRIBUTION

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study's results were interpreted upon publication.

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