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DETECTION OF GENE ALG8 AND ALG 44 IN CLINICAL ISOLATES PSEUDOMONAS AERUGINOSA USING PLYMERASE CHAIN REACTION METHOD

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ABSTRACT

The *alg8* and *alg44* genes are one of the genes that control alginate production in *Pseudomonas aeruginosa* bacteria, these genes are one of the main virulence factors causing chronic infections in the human body. *Pseudomonas aeruginosa* is a bacterium that causes infections in several cases in various parts of the body. The purpose of this study was to detect the presence of *alg8* and *alg44* genes in several isolates of *Pseudomonas aeruginosa* from several clinical samples (urine, sputum, and pus) using the Polymerase Chains Reaction method. The study was initiated by characterizing and purification of 6 isolates of *Pseudomonas aeruginosa* from urine, sputum, and pus samples (2 isolates each), identification of isolates was carried out by biochemical tests. Bacterial DNA isolation was carried out using the DNeasy Blood and Tissue Kit, the results of the isolation were tested by electrophoresis. Six samples of *Pseudomonas aeruginosa* DNA were tested for the presence of *alg8* and *alg44* genes by PCR method. The primary design was carried out using the website https://www.ncbi.nlm.nih.gov. The *alg8* gene as a whole consists of 1214 nitrogenous bases, the primer used produces an amplicon of 882 bp (72.6%), alg44 gene consists of 818bp, the primer used amplifies 316 bp (36%). *alg8* and *alg44* genes were found in all isolates of *Pseudomonas aeruginosa*.

Keywords: *alg8*; *alg44*; *pdomonas aeruginosa*; pcr

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INTRODUCTION

Pseudomonas aeruginosa is an opportunistic bacterial pathogen that is the main cause of nosocomial infections such as pneumonia, urinary tract infections (UTI), infections of various organs, and bacteremia (Jurado-Martín et al., 2021), especially in patients who have a weakened immune system (Martínez-Ortiz et al., 2020). P. aeruginosa has very unique properties, under certain conditions it forms biofilms on body tissues, colonizing to form complex and specific micro-niches (Brandenburg et al., 2019). These conditions cause the bacteria to change in character, making the infection difficult to treat (Vetrivel et al., 2021).

Alginate is an extracellular component of Pseudomnas aeruginosa that acts as a virulence factor and extracellular matrix, which protects bacterial cells from interference by external factors (Whitney et al., 2011). Wolska et al., 2012 stated that alginate is the main component of biofilms. The presence of biofilms in bacterial cells contributes to bacterial resistance to antibiotics (Wahyudi et al., 2019)

Alginate is composed of several genes that work continuously (Valentine et al., 2020), Romero et al., 2022 mentions that the genes that regulate the main alginate coordination are alg44 and alg8. Rocha et al., 2019 stated that there was a relationship between the presence of alginate and resistance to antimicrobial agents. Rekadwad et al., 2019 wrote that several genes that make up the extracellular matrix (one of which is alginate) contribute to virulence factors and resistance to antibiotics.

Polymerase Chain Reaction (PCR) is an examination method that is known to be effective for detecting the presence of genetic material from cells, bacteria, or viruses. The genetic material in each cell can be either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid). PCR is a method that is quite simple and easy to do to detect genes in a microorganism (Muhammadi & Shafiq, 2019). Detection of alg genes as genes controlling alginate production in Pseudomonas aeruginosa by PCR is an effective method and has been referred by many researchers (Wahyudi et al., 2019, Dimitriou, 2020 and Davarzani et al., 2021). The purpose of this study was to detect alg8 and alg44 genes against several clinical isolates of Pseudomonas aeruginosa isolated from patient samples at the hospital.

METHOD

Pseudomonas aeruginosa was obtained from clinical samples (urine, sputum and pus), identification and identification was carried out by Gram staining and Biochemical Test (Franco-Duarte et al., 2019). DNA isolation was carried out using the DNeasy Blood and Tissue Kit. Gene detection was carried out using the Polymerase Chain Reaction method (Jurado-Martín et al., 2021). The primers used in this study were the following alg8 gene: Forward GTC TAT CGC TCG GTG ATC CG, Reverse CAG GTA CAC CAG CAG GAA GG, R- (5'----3,), alg44 gene, with primer: forward AGT TCC AGA ACC TCA AGC CG, and reverse ATG GTG ATC TGC TGG TTG GG (Schoch et al., 2020). PCR products were visualized using electrophoresis (Liu et al., 2020; Muhammadi & Shafiq, 2019). The population of this study was Pseudomonas aeruginosa isolates. The samples of this study were 6 isolates of Pseudomonas aeruginosa, consisting of 2 isolates from urine samples, 2 isolates from sputum samples, and 2 isolates from pus samples. Research variables are as follows the dependent variable is the presence of alg8 and alg44 genes in clinical samples which were detected by the Polymerase Chain Reaction method. The independent variable was the type of clinical sample (urine, sputum and pus) from which Pseudomonas aeruginosa was isolated.

RESULTS

Results Identification and phenetic characterization of 6 clinical isolates (Pa.01, Pa.02.Pa.03, Pa.04, Pa.05 and Pa.06) derived from various clinical samples of hospital patients (urine, sputum, pus) were P. aeruginosa, a bacterium with shiny circular colonies, gram-negative rods, no spores, and single flagella, was able to grow at a temperature of 25° - 42° C with specific characteristics (Figure 1). The results of the isolation of P. aeruginosa from 6 clinical samples came from various cases of infection and different types of samples, namely: urine samples (Pa.01 and Pa.02), sputum samples (Pa.03 and Pa.04), pus (Pa. Pa.05 and Pa.06).

A B

Figure 1. Overview of P. aeruginosa colonies on Trypticase agar (A). Microscopic view of P. aeruginosa by Gram stain, Microscopic magnification (B).

The results of the biochemical test of 6 isolates of Pseudomonas aeruginosa had the same characteristics, fermentation in alkaline/alkali TSIA media, all results of the biochemical test were negative except for citrate and motile on SIM media (Table 1).

Table 1.
Biochemical Test Results of Pseudomonas aeruginosa . isolates

Kode	TSIA			SIM			Urea	Citrat	MR	VP	Fermantasi Karbohidrat			
	ferm	H2S	Gas	Indol	H2S	Motil					Glu	Man	Mal	Lakt
Pa.01	Alk/Alk	-	-	-	-	+	-	+	-	-	-	-	-	-
Pa.02	Alk/Alk	-	-	-	-	+	-	+	-	-	-	-	-	-
Pa.03	Alk/Alk	-	-	-	-	+	-	+	-	-	-	-	-	-
Pa.04	Alk/Alk	-	-	-	-	+	-	+	-	-	-	-	-	-
Pa.05	Alk/Alk	-	-	-	-	+	-	+	-	-	-	-	-	-
Pa.06	Alk/Alk	-	-	-	-	+	-	+	-	-	-	-	-	-

The results of DNA isolation using the Qiagen DNeasy Blood and Tissue Kit reagent showed the presence of DNA in the isolates examined (Figure 2). indicated by the appearance of bands on the visualization results by electrophoresis

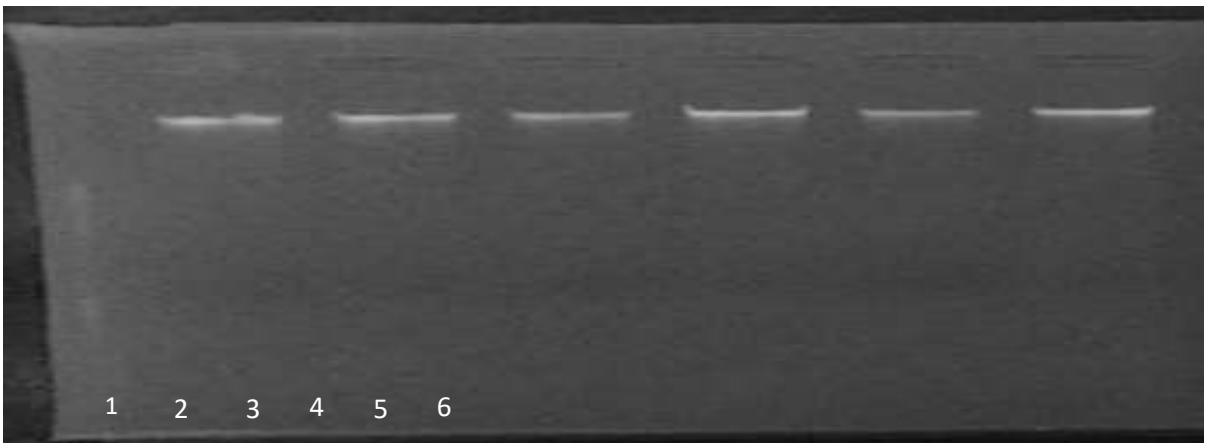

Figure 2. The results of DNA isolation of Pseudomonas aeruginosa isolates. Note: 1= Pa01, 2= Pa02, 3= Pa03, 4=Pa04, 5=Pa05, 6=Pa06

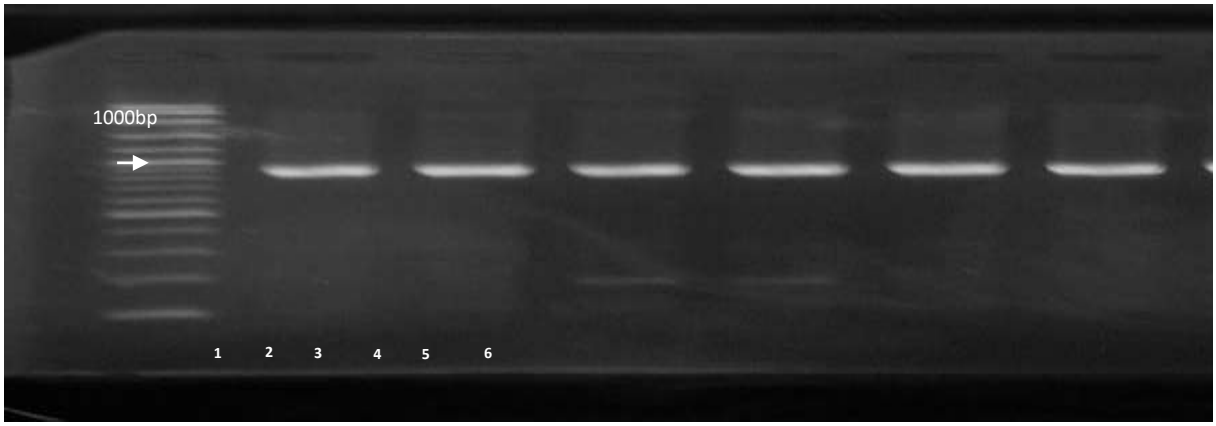

Figure 3. Visualization of the alg8 gene PCR product on clinical isolates of Pseduomonas aeruginosa by electrophoresis. Note: 1= Pa01, 2= Pa02, 3= Pa03, 4=Pa04, 5=Pa05, 6=Pa06

The results of primary visualization of the alg44 gene showed that the gene was found in all isolates of Pseudomonas aeruginosa both in urine, sputum and pus samples. This gene triggers an increase in n-mannose rich. The alg44 gene-specific primer produced a 316bp PCR product (Figure 4).

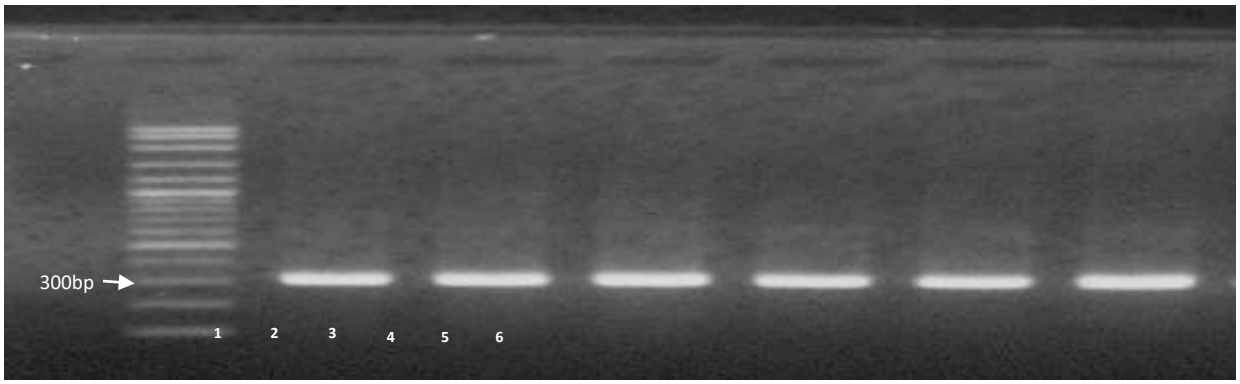

Figure 4. Visualization of the PCR product of the alg44 gene on clinical isolates of Pseduomonas aeruginosa by electrophoresis. Note: 1= Pa01, 2= Pa02, 3= Pa03, 4=Pa04, 5=Pa05, 6=Pa06

DISCUSSION

All clinical isolates of P. aeruginosa in this study had the same colony morphology, namely on solid TSA media they formed S-type (smooth) colonies, irregular, some isolates formed pale green pigments and some were bluish green. The results of Gram staining showed that P. aeruginosa isolates were Gram negative, red rod-shaped. All isolates of P. aeruginosa grew on liquid trypticase media to form a biofilm on the surface of the medium. Several isolates grew on agar and liquid media after incubation for 24-48 hours at 37°C to form a blue-green pigment (pyocyanin) with varying intensity (Figure 1).

P. aeruginosa isolates from different samples or specimens had different biochemical, enzymatic and antimicrobial susceptibility activities (Dimitriou, 2020; Romero et al., 2022). P. aeruginosa culture has a specific aroma, smells like wine, because it produces aminoacetophenone (Liu et al., 2020; Rocha et al., 2019). These bacteria do not actively ferment all carbohydrates, produce only acid, but do not release gas, do not ferment lactose; have oxidase and catalase.

The results of primary visualization of the alg8 gene showed that the gene was found in all P. aeruginosa isolates both in urine, sputum and pus samples. This gene triggers an increase in n-mannose rich. The specific primer alg8 gene produces 882bp PCR product (Figure 3) Alg8 cooperates systemically with other genes in forming alginate in P. aeruginosa. The specific primer alg8 gene produced a PCR product of 882bp, is a gene that controls the production of alginate polysaccharides, which is one of the components of the extracellular matrix, this gene triggers an increase in n-glucose rich. In general, there was no variation in the presence of pelD from the existing isolates, so the presence of this gene could not be used as an indicator of the strength and weakness of biofilm synthesis, especially in P. aeruginosa.

The alg44 gene as a whole consists of 818bp (Schoch et al., 2020), the primer used for identification can amplify 316 bp, 36% of the total length of the alg44 gene. Alginate is an exopolysaccharide that is frequently and mainly produced by clinical isolates of P. aeruginosa from the lungs of Cistic Fibrosis (CF) patients (Martínez-Ortiz et al., 2020; Powell et al., 2018; Rocha et al., 2019). The characteristic mucoid phenotype is due to the overproduction of this polysaccharide, which protects P. aeruginosa from the harsh environment in the CF lung by providing an extracellular matrix within the biofilm. However, it is not absolutely necessary during the formation of non-mucoid biofilms in vitro (Pournajaf et al., 2018; Romero et al., 2022). Alginate has an important role in maintaining structural stability and biofilm protection of the immune system in the human body. It is necessary for water and nutrient retention in biofilms (Jurado-Martín et al., 2021; Protects, 2020). Mucoid P. aeruginosa also uses Psl to form biofilms (Romero et al., 2022; Vetrivel et al., 2021). Alginate has been identified to have a function in the protection of the immune system (Valentine et al., 2020). Increased alginate production can provide resistance to antibiotics as well as phagocytosis (Powell et al., 2018; Rekadwad et al., 2019). Alginate also has the ability to bind free radicals released from neutrophils and activate macrophages in vitro which are usually used to kill pathogens (Davarzani et al., 2021).

The results showed that from 6 clinical isolates of Pseudomonas aeruginosa¸both from urine samples (Pa.01 and Pa.02), Sputum samples (Pa.03 and Pa.04), and Pus samples (Pa.05 and Pa.06) were detected alg8 and alg44 genes. The presence of these two genes could not be influenced by the type of clinical sample from which the bacteria were isolated. So it is necessary to identify other genes or measure the expression of the two genes, whether they are different if measured from different types of clinical samples. However, further information is also needed regarding the characteristics of the isolate related to its sensitivity to antibiotics and the ability to form biofilms, this is because alginate is an extracellular product that contributes to virulence factors, biofilm formation, so that it affects its sensitivity to antibiotics. The existence of the alg8 and alg44 genes cannot be separated from the regulatory system, this is because the two genes work continuously, and control alginate production, further research is needed on the characteristics of these two genes in Pseudomonas aeruginosa isolates based on their level of pathogenicity or their ability to form biofilms.

CONCLUSION

The results showed that the alg8 and alg44 genes were found in all isolates examined by the polymerase chain reaction method, namely Pseudomonas aeruginosa isolated from pure samples, sputum, and pus.

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