KARAKTERISASI ISOLAT KLINIK *Pseudomonas aeruginosa* PEMBENTUK BIOFILM DAN FAKTOR-FAKTOR PEMBENTUKAN BIOFILM

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INTISARI

Pseudomonas aeruginosa merupakan bakteri patogen oportunistik, penyebab infeksi nosokomial, seperti pneumonia, infeksi saluran kemih (ISK) serta bakteremia. Bakteri tersebut sangat unik karena mampu membentuk biofilm dengan karakteristik fenotipik dan biokimia yang spesifik, dan menyebabkan infeksi berbasis biofilm yang sulit diobati. Biofilm *P. aeruginosa* berkembang pada infeksi kronis dan sangat terkait dengan persistensi dan resistensi terhadap antibiotik. Penelitian ini bertujuan untuk melakukan karakterisasi isolat klinis *P. aeruginosa* berdasarkan kemampuan membentuk biofilm dan kepekaannya terhadap berbagai antibiotik, mengkaji pengaruh faktor lingkungan (komposisi media kultur, suhu inkubasi, pH media, dan antibiotik) terhadap pembentukan biofilm *P. aeruginosa*, dan mendeteksi gen yang mengendalikan produksi biofilm. Isolat *P. aeruginosa* diperoleh dari Rumah Sakit, berasal dari berbagai jenis sampel klinis antara lain; darah, sputum, urin, cairan telinga, cairan pleura, pus, *feces*, dan cairan jaringan. Isolat bakteri dipurifikasi menggunakan teknik koloni sel tunggal. Karakterisasi kultur murni isolat dikerjakan berdasarkan kepekaan terhadap antibiotik dengan metode pelarutan menggunakan *Vitek 2 Compact*. Uji kemampuan isolat dalam membentuk biofilm dilakukan dengan menumbuhkannya pada medium cair *trypticase* selama 24 jam, massa biofilm yang terbentuk diukur dengan *microtiter plate culture technique*, secara spektrofotometri (λ 570 nm) dengan *microplate reader*, isolat diseleksi berdasarkan 3 kriteria pembentukan biofilm (lemah, sedang, dan kuat), Kelompok isolat (lemah, sedang, dan kuat) terpilih (masing–masing 8 isolat yang representatif) diuji lebih lanjut terhadap faktor lingkungan yang mempengaruhi pembentukan biofilm. Uji Pengaruh faktor lingkungan dilakukan melalui percobaan kultivasi pada medium *trypticase* cair dengan sumber C (glukosa, mannosa) sumber N (lisin, tryptophan) dengan konsentrasi, suhu inkubasi, dan pH media berbeda. Biofilm yang terbentuk diamati dengan pewarnaan crystal violet 0,1%. Pengaruh antibiotik (ciprofloxacin dan amikacin) konsentrasi 1, 2, 4, 8. 16. 32. 64. dan 128 µg/ml ditentukan dengan menggunakan *3-(4,5-dimethythiazol-2-yl)-2, 5- diphenyl tetrazolium bromide* (MTT). Deteksi gen pengendali biofilm dilakukan dengan *Polymerase Chain Reaction* (PCR), dilanjutkan *sequensing*. Hasil Penelitian menunjukkan bahwa 64 isolat *P. aeruginosa* memiliki bentuk batang berukuran 0,5-0,8 µm x 1,5-3,0 µm, Gram negatif. Beberapa isolat (72%) sensitif terhadap piperasilin, ceftazidime, cefepime, oztreonam, meropenem, amikacin, gentamicin, dan ciprofloxacin. Berdasarkan kemampuan pembentukan biofilm, isolat *P. aeruginosa* meliputi tiga kriteria: kelompok pembentuk biofilm lemah (14 isolat), kelompok pembentuk biofilm

sedang (32 isolat), dan kelompok pembentuk biofilm kuat (18 isolat). Penambahan glukosa pada konsentrasi 0,3mM dan manosa pada konsentrasi 0,5 mM selama 24 jam telah memacu produksi biofilm, baik pada kelompok biofilm yang lemah, sedang, maupun yang kuat; sedangkan penambahan lisin tidak mempengaruhi pembentukan biofilm, dan tryptophan menghambat pembentukan biofilm. Semua isolat *P. aeruginosa* mampu membentuk biofilm pada suhu 28^{o} dan 37^{o}C, sedangkan pada suhu 40^{o}C pembentukan biofilm lamban. Pembentukan biofilm terjadi dengan cepat pada pH media 7,0 dan 9,0; dan sangat lamban pada pH 5,0. Amikacin (16µg/ml) dan ciprofloxacin (64µg/ml) mampu menghambat sel *P. aeruginosa* dalam biofilm sebesar 50% (MBIC 50). Gen *pslA, alg44,* dan *pelD* terdapat pada semua kelompok isolat (kuat, sedang, dan lemah). Kesimpulan, isolat klinik *P. aeruginosa* memiliki kepekaan yang berbeda-beda terhadap antibiotik, dan memiliki kemampuan yang bervariasi dalam membentuk biofilm. Pembentukan biofilm dipengaruhi oleh komposisi media kultur, suhu inkubasi, pH media, dan antibiotik. Semua isolat tedeteksi adanya gen *pslA*, *alg44*, dan *pelD.* Pada kondisi planktonik, isolat yang lebih kuat dalam membentuk biofilm lebih resisten terhadap antibiotik, dan ketika berada di dalam biofilm isolat dengan kemampun lemah, sedang, dan kuat dalam membentuk biofilm memiliki kemampuan bertahan hidup yang sama ketika terpapar dengan antibiotik.

Kata kunci*: P. aeruginosa, biofilm, medium, suhu, pH, antibiotik, gen.*

CHARACTERIZATION OF BIOFILM-FORMING *Pseudomonas aeruginosa* CLINICAL ISOLATES AND FACTORS INFLUENCING BIOFILM FORMATION

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogenic bacterium, causing nosocomial infections, such as pneumonia, urinary tract infections (UTI) and bacteremia. These bacteria are very unique because they are able to form biofilms with specific phenotypic and biochemical characteristics, causing biofilm-based infections that are difficult to treat. *P. aeruginosa* biofilms developed in chronic infections and were strongly associated with persistence and resistance to antibiotics. The aims of the study were to characterize *P. aeruginosa* clinical isolates based on their ability to form biofilms and their sensitivity to various antibiotics, to assess the influence of environmental factors (culture media composition, incubation temperature, media pH, and antibiotics) on the formation of *P. aeruginosa* biofilms, and to detect genes that controlling biofilm production. *P. aeruginosa* isolates were obtained from the hospital, and originated from various types of clinical samples, among others; blood, sputum, urine, ear fluid, pleural fluid, pus, feces, and tissue fluid. Bacterial isolates were purified using a single cell colony technique. Identification of pure culture of isolates was carried out based on sensitivity to antibiotics through the dissolution method using *Vitek 2 Compact.* The biofilm formation ability of pure isolates culture was done by growing isolates on trypticase liquid medium for 24 hours, the mass of biofilms formed was measured by microtiter plate culture technique, spectrophotometrically (λ 570 nm) with a microplate reader, isolates were selected based on 3 biofilm formation criteria (weak, moderate , and strong), selected isolates (weak, medium, and strong) groups (each of 8 representative isolates) were further tested for environmental factors affecting biofilm formation. The test of the influence of environmental factors was carried out through cultivation experiments on liquid trypticase medium with a C source (glucose, mannose) N source (lysine, tryptophan) with different concentrations, incubation temperatures, and pH. Biofilms formed were observed with 0.1% crystal violet staining. The effect of antibiotics (ciprofloxacin and amikacin) concentrations of 1, 2, 4, 8. 16. 32. 64. and 128 µg / ml was determined using 3- (4,5-*dimethythiazol-2-yl) -2, 5- diphenyl tetrazolium Bromide* (MTT). Detection of biofilm controlling genes is done by Polymerase Chain Reaction (PCR), followed by sequensing. The results showed that 64 *P. aeruginosa* isolates had rod shapes of 0.5-0.8 µm x 1.5-3.0 µm, Gram negative. Some isolates (72%) were sensitive to piperacillin, ceftazidime, cefepime, oztreonam, meropenem, amikacin, gentamicin, and ciprofloxacin. Based on the ability of biofilm formation, *P. aeruginosa* isolates included three criteria: weak biofilm forming groups (14 isolates), moderate biofilm forming groups (32 isolates), and strong biofilm

forming groups (18 isolates). The addition of glucose at a concentration of 0.3 mM and manosa at a concentration of 0.5 mM for 24 hours had stimulated biofilm production, in the weak, moderate, or strong biofilm group; whereas the addition of lysine did not affect the formation of biofilms, and tryptophan inhibited the formation of biofilms. All of *P. aeruginosa* isolates were able to form biofilms at 28^{o} and 37^{o}C, but at 40^{o}C the formation of biofilms was slow. Biofilm formation occurs rapidly at pH media 7.0 and 9.0; and very slow at pH 5.0. Amikacin (16 µg / ml) and ciprofloxacin (64 µg / ml) were able to inhibit P. aeruginosa cells in biofilms by 50% (MBIC 50). *pslA, alg44*, and *pelD* genes were present in all isolate groups (strong, moderate, and weak). Conclusion, *P. aeruginosa* clinical isolates had different sensitivity to antibiotics, and had varying abilities in forming biofilms. The formation of biofilms was influenced by the composition of culture media, incubation temperature, pH of the media, and antibiotics. All isolates detected the *pslA, alg44*, and *pelD genes*. In planktonic conditions, isolates that are stronger in forming biofilms are more resistant to antibiotics, and when in biofilms, isolates with weak, moderate, and strong abilities had the same survival ability when exposed to antibiotics.

Keywords: *P. aeruginosa*, biofilm, medium, temperature, pH, antibiotic, genes.