Karakterisasi molekuler gen resistensi DHPS (Dihidropteroat Sintase) dan Polimorfisme gen mtLSU (Mitochondrial Large Subunit) Pneumocystis jirovecii pada ODHA terduga pneumonia di Jakarta = Molecular resistant characterization of DHPS (Dihydropteroate Synthase) and Polymorphism of mtLSU (Mitochondrial Large Subunit) gene in Pneumocystis jirovecii from HIV-AIDS suspected.

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Abstrak

Latar belakang. Meningkatnya kasus HIV-AIDS human immunodeficiency virus-acquired immunodeficiency syndrome secara global memicu kewaspadaan akan peningkatan infeksi oportunistik, salah satunya infeksi Pneumocystis jirovecii yang mengakibatkan pneumonia PjP. Infeksi PjP merupakan kasus yang sulit ditangani terkait rendahnya sensitivitas uji diagnostik diiringi dengan peningkatan kasus resistensi terhadap antibiotik. Di Indonesia belum terdapat data demografis, epidemiologi molekuler maupun data resistensi mengenai kasus infeksi PjP. Mengantisipasi masalah tersebut, dalam penelitian ini dikembangkan uji diagnostik PjP pada ODHA Orang Dengan HIV-AIDS terduga pneumonia melalui pendekatan molekular terhadap gen MSG Major Surface Glycoprotein disertai dengan karakterisasi gen DHPS dihidropteroat sintase dan gen mtLSU mitochondrial large subunit yang berkorelasi dengan genotipe resisten dan virulensi P. jirovecii.
Tujuan penelitian. Memperoleh suatu uji deteksi infeksi PjP, data genotipe resistensi dan virulensi PjP melalui pendekatan secara molekuler yang dapat dimanfaatkan sebagai dasar data demografi dan epidemiologi molekuler PjP di Indonesia.
Metode penelitian. Pengembangan uji diagnosis molekuler PjP terhadap gen MSG dilakukan dengan metode real- time PCR yang diujikan terhadap 100 sampel sputum. Pola genotipe resistensi dilakukan melalui amplifikasi gen DHPS dilanjutkan dengan restriction fragment length polymorphism RFLP . Virulensi daerah hot spot gen mtLSU dianalisis dengan metode PCR dan sekuensing DNA.
Hasil. Secara demografi, diketahui prevalensi PjP pada ODHA terduga pneumonia di Jakarta mencapai 20,0, laki-laki 75, rentang usia terbanyak 31-40 tahun 35, dominan 80 pada kisaran sel limfosit T CD4 200-349 sel/L. Sebanyak 12 pasien menunjukkan gen DHPS positif, lima pasien 41,66 merupakan genotipe wild type WT dan 7 pasien lainnya 58,32 merupakan genotipe resisten, terdiri dari 16,67 genotipe-3 dan 41,66 genotipe campuran WT dan genotipe 1. Analisis virulensi berdasarkan gen mtLSU diperoleh 30 strain PjP positif yang didominasi oleh variasi-3. Status imun pasien lebih berkaitan dengan genotipe resistensi dibandingkan dengan jenis varian.
Kesimpulan. Uji real-time PCR yang dikembangkan mampu memberikan nilai diagnostik yang lebih baik dibandingkan pewarnaan Giemsa. Terdapat 3 genotipe gen resistensi WT, genotipe 1 dan 3 dan 7 varian P. jirovecii yang bersirkulasi di Jakarta. Genotipe resistensi lebih berkaitan terhadap kondisi klinis pasien dibandingkan dengan jenis varian.
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Background. The global rise of HIV-AIDS cases increase the alertness against oportunistic infections, one of them is Pneumocystic jirovecii pneumonia PjP. PjP infection is a one of a tough infection to be cured due

to low sensitivity of its diagnostic method following the escalation of PjP resistance against antibiotics. There is no demografic, molecular epidemiology nor antibiotics resistance data were available related to PjP infection in Indonesia. Thus, this study was conducted to develop a molecular test to diagnose PjP infection in HIV-AIDS suspected pneumonia patients based on MSG Major Surface Glycoprotein gene detection, followed by characterization of DHPS dihydropteroat syinthetase and mtLSU mitochondrial large subunit genes represent genoype resistance and P. jirovecii virulence.

Research objective. To obtain a molecular test in diagnosing PjP infection and information of P. jirovecii genotype resistance and virulence based on molecular characteristics, which can be used further as demographic and molecular epidemiology basis data of PjP in Indonesia. Research methods. Molecular diagnostic test aimed for MSG gene of P. jirovecii detection was done through real-time PCR against 100 sputum samples. Genotype resistance and P. jirovecii polymorphism patterns was done through DHPS and mtLSU genes amplification followed by restriction fragment length polymorphism RFLP and DNA sequencing analysis. Virulence of the hot spot area are of the mtLSU gene was analyzed by PCR method and DNA sequencing.

Results. The prevalence of PjP infection in HIV-AIDS suspected pneumonia patients in Jakarta was 20.0, male 75 within 31-40 y.o 35, dominant 80 from patients with CD4 T-lymphocytes of 200-349 cells/L. Molecular real-time PCR methods give five times sensitivity higher than Giemsa stain. Twelve patients showed positive DHPS gene, five patients 41.67 were wild type WT genotypes and 7 other patients 58.32 were resistant genotypes, with 16.66 was genotype-3 and other 41.66 was mixed genotypes WT and genotype 1. Virulence analysis based on mtLSU gene show 30 positive strains which dominated by variant-3. The patients immune status is more related to the resistance genotype compared to the variant type.

Conclusion. The developed real-time PCR method is proven to able to give better diagnostic value than Giemsa stain. There are 3 genotypes of resistance genes WT, genotypes 1 and 3 and 7 variants of P. jirovecii circulating in Jakarta. Resistance genotypes are more related to the clinical condition of patients compared to variant types.