Optimasi uji multiplex real time PCR untuk deteksi bakteri atipik pada sputum pasien Community Acquired Pneumonia (CAP) = Optimization of multiplex real time PCR to detect atipycal bacteria in sputum of patients with Community Acquired Pneumonia (CAP)

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Abstrak

Latar Belakang: Community Acquired Pneumonia (CAP) merupakan salah satu penyebab utama morbiditas dan mortalitas di dunia. Bakteri atipikal (Chlamydia pneumoniae, Mycoplasma pneumaniae, Legionella pneumophila) sebagai penyebab penting CAP. Sejauh ini belum ada pemeriksaan mikrobiologi yang rutin dilakukan sehingga perlu pengembangan uji, salah satunya metode molekuler multiplex real time PCR. .
Tujuan: Melakukan optimasi uji multiplex real time PCR untuk mendeteksi secara simultan dan cepat C.pneumoniae, L.pneumophila dan M.pneumoniae pada sputum pasien CAP.
Metode: Penelitian ini merupakan uji eksperimental laboratorium yang terdiri atas 3 tahap. Tahap 1 meliputi optimasi suhu penempelan, primer, probe, volume elusi akhir dan cetakan DNA. Tahap 2 untuk menentukan batas ambang deteksi DNA dan reaksi silang. Tahap 3 adalah penerapan uji multiplex real time PCR pada spesimen sputum pasien CAP.
Hasil: Uji multiplex real time PCR telah berhasil dioptimasi dengan ambang batas minimal deteksi DNA untuk Chlamydia pneumoniae, Legionella pneumophila dan Mycoplasma pneumaniae adalah 1855, 3185 dan 130 kopi DNA. Uji ini tidak bereaksi silang dengan mikroorganisme yang berpotensi menimbulkan reaksi positif palsu. Sebanyak 134 sputum telah diuji dan ditemukan positif M.pneumoniae sebanyak 1 spesimen (0,74 %).
Kesimpulan: Uji multiplex real time PCR dapat mendeteksi C.pneumoniae, M.pneumoniae, dan L.pneumophila secara simultan pada sputum pasien CAP.
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Background: Community Acquired Pneumonia (CAP) is one of the leading causes of morbidity and mortality in the world. Atypical bacteria (Chlamydia pneumoniae, Legionella pneumophila, Mycoplasma pneumaniae) are the important causes of CAP. In daily clinical practice, detection of atypical bacteria are sometimes neglected due to the limited standard test available. The real time multiplex PCR methode can be used as an alternative test for the detection of atypical bacteria.
Objective: Optimization of the multiplex real time PCR test to simultaneously detect C.pneumoniae, L.pneumophila and M.pneumoniae in CAP patients.
Methods: This study is experimental laboratory test that conducted in three phases. The first is optimization of annealing temperature, primers dan probe concentration, final elution of DNA extraction and volume of PCR templete. The second is determination of minimal detection of DNA and cross reaction of optimized real time PCR multiplex. The third is application of real time PCR multiplex in sputum clinical specimen patient with CAP.
Results: The multiplex real time PCR test was successfully optimized for annealing temperature, concentration of primer both forward and reverse, probes concentration and inhibitor. Limit detection of the DNA Chlamydia pneumoniae, Legionella pneumophila and Mycoplasma pneumaniae were 1855 copies,

3185 copies and 130 copies DNA. This test also showed no cross reaction to microorganisms that have potential to cause false positives. A total of 134 sputum clinical specimens have been tested with this method and only one sample (0,74%) was positive M.pneumoniae.
Conclusion: The multiplex real time PCR assay can detect C. pneumoniae, M. pneumoniae, and L. pneumophila simultanously in sputum of patients with Community Acquired Pneumonia (CAP)