

RINGKASAN

ANALISIS POLA DISTRIBUSI PENGULANGAN SEKUENS NUKLEOTIDA TTC *Mycobacterium leprae* DARI PENDERITA KUSTA DAN SUMBER AIR PENDUDUK DI PULAU POTERAN SUMENEP, MADURA

Mudatsir

Keanekaragaman hayati (*biodiversity*) merupakan bagian dari kehidupan alam semesta, dimana berbagai jenis mikroorganisme saling berinteraksi dan terus berubah. Beberapa mikroorganisme ada yang bersifat patogen pada manusia dan menyebabkan penyakit.

Kusta disebabkan oleh *Mycobacterium leprae* dan sampai sekarang masih menjadi masalah kesehatan bagi negara-negara endemik kusta termasuk Indonesia. Beberapa studi menunjukkan bahwa *Mycobacterium leprae* ditemukan di tubuh penderita dan alam sekitar daerah endemik kusta tapi belum jelas apakah eksistensi basil tersebut berhubungan dengan masalah kusta di daerah tersebut atau berdiri sendiri sebagai keanekaragaman hayati yang hidup di lingkungan tersebut.

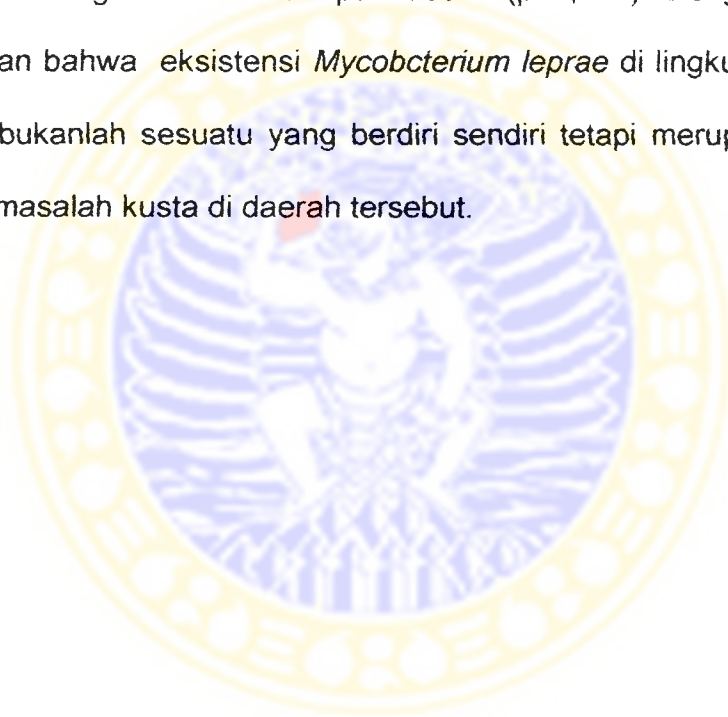
Genomic mapping Mycobacterium leprae telah selesai dilakukan pada tahun 2000 dan sekarang banyak digunakan untuk membedakan strain atau sub-strain karena basil ini tidak dapat dibiakkan. *Variable Number of Tandem Repeat (VNTR)* digunakan dan salah satu teknik yang sering digunakan untuk menganalisis variasi genetik *Mycobacterium leprae* adalah pengulangan sekuens nukleotida TTC.

Penelitian ini bertujuan untuk menganalisis pola distribusi TTC *Mycobacterium leprae* antara isolat 1) sumber air penduduk dari lingkungan endemik kusta di Pulau Poteran; 2) hapusan hidung narakontak sehat di daerah yang sama dan 3) sayatan kulit penderita kusta di daerah tersebut.

Telah dilakukan penelitian terhadap 201 sampel dari ketiga kelompok yaitu 91 sampel sumber air penduduk, 42 hapusan hidung narakontak dan 68 sayatan kulit penderita kusta dengan teknik biologi molekuler. *Polymerase Chain Reaction* (PCR) digunakan untuk memastikan adanya *Mycobacterium leprae* dengan menggunakan *nested primer* TTC. Dilakukan uji PCR untuk mengamplifikasi regio TTC yang akan diperiksa sekuensnya. Hasil positif PCR dipurifikasi dan diteruskan dengan sekuensing untuk melihat urutan nukleotidanya.

Dari seluruh sampel yang diperiksa, 74 isolat menghasilkan PCR positif terdiri dari 24 (26,37%) isolat dari sumber air penduduk, 26 (61,9%) isolat hapusan hidung narakontak sehat dan 24 (35,29%) isolat sayatan kulit penderita kusta. Dari analisis sekuensing menunjukkan bahwa variasi pengulangan sekuens nukleotida TTC dari sampel sumber air penduduk membentang antara 10-49 kali dengan frekuensi tertinggi terdapat pada pengulangan TTC 11 dan 14 kali. Dari sampel hapusan hidung variasi TTC membentang antara 9-44 kali dengan frekuensi tertinggi terdapat pada pengulangan TTC 11, 14 dan 10 kali. Sedangkan isolat dari sayatan kulit variasi TTC membentang antara 10-60 kali dengan frekuensi tertinggi terdapat pada pengulangan TTC 10 dan 11 kali.

Berdasarkan analisis statistik *Kolmogorof-Smirnov* tidak ada perbedaan yang bermakna pada pola distribusi pengulangan TTC antara isolat *Mycobacterium leprae* yang berasal dari sayatan kulit penderita kusta dengan hapusan hidung narakontak ($p=0,594$). Tidak ada perbedaan yang bermakna pada pola distribusi pengulangan TTC antara isolat *Mycobacterium leprae* yang berasal dari sayatan kulit penderita kusta dengan sumber air penduduk ($p=0,441$). Demikian juga tidak ada perbedaan yang signifikan pola distribusi pengulangan TTC antara isolat *Mycobacterium leprae* yang berasal dari narakontak kusta dengan sumber air penduduk ($p=0,906$). Dengan demikian dapat disimpulkan bahwa eksistensi *Mycobacterium leprae* di lingkungan daerah endemik kusta bukanlah sesuatu yang berdiri sendiri tetapi merupakan bagian dari rangkaian masalah kusta di daerah tersebut.



SUMMARY

PATTERN ANALYSIS OF TTC SEQUENCE FROM *Mycobacterium leprae* ISOLATES OBTAINED FROM LEPROSY PATIENTS AND PUBLIC WATER RESOURCES IN POTERAN ISLAND SUMENEP, MADURA

Mudatsir

Biodiversity is a natural characteristic of wild life. As a part of biodiversity, microorganisms are always going forward as an evolution. Some microbes are pathogenic for human.

Mycobacterium leprae has been known as a causative agent of Leprosy, a disease that still a health problem in some countries, including Indonesia. Recent studies showed that *Mycobacterium leprae* is also found in the environment of endemic leprosy areas. It is still unclear whether the existence of the bacilli in the environment is related to leprosy problem in that area or it is non-related and only a part of biological diversity that live in the environment.

Genomic mapping of *Mycobacterium leprae* has been completely documented since the year 2000 and it has been used for the study of strains and sub-strains, due to uncultivable of the bacilli. The Variable Number of Tandem Repeat (VNTR) methods have been used for this purpose and one of this, analysis of variation number in TTC repeat is used for genomic studies of *Mycobacterium leprae*.

The aim of this study is to explore the variation number of TTC repeat of *Mycobacterium leprae*, isolate from 1) water resources of Poteran island, an endemic leprosy area in Sumenep regency; 2) nasal swabs of healthy inhabitants

live in the same area and 3) skin slit smears from leprosy patients live in that area.

Two hundreds and one specimens were collected from the 3 groups and examined by Polymerase Chain Reaction (PCR) test using TTC nested primers to amplify the TTC target area of *Mycobacterium leprae* DNA. After purification, DNA sequencing was performed and the number of TTC repeat were analyzed.

Seventy four DNA sequences of *Mycobacterium leprae* isolates were documented from water resources 24 specimens, from healthy inhabitants 26 nasal swab, and 24 from leprosy patients. The results showed that *Mycobacterium leprae* originated from water environment has a range between 10 – 49 times of TTC repeats, with two peaks of frequencies in 11 and 14. The nasal cavity group showed the range between 9 – 44 , with three peaks at 11, 14 and 10 ; while the skin slit smears of leprosy patients showed a range between 10 – 60 with two peaks of frequencies at 10 and 11. No statistical difference in the pattern distribution repetition of nucleotide TTC *Mycobacterium leprae* between skin slit smear and healthy nasal cavity ($p=0,594$); There is also no difference distribution pattern between isolate skin slit smear from leprosy patients with water resources ($p=0,441$). There is also no difference distribution pattern between isolate from nasal cavity with water resources ($p=0,906$).It could be concluded that the existence of *Mycobacterium leprae* in the environment is not standing alone, but it is a part of the chains in leprosy problems in this endemic area.

ABSTRACT

Mycobacterium leprae has been known as a causative agent of Leprosy, a disease that still a health problem in some countries, including Indonesia. Recent studies showed that *Mycobacterium leprae* is also found in the environment of endemic leprosy areas. It is still unclear whether the existence of the bacilli in the environment is related to leprosy problem in that area or it is non-related and only a part of biological diversity that live in the environment.

Genomic mapping of *Mycobacterium leprae* has been completely documented since the year 2000 and it has been used for the study of strains and sub-strains, due to uncultivable of the bacilli. The Variable Number of Tandem Repeat (VNTR) methods have been used for this purpose and one of this, analysis of variation number in TTC repeat is used for genomic studies of *Mycobacterium leprae*.

The aim of this study is to explore the variation number of TTC repeat of *Mycobacterium leprae*, isolate from 1) water resources of Poteran island, an endemic leprosy area in Sumenep regency; 2) nasal swabs of healthy inhabitants live in the same area and 3) skin slit smears from leprosy patients live in that area.

Two hundreds and one specimens were collected from the 3 groups and examined by Polymerase Chain Reaction (PCR) test using TTC nested primers to amplify the TTC target area of *Mycobacterium leprae* DNA. After purification, DNA sequencing was performed and the number of TTC repeat were analyzed.

Seventy four DNA sequences of *Mycobacterium leprae* isolates were documented from water resources 24 specimens, from healthy inhabitants 26 nasal swab, and 24 from leprosy patients. The results showed that *Mycobacterium leprae* originated from water environment has a range between 10 – 49 times of TTC repeats, with two peaks of frequencies in 11 and 14. The nasal cavity group showed the range between 9 – 44 , with three peaks at 11, 14 and 10 ; while the skin slit smears of leprosy patients showed a range between 10 – 60 with two peaks of frequencies at 10 and 11. No statistical difference in the pattern distribution repetition of nucleotide TTC *Mycobacterium leprae* between skin slit smear and healthy nasal cavity ($p=0,594$); There is also no difference distribution pattern between isolate skin slit smear from leprosy patients with water resources ($p=0,441$). There is also no difference distribution pattern between isolate from nasal cavity with water resources ($p=0,906$). It could be concluded that the existence of *Mycobacterium leprae* in the environment is not standing alone, but it is a part of the chains in leprosy problems in this endemic area.

Keywords: *Mycobacterium leprae*, Polymerase Chain Reaction (PCR), DNA Sequencing, TTC repeats