DOI Number: 10.5958/0976-5506.2018.00592.2

# The Determinants of the Geographical Distribution and Transmission of 16S rRNA of *M.leprae* in Endemic Areas, Indonesia

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## ABSTRACT

The prevalence of leprosy disease remains a worldwide public health problem including in Indonesia. There are five main countries as the foremost category of leprosy cases that include Brazil, India, Indonesia, Nigeria and the Democratic Republic of Congo. The research method used in this study was the observational study using a case-control research design. The total samples were 81 individuals that consisted of 27 leprosy patients (the case group) and 54 non-leprosy patients (the control group). Analysis of 16S rRNA gene expression was conducted using the PCR method to detect the risk of leprosy.

Distribution of leprosy disease at the endemic areas was caused by dwelling density and unhealthful environment, and moreover the distance among houses was only around 10 m as the causal factors of *M.leprae* transmission.

***Keywords***: *leprosy patients, 16S rRNA gene, case-control.*

## INTRODUCTION

There are five countries in the foremost category of leprosy cases that include Brazil, India, Indonesia, Nigeria and the Democratic Republic of Congo. These countries have high endemic of leprosy disease. The estimated number of leprosy cases at the global level was 436,246 cases in the year 2008 cases in which (WHO, UNICEF, UNFPA, 2014). In 2008, leprosy cases were 436.246 worldwide, in which India was the first foremost category of leprosy with 83.041 cases, Brazil was the second foremost category of leprosy with 29.761 cases and Indonesia was in the third foremost category of leprosy with 19.785 cases respectively.[1]

*M. leprae* identification is difficult due to the inability of the bacillus to grow in vitro, this leprosy diagnosis is based on microscopic detection of the bacilli combined with clinical assessment. DNA studies using polymerase chain reaction (PCR) have been used for the molecular diagnosis of *M. leprae*.[7]

Clinical manifestation of the disease varies widely among individuals. The transmission of *M. leprae* not only consider the cycle of transmission in the form of the source of leprosy disease, contact with the host, but also it is important to consider the source of infection., bacterial virulence, frequency of contacts and the characteristics of the host, including his/her immunity, specific immunity, age, sex and nutritional status and other factors.[3]

16S rRNA gene is commonly found in species of mycobacterium and consequently 16S rRNA gene can be used to identify different species of mycobacterium.[5]

16S rRNA gene encodes a ribosomal RNA in a small sub-unit of the ribosome and it has a distinct nucleotide sequence in each bacterium.[5] In addition, 16S rRNA gene is more stable and it is suitable to be used a specific molecular marker for the identification[6] of 16S rRNA gene in bacteria (its existence is always retained in any conditions) and has identical characteristics in all

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organisms. Therefore, the use of 16S rRNA is suitable in identifying and analyzing a species at a molecular level.

Based on the background of problems, this study aims to assess the determinants of geographical distribution in epidemiology of leprosy disease in Indonesia.

## MATERIALS AND METHOD

### Samples of the study

The study was classified as population in this study as the observational study using a case-control research design. The population in this study consisted of both the case group and control group. The case group was leprosy patients (BTA+), whereas, the control group was non-leprosy patients in Makassar municipality. Statistical analysis for both the case group and the control group was done using the Lameshow formula.

This study was classified as the observational study in which the authors did not control the assignment of treatments by using a case-control research design where the exposures to risk factors for the case group were compared to exposures for the control group. Samples in this study were leprosy patients (BTA+) and non-leprosy patients (BTA-) in Makassar municipality. The total samples were 81 individuals that consisted of 27 leprosy patients as the case group and 54 non-leprosy patients as the control group.

The sources of data in this study were collected from primary data through direct observations that include the prevalence of leprosy cases and dwelling distance among leprosy and non-leprosy individuals.

The study instruments were observation sheets, Global Positioning System(GPS)and Corel Draw software X7. Observation sheets were used during direct observations or field surveys to date direct observations or field surveys were conducted at dwelling clusters and spatial distribution of leprosy disease. Global Positioning System (GIS) was used to digitization based on the coordinates of X and Y points of cluster and spatial distribution of leprosy disease. Global Information System(GPS)of individuals in the leprosy disease and risk factors of environment such as dust distribution by air. The study data were then analyzed and processed using the Corel Draw X7 software,

### Statement of Ethics

All experimental procedures for the treatment of individuals as the samples in this study were reviewed and approved by the Research Ethics Committee of Medicine Faculty, Hasanuddin University, as stated in the Recommendation of Research Ethics issued in the registration no. 311/H4.8.4.5.31/PP36-Kometik/2017 with written permission from the respondents.

## RESULTS

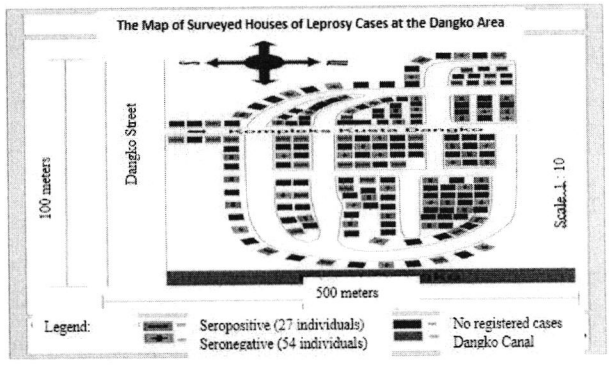


**Figure 1. Map of surveyed houses of leprosy cases in Makassar Municipality.**

The above map indicates dwelling density in the study areathat leads to the transmission of*M.leprae*. Other factors that affect the distribution and transmission of *M.leprae* were poor conditions of dwelling. Most houses of leprosy individuals were semi-permanent and there were houses built on stilts, distances among houses were separated by wall or with house distance was ±10 m.Close interactions between leprosy individuals and non-leprosy individuals under the same dwelling as "the port of entry"of leprosy bacteria transmitted by air when leprosy individuals having coughing and sneezing that could release around 110.000 bacilli of *M.leprae*. Transmission through droplets increases the infection as well as skin contacts as the main route of transmission.

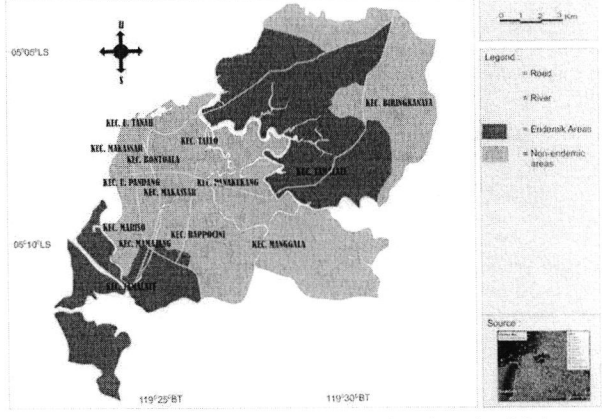


**Figure 2. The map of leprosy distribution in Makassar Municipality.**

Based on the map in Figure 2, the main clusters of distribution of leprosy cases in Makassar Municipality were found at Tamalate dan Biringkanaya subdistricts, as indicated in colored gradation.

## DISCUSSION

In the geographic distribution analysis of PCR positivity among the cases with a positive bacciloscopy index in Fortaleza, individuals were found clustered in certain small areas. As expected, the areas surrounding those clusters were found clustered in certain small areas. These situaions suggest that MB cases harbouring *M.leprae* in nasal cavities could be a major potential pathway for transmission of the bacillus within the population[16].

Based on direct observations at dwelling areas of respondents, there were 98.5% of respondents' houses were in poor conditions to fulfill suitable healthy dwelling, Most houses of respondents have unsuitable physical components such as clay-floor and walls of their houses were permeable to water, poor ventilation and windows (floor areawas <10%), poor ventilation for light rays that lead to high moisture. Such conditions cause adverse effects to dwellers and prompt to the growth of bacteria and other microorganisms as the source of transmission of leprosy[17]*Kerr-Pontes* (2006) [8] reported that water or ground were reservoirs for the growth of *M.leprae*. Other conditions that induced the risk of leprosy transmission at the endemic areas werethe daily habits of the local people at the endemic areas did not use footwear that lead to the transmission of *M.leprae* through skin contact at wound area on the skin and ignore their wounded legs.

Local people at the endemic areas had close social interactions among their relatives and neighboors in daily activities. High intensity of touch between leprosy individuals and non-leprosy individuals could increase the transmission of leprosy disease. Poverty condition could intensify the transmission of *Mycobacterium leprae* bacilli which compels relatives and others to live together for long periods of time, especially young married couples and their children, typically under precarious sanitation conditions.

The average household density was higher in the residences with a leprosy case, Considering its total area, the main area of leprosy cases in Makassar Municipality has the highest household density in the residence with a leprosy case and poor sanitation due to a direct result of poverty. Such conditions facilitate the transmission of infectious disease. Social interactions among local families at the study area commonly occur at afternoon as the spare times as observed in their daily activities,

This study is in line with a study conducted in Bangladesh in which people with poor socioeconomic conditions susceptible to leprosy[1]. Distance of road is insignificantly correlated with the transmission of leprosy in Bangladesh[1], whereas, distance of dike and dwelling is significantly correlated with leprosy case. A previous study reported that there was significant correlation between people who live around pond with the higher prevalence of leprosy cases [3]. Another study showed that infected individuals generally have *Multibasiller/MB* who live under the same house with leprosy patients[9].

Global standardized guidelines for the diagnosis and treatment of leprosy with a goal of elimination have been available and in practice for more than 2 decades[9] Classical epidemiology and leprosy control program have frequently identified household contacts of patient as being at higher risk of infections than the general populations,[10,11,4], explanation or tools are not routinely in place for tracing the origins and relationship of these and remaining new cases[12,13]

## CONCLUSION

Based on results of the study, the determinants on the distribution and transmission of leprosy disease at the study areas were close density of dwelling, poor physical houses and semi-permanent houses which made of clay-floor and houses on stilts, close distance of houses separated by dividing wall with common distance of houses at the endemic areas was ±10 m. It is suggested to conduct strict control of the distribution of leprosy to target intervention more systematically to areas where the risk of leprosy is high, and there were two main clusters of leprosy in Makassar Municipality, i.e., Tamalate and Biringkanaya subdistricts,

**Source of Funding-** From Authors their selves

**Conflict of Interest** – Nill

**Ethical Clearance:** Obtained from the University committee

## REFERENCES


1. World Health Organization (2009). Guedeliness for Global Survailance of Drug Resistance in Leprosy, New Delhi.
2. BakkerMI, Scheelbeek PF, Van Beers SM. The use of GIS in leprosy control. Lepr Rev. 2009 Sep 1:80 (3):327-1
3. Barreto JG, Bisanzio D, de Souza Guimareas L, Spencer JS, Vazquez-prokepec GM, Kitron U, Salgado CG, Spatial analysis spotlighting early childhood leprosy transmission in a hyperendemic municipality of the Brazilian Amazon Region. PloS neglected tropical diseases. 2014 Feb 6;8(2):e2665
4. Bear SM, van, Wit MYL de, Klatser PR. 1996. The epidemiology of Mycobcaterium leprae:recent insight FEMS Microbiol Lett. 136:221-230.
5. Yoshua Y, Yan W, Yuangang Y, Yan X, Huanying L, Xioman W, Nan W, Shuang L, Shanshan Z, Wenhong Z, Ying Z, (2015), Characterization of Mycobacterium leprae genotypes in China-Identification of a new Polymorphism C251T in the 16S rRNA Gene. Plos One 10: 1-10.
6. Singh, V., Chaudhary, D.K., Mani. L (2012). "Molecular characterization and modeling of secondary structur of 16S rRNA from Aeromonas veronii." International journal of applied biology and pharmaeutical technology
7. Santos AR, Degrave WM, Suffys PN 1999. Use of polymerase chain reaction (PCR) in leprosy research . Indian J Lepr 71: 101-110
8. Kerr-Pontes LR, Barreto ML, Evangelista CM, Rodrigues LC, Heukelbech J, Feldmer H. 2006. Socioeconomic, environmental and behavioural risk factors for leprosy in Northeast Brazil : results of case-control study. Int J Epedemiol 35:994-1000
9. Izumi S, 1999. Subclinical Infection by Mycobacterium leprae. International Journal of leprosy, 67(4): 67-71.
10. Bear SM van, Hatta. M., Klatser PR (1999). "Patient contact is the major determinant in incident leprosy. Implications for future control " Int. J. Leprosy67: 119-128
11. Cardona-Castro, N.J. Beltran -Alzate, and R. manrique- Harnandez, 2008. Survey to identifity Mycobacterium leprae- infected household contacts of patients from prevalent regions of leprosy in Colombia. Mem. Inst. Oswaldo Cruz 103:332-336
12. Cellona R.V..,G.P. Walsh, T. T. Fajardo, Jr.,R.M. Abalos, E.C. dela Cruz, L. Guido-Villahermosa, M.V. Felicio Belago, G.J. Steen bergen, and J.T Douglas. 1993. Croos- Sectional assesment of ELISA reactivity in leprosy patient, contacts, and normal populations using the semisynthetic antigen natural disaccharide octyl bovine serum albumin (ND-O_BSA) in Cebu, the Philippines. Int.J. Lepr. Other Mycobcat. Dis 61:192-198
13. Deeps, P.D., B.V. Guedes, J.B. Filho, M.K. Andreatta, R.S. Marcari, and L.C. Rodrigues, 2006. Delay in the diagnosis of leprosy in the metropolitan region of Vitoria Brazil. Lepr. Rev. 77:41-47
14. Tadese Argaw, A.,E.J. Shannon, A. Assefa,F.S, Mikru, B. K. Mariam, and J.B. Malone, 2006. A geospatial risk assesment model for leprosy in Ethopia based on environmental thermal-hydrological regime analysis. Geospat Health. 1:105-113
15. Wourld Health Organization 2008, Global leprosy situation, begening of 2008, Wkly. Epidemiolo. Rec. 83:293-300
16. LuanaNeomuceno G.,C.,L, Cristiane C.,F, Rosa Maria S.,M, Rosa Livia F.,A, Maria Araci de A.,P, Heitor de Sa G.,Laura C.,R, Carl Kendall, Ligia Kerr, Widespread nasal carriage of Mycobacterium leprae among a healthy population in a hyperdemic region of northeastern Brazil, Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol 110 (7):898-905, November 2015
17. Grould Brooker, 2003. Disability Prevention and Management in leprosy: a field experience, Vol, 69 (6): 369-374.