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STUDY OF RISK FACTORS AND LEPTOSPIRA DETECTION OF SANITARY WORKERS IN JAKARTA, INDONESIA

STUDI FAKTOR RISIKO DAN DETEKSI LEPTOSPIRA PADA PEKERJA SANITASI DI JAKARTA, INDONESIA

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ABSTRACT

Leptospirosis is zoonotic disease, caused by spirochete-bacteria Leptospira, transmitted by excreted urine of rodent into the environment. Sanitary workers had high-risk of Leptospira infection due to frequent exposure to contaminated environment, which could cause asymptomatic leptospiruria (the presence of leptospira in urine) and severe complications, such as chronic kidney disease. The aim of this study was to find leptospiruria in sanitary workers, using PCR method, targeting specific genes of Leptospira. Urine samples and questionnaires were obtained from fifteen sanitary workers. Samples were cultured in EMJH-broth with addition of 5-fluorouracil, incubated for 3 months and observed for growth of bacteria using dark-field microscope. Identification of bacteria was performed by PCR, targeting lipl32, rrl, flaB, ompL1 genes, followed by sequencing using Sanger method, alignment using ClustalW and BLAST. The questionnaires result showed that 26,7% of respondent had medium level of risk factors, and 53,3% of respondent had applied good prevention for leptospirosis. Pearson correlation analysis showed that there was relationship between risk factors and prevention. Culture result showed growth in four samples, and analysis by PCR only showed rrl-PCR had expected amplicon. However sequencing result showed that the amplicon had 99% homogeneity to Pseudomonas stutzeri. In conclusion, Leptospira was not found in the urine of sanitary worker, might be due to applied good prevention for leptospirosis.

Keywords: *Leptospira, Leptospiruria, Sanitary Worker, Risk Factors*

ABSTRAK

Leptospirosis adalah penyakit zoonosis, yang disebabkan oleh bacteria Leptospira Sp., ditularkan melalui urin hewan pengerat yang dikeluarkan ke lingkungan. Pekerja sanitasi memiliki risiko tinggi terinfeksi Leptospira karena sering terpapar lingkungan yang terkontaminasi, yang dapat menyebabkan leptospiruria (adanya liptospira dalam urin) tanpa gejala, dan komplikasi berat, seperti penyakit ginjal kronis. Tujuan dari penelitian ini adalah untuk menemukan leptospiruria pada petugas kebersihan, menggunakan metode PCR, menargetkan gen spesifik Leptospira. Sampel urin dan kuesioner diperoleh dari lima belas petugas kebersihan. Sampel dikultur dalam EMJH-broth dengan penambahan 5-fluorouracil, diinkubasi selama 3 bulan dan diamati pertumbuhan bakterinya menggunakan mikroskop medan gelap. Identifikasi bakteri dilakukan dengan PCR, menargetkan gen lipl32, rrl, flaB, ompL1, dilanjutkan dengan sequencing menggunakan metode Sanger, alignment menggunakan ClustalW dan BLAST. Hasil kuesioner menunjukkan bahwa 26,7% responden memiliki faktor risiko tingkat sedang, dan 53,3% responden telah menerapkan pencegahan leptospirosis yang baik. Analisis korelasi Pearson menunjukkan bahwa ada hubungan antara faktor risiko dan pencegahan. Hasil kultur menunjukkan pertumbuhan pada keempat sampel, dan analisis dengan PCR hanya menunjukkan rrl-PCR memiliki amplikon yang diharapkan. Namun hasil sekuensing menunjukkan bahwa amplikon memiliki homogenitas 99% terhadap Pseudomonas stutzeri. Kesimpulannya, Leptospira tidak ditemukan dalam urin petugas kebersihan, mungkin karena penerapan pencegahan leptospirosis yang baik.

Kata kunci: *Leptospira, Leptospiruria, Petugas Kebersihan, Faktor Risiko*

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BACKGROUND

Leptospirosis is zoonotic disease, caused by Leptospira, which belongs to Spirochaetes class. Leptospira has become a neglected infectious disease for long period, particularly in developed countries, which had increased the standard of hygiene and health (Ko *et al.*, 2019). However, this disease has become a re-emerging disease, because of some outbreaks occurred after natural disasters. Indonesia has been estimated as country with high cases of leptospirosis (Pappas *et al.,* 2008). Morbidity of leptospirosis in Indonesia is estimated as 39.2% for 100.000 populations, which is higher than other Asian countries, such as India (19.69%) and Filipina (14.98%) (Costa *et al.,* 2015). Outbreaks of leptospirosis had been occurred in several provinces in Indonesia. Leptospirosis cases has been increasing in Indonesia with case fatality rate (CFR) 7.35 – 16.65% (Kemenkes, 2019). The highest CFR is recorded in 2018 in Banten and Jawa Tengah, 26.96% and 20.84% respectively (Kemenkes, 2019).

Leptospira is transmitted directly by direct contact from Leptospira-infected animal, particularly rodents, to human. Sometimes, indirectly by intermediary of environment contaminated by Leptospira-infected animal urine (Adler and de la Peña Moctezuma, 2010). Leptospira could penetrate the human body through skin abrasion and mucous membrane, such as mouth, conjunctiva and nose. Leptospira could survive for long time in environment, particularly soil and water, also could cause disease for human, which contact with Leptospira-contaminated environment.

Human is known as incidental host for Leptospira and excreted for several months in urine. However, study of Ganoza *et al.,* (2010) had found long-term leptospiruria excreted from human in endemic area, which show no symptoms and also seronegative. Another study found 20% of population in leptospirosis endemic area showed leptospiruria without any symptoms and fever before (Sivasankari *et al.,* 2016). These studies showed that Leptospira might adapt and survive in kidney of human for long-term (years) in endemic area of leptospirosis, without any symptoms. Frequent exposure of Leptospira might cause adaptation of this bacterium to immune system. The clinical manifestation of this disease is ranging from the mild one, i.e flu-like syndrome, until jaundice, pulmonary hemorrhage, or even death. Asymptomatic leptospirosis could cause severe complication into chronic kidney disease (Yang,

2018).

Jakarta, the capital city of Indonesia, is located in tropical area which prone to flood due to high rainy season and estuary of thirteen rivers. Outbreak of leptospirosis had been also occurred in this city (Kemenkes, 2019). Leptospira also had been found in the environment of Jakarta (Widiyanti et al., 2019). These conditions cause Jakarta to become endemic area of leptospirosis. Human, who work or had frequent contact with this city environment, particularly sanitary workers, is prone to the infection. The highest risk factors of Leptospira infection are frequent contact with contaminated environment, particularly without using personal protective equipment while working (Kamath et al., 2014) . Study about risk factor of leptospirosis in the sanitary worker and detection of leptospiruria is necessary to be carried out, in order to detect leptospiruria and its relation with personal hygiene.

METHOD

Research design and Sample collection

The research design is descriptive analytic, which gathering data from laboratory examinations and questionnaires. The questionnaire method used was a dichotomous scale questionnaire. Samples were collected from urine of fifteen sanitary workers in Jakarta, Indonesia. This location was chosen because it is included in the area that is flood-prone and close to the laboratory. The workers were chosen based on the inclusion character as PPSU officers in region X Jakarta, while the exclusive character was not having fever and never contact with an environment that is likely to be contaminated (water/gutters, waste).

Urines were collected in the morning and transported immediately to Microbiology Laboratory of Universitas YARSI. Questionnaires of personal hygiene and informed consent were obtained from the sanitary workers prior to sample collection.

Bacteria culture

One hundred µL of urine samples were inoculated into the Ellinghausen–McCullough–Johnson–Harris (EMJH) broth with addition of 5 fluorouracil (5FU) and incubated for 1-2 months at 30^{o} C. The samples were observed every week for any growth of bacteria using dark-field microscope.

Molecular identification

The samples culture, which showed any growth of bacteria, were then analysis for the rrl, lipl32, flaB and ompL1 genes using PCR. The cultures were then extracted for DNA using

Purelink Genomic DNA mini kit (Invitrogen) according to manufacturer manual. The primers used in this study were listed in Table 1. The condition of PCR was following each references. PCR product was electrophoresed in 1% of agarose gel and visualized using SYBR@safe DNA gel stain (Thermo Fisher Scientific, US).

Table 1. Primer used in the study

No	Gene	Primer sequence	Reference
1	flaB1 (forward)	5'TCTCACCGTTCTCTAAAGTTCAAC-3'	Kawabata *et al.* (2011)
2	flaB1 (reverse)	5'CTGAATTCGGTTTCATATTTGCC-3'	Kawabata *et al.* (2011)
3	flaB2 (forward)	5' TGTGCACAAGACGATGAAAGC-3'	Koizumi *et al.* (2013)
4	flaB2 (reverse)	5' AACATTGCCGTACCACTCTG-3'	Koizumi *et al.* (2013)
5	lipl32 (forward)	5'-CGCTGAAATGGGAGTTCGTATGAT T-3'	Patricia *et al.* (2014)
6	lipl32 (reverse)	5'-CCAACAGATGCAACGAAAGATCCTTT-3'	Patricia *et al.* (2014
7	ompL1 (forward)	5'-TTGATTGAATCTTAGAGTTCGTGTTTATA-3'	Patricia *et al.* (2014
8	ompL1 (reverse)	5'-AAGGAGAAGCTTATGATCCGTAACATAAGT-3'	Patricia *et al.* (2014
9	rrl (forward)	5′-GACCCGAAGCCTGTCGAG-3	Saito *et al.* (2013)
10	rrl (reverse)	5′-GCCATGCTTAGTCCCGATTAC-3′	Saito *et al.* (2013)

16SrRNA identification

The sample which showed suspected amplicon was isolated using EMJH + 5FU soft agar. The colony was the extracted for the DNA using Purelink Genomic DNA mini kit (Invitrogen) according to manufacturer manual. The extracted DNA was then amplified for 16SrDNA using universal primer P16S-8UA 5'AGAGTTTGATCMTGGCTCAG-3' and P16S-1485R 5'TACGGYTACCTTGTTACGACTT-3' with condition 30 *cycles denaturing* at 96º C for 1 minute; *annealing* at 55º C for 1 minute; *extension* at 72º C for 1 minute (Saito *et al.,* 2013). PCR product was electrophoresed in 1% of agarose gel and visualized using SYBR@safe DNA gel stain (Thermo Fisher Scientific, US). PCR product was then purified using *Purelink Quick Gel Extraction and PCR Purification Combo Kit* (Invitrogen) and sequenced using the Sanger method. The DNA sequence was aligned and analyzed for homology using CLUSTALW and BLAST.

Ethical clearance

The Research Ethic Committee of Universitas YARSI had reviewed and given the approval ethic under the statement letter No 044/KEP-UY/BIA/V/2019.

RESULTS AND DISCUSSION

Questionnaires analysis

List of questions in questionnaires were listed in Table 2. Analysis showed that most of the sanitary worker had low risk infection of leptospirosis (73%), while the remaining has

middle risk of infection (27%). No respondents showed high infection risk of leptospirosis. For the prevention effort, more than half of respondents (53%) had showed high level of efforts, while the remaining showed middle level (47%). None of the respondents showed low level of prevention effort. Pearson analysis showed the relation of risk infection and prevention efforts, with ρ value was -0.768. It showed that the respondent with high infection risk of leptospirosis had smaller prevention effort or vice versa.

Table 2. List Of Leptospirosis Risk Factors Questions

No	Question	Yes (%)	No (%)
1	Wound in foot	26.67	73.33
2	Wound in hand	26.67	73.33
3	Long working hours	40.00	60.00
4	Working at flood area	26.67	73.33
5	Working at garbage disposal	60.00	40.00
6	Working at river area	60.00	40.00
7	Using boots while working	100.00	0.00
8	Using gloves while working	40.00	60.00
9	Using mask while working	53.33	46.67
10	Cleaning body using soap	100.00	0.00
11	Cleaning body using tap water	53.33	46.67
12	Cleaning body using well water	73.33	36.67
13	Cleaning body using river water	6.67	93.33

Isolation and molecular identification

Incubation of urine samples in EMJH + 5 fluorouracil (5FU) broth had showed that four samples had growth of bacteria. PCR result for detection of lipl32, ompL1 and fla B genes showed no amplicon, while rrl showed amplicon in one sample (Figure 1).

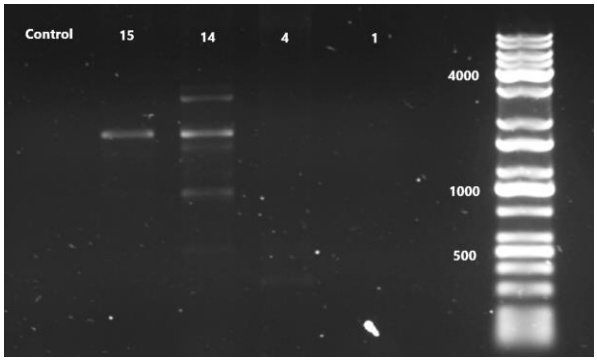

Figure 1. PCR-rrl results. Lane 1: positive control, lane 2-4 : samples, lane 5: DNA marker

16SrDNA identification.

The use of 16srdna was done to identify species/serovar from isolates. Identification using 16SrDNA, alignment and sequencing had resulted that the colony LT2 (Genbank ID MW832378.1) was belong to Pseudomonas group (Figure 2). It showed 99% homogeneity to *Pseudomonas stutzeri*.

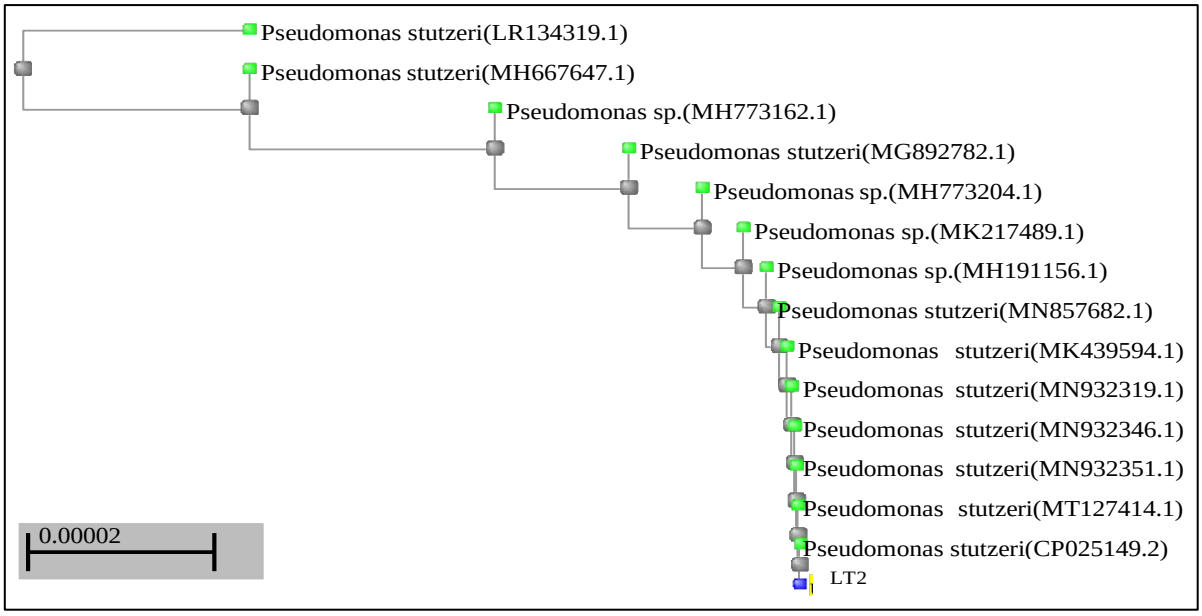

Figure 2. Phylogenetic tree

Sanitary workers are an occupation, which have high risk of leptospirosis infection, due to continuous exposure from contaminated environment. Knowledge about infection risk and prevention during work is necessary to avoid the infection. Based on the questionnaires result, most of sanitary workers in region X Jakarta (60%) worked in area, which were the habitat of rodents such as flooding area, garbage disposal and river. However, most of them used the personal protection equipment (PPE), such as boot, mask and glove, even 100% of them used boots while working. Personal hygiene of respondents was also good, because all of them were cleaning their body using soap or disinfectant after contacting with contaminated environment. These behaviors had lowered the risk of leptospirosis infection. Lau *et al.* (2018) and Fernandez *et al.* (2019) stated that the utilization of personal protection in sanitary worker and good personal hygiene were important for avoiding exposure of Leptospira or lowering the infection risk. The relation of infection risk and prevention effort had been analyzed using Pearson correlation, and resulted that both variable had significant correlation. The coefficient correlation showed the higher infection risk is, the lower prevention effort is. This showed that some of the sanitary workers might not fully understand the usability of personal protection during working. For example, the respondent, which had medium infection risk (work in flood area or garbage disposal) only used boots as personal protection, but did not use glove or mask. Therefore, the comprehension of the usability of personal

protection and hygiene should be realized throughout in sanitary workers, by explanation or counseling.

PCR of lipl32, ompL1 and flaB showed no amplicons. These genes were applied to detect the pathogenic Leptospira. Negative result of these genes showed that no pathogenic Leptospira were found in the sample. PCR of rrl gene showed the amplicon in range size of expected band (482 bp) in one sample (Figure 1). This gene was used to detect 23S rRNA of bacteria and could detect the genus of Leptospira. Confirmation of this amplicon was performed by isolating the sample and performing 16SrDNA PCR, followed by sequencing. The result showed that the isolate (Genbank ID MW832378.1) had 99% homogeneity to *Pseudomonas stutzeri*. The discrepancy of the result of rrl gene and sequencing result might be caused by the amplicon in rrl PCR actually did not showed the exact size of expected band. *Pseudomonas stutzeri* is ubiquitous and rarely cause infections (Lalucat *et al.*, 2006; Park *et al.*, 2013) . This bacteria has been reported as an oportunistic pathogen, which cause some infection, such as hospital-acquired infection (HAI) (Noble and Overman, 1994), septicemia (Bello, 2007), peritonitis (Park *et al.*, 2013), endocarditis (Alwazzeh *et al.*, 2020) and urinary tract (Taneja *et al.*, 2004). Based on the result, it could be concluded that no Leptospira was found in urine samples of sanitary worker. This might be caused by the application personal protective and hygiene, which is quite good in avoiding Leptospira infection. The number of samples, which was few, might cause the non-detected Leptospira, therefore a bigger samples might be necessary to be obtained.

CONCLUSION

Leptospira was not detected in the urine of sanitory workers, might be due to good personal hygiene and utilization of PPE of sanitary workers and small number of samples. Further study could be carried out using bigger sample number for population in rural and urban area.

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