SPECIFIC GYRB SEQUENCE OF MYCOBACTERIUM TUBERCULOSIS CLINICAL ISOLATED FROM SPUTUM OF PULMONARY TUBERCULOSIS PATIENTS IN INDONESIA

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Background: Indonesia have many different geographic areas which could be various on the variant strains of Mycobacterium tuberculosis. The gyrB gene codes GyrB protein as sub unit compound of Gyrase enzyme that functioning in multiplication of bacteria. Detection of gyrB gene could be a marker of active multiplication of viable bacteria in the specimen from patients; and some of the DNA sequence regions were conserved and specific in the strain of Mycobacterium tuberculosis that would be a marker for identification. This research aims to analyze the sequence of gyrB gene of Mycobacterium tuberculosis clinical isolates from sputum of pulmonary TB patients in Indonesia, and determine the specific region. Method: Mycobacterium tuberculosis clinical isolates have been collected from sputum of the patients with pulmonary TB that live in some area in Indonesia. Isolation and identification of Mycobacterium tuberculosis clinical isolates standard culture method; sequence analysis using PCR-direct sequencing of the part using bases region of gyrB. Results: this study revealed that nucleotide sequence on a fragment 764 bases of gyrB gene Mycobacterium tuberculosis strains among clinical isolates almost identically to a wild type strain Mycobacterium tuberculosis H37Rv and subspecies member of Mycobacterium tuberculosis complex (MTBC), with a little difference of SNPs; there are many difference nucleotide sequence with MOTT and Gram positive or negative bacteria, except Corynebacterium diphtheriae identically with MTBC. Conclusion: the gyrB sequence in Mycobacterium tuberculosis strains among these clinical isolates from sputum of pulmonary TB patients in Indonesia have the conserved specific DNA region that almost identically with wild type strain H37Rv and MTBC.

Keywords: sequence; gyrB gene; Mycobacterium; tuberculosis; sputum.

INTRODUCTION

Tuberculosis (TB) is a major health problem in Indonesia and in the world. The WHO global TB control report of 2009, mentions in Indonesia noticed 528.063 new TB cases and 102 new sputum smear positive cases per 100.000 population, with the total population 226 million people in 2007 as reported by Indonesian Ministry of Health; the prevalence of TB in Indonesia differs per geographical areas, Java-Bali having the lowest prevalence (67 per

Address of Correspondence: Ni Made Mertaniasih Department of Clinical Microbiology, Faculty of Medicine Airlangga University/Dr. Soetomo Academic Hospital, Surabaya-Indonesia Email: mniasih@gmail.com 100.000 population), and Eastern Indonesia the highest (198 per 100.000 populations).¹ The other period reported at 2011 in Indonesia there are AFB positive 197.797 cases which the highest cases in Java; on the other hands at 2010 estimated the prevalence of pulmonary TB 725 per 100.000 population with the highest TB prevalence in Papua province 1.441 per 100.000 population. Batan 1.282/ 100.000, north Sulawesi 1.221/ 100.000, and lower in Lampung (270/ 100.000), Bali (306/100.000), DI. Yogyakarta (311/100.000) (Indonesia MOH, 2012).² At 2014, WHO reported the estimates of TB disease problem in Indonesia at 2012 noticed in 246.864.000 population with incidence rate 185 (153 - 220) per 100.000 population (95% CI); prevalence rate 297 (144 -506); and mortality rate 27 (12 - 48). In South

East Asia at 2012 estimate TB global incidence 8.300.000 –9.000.000 cases of all forms of TB. In global TB report at 2013 estimated that total TB cases 39 % prevalent in South East Asia region of all TB cases in the world, and mortality estimated 47,6 % from totally in the world, estimated global TB mortality 790.000 – 1.100.000 cases of all form of TB.³

The effective control of TB case finding is of utmost importance. Accurate and rapid diagnostic methods are needed for determined TB diagnosis. Until now the culture method is the gold standard for TB diagnosis, on the other hands the culture method constrains with takes the time consuming, more than 3 weeks up till 2 months. Nucleic acid amplification is a rapid diagnostic methods that is sensitive and specific.⁴⁻⁶ Nucleic acid amplification tests have as the for identification, i.e. region target gene insertion sequence IS 6110, 16S rRNA, hsp60, gyrB, rpoB, 16-23S rRNA etc., which have the and gene regions conserved specific of *Mycobacterium* tuberculosis.⁷ High copy number of gyrB gene have the important role to distinguish the specific nucleotide sequence with MOTT species or other Gram positive bacteria.8 Based on the advantages of the role for the specific region identification in gyrB gene, as a basis primer design, the sequence of gyrB gene of Mycobacterium tuberculosis by DNA sequencing must be determine among clinical isolates from TB patients.

This study aims to analyze the sequence of *gyrB* gene *Mycobacterium tuberculosis* clinical isolates from sputum of pulmonary TB patients that live in some area in Indonesia. Some isolates from TB patients in many different geographic areas in Indonesia could be various strains of *M. tuberculosis*, however the *gyrB* gene nucleotide sequence would have the conserved and specific nucleotide sequence in the DNA regions.

MATERIALS AND METHOD

Bacterial Strains of Mycobacterium tuberculosis

Mycobacterium tuberculosis strains 102 isolated from sputum of 307 suspected pulmonary TB patients that collected in 2010, and 170 from 536 suspected pulmonary TB patients at 2013, patients from some area in Indonesia. Isolation and identification of *Mycobacterium tuberculosis* were carried out using standard culture method -MGIT 960 System (BD) and conventional standard on L-J medium (WHO). A11 of t h e s e were conducted in TB Laboratory in Department of Clinical Microbiology, Dr Soetomo Academic Hospital, Surabaya, Indonesia.

Analysis of DNA sequence of gyrB gene of Mycobacterium tuberculosis clinical isolates

Molecular study of DNA sequencing

conducted in Institute of Tropical Diseases, Airlangga University, Surabaya, Indonesia; eight isolates of TB patients (in 2010) have been done sequencing on a part of the target the 1.020 bases, and three isolates of TB patients (in 2013) with target a part of the almost whole gene sequence. Extraction and purification of DNA from *Mycobacterium tuberculosis* clinical isolates, as is follow, one loop full of fresh colonies of *Mycobacterium tuberculosis* (3 – 4 weeks old on Lowenstein-Jensen medium) was suspended in a buffer solution, extraction and purification of DNA using DNeasy kit (QIAGEN).

PCR to detect and amplify the DNA region of gyrB gene Mycobacterium tuberculosis, 41 isolates that collected at 2010 were conducted PCR for gene target 1.020-bp of gyrB. The suspension of PCR mix Dream Taq "Green PCR Master Mix (Fermentas) was added by primer target 1.020-bp region as is MTUB-f 5'-TCGGACGCGTATGCGATATC-3' and MTUB-r 5'-ACATACAGTTCGGACTTGCG-3' each 1,0 μ M, and DNA template 2 μ l, reaction volume 50 μ l. Amplification reaction is 98° C 2 minutes; 96° C 20 seconds; 58° C 20 seconds; 72° C 1 minute; and 72° C 7 minutes, and the reaction 40 cycles.^{7,8}

Optimized PCR for target the sequence next to the whole genome of *gyrB* gene were done in 3 isolates from TB patients in 2013 using the five primer settings, Using *Mycobacterium tuberculosis* H37Rv as a positive control and mix reaction suspension without DNA as a negative control.

DNA sequencing and analysis of DNA sequence, PCR product is purified using QIAquickspin PCR purification kit (QIAGEN). Eight isolates of *Mycobacterium tuberculosis* strains were conducted sequence analysis of 1.020 base region of *gyrB*, and three isolates sequenced of the next to the whole gene using ABI PRISM 310 System Sequencer. Alignment analysis was carried out using Genetix WIN V.10 program.

RESULTS

All of the 41 isolates (100%) *Mycobacterium tuberculosis* could be detect with PCR 41 strains of clinical isolates were positive *gyrB* conserved region at 1.020-bp (Figure 1, with example of five isolates).

Isolates P-1, P-2, P-3, P-4, P-5, P-6, P-7, P-8 from eight TB patients collected in 2010 have been sequenced analysis on alignment and phylogeny tree, revealed that all of these isolates were almost identically, more than 97 % with homolog nucleotide sequence in 764 bases that have been sequenced, with strain *Mycobacterium tuberculosis* H37Rv and other member of MTBC i.e. *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium canettii*, *Mycobacterium microti*, *Mycobacterium caprae*; on the other hands also closed related to Corynebacterium diphtheriae. These six strains are not identical to Mycobacterium kansasii and other MOTT i.e. *Mycobacterium* avium. *Mycobacterium* intracellulare, *Mycobacterium* scrofulaceum, Mycobacterium asiaticum, **Mycobacterium** smegmatis.

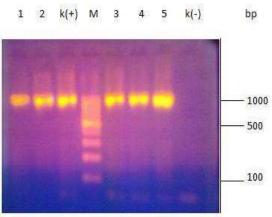


Figure 1

PCR products of *gyrB* gene *Mycobacterium tuberculosis* clinical isolates from sputum of pulmonary TB patients. Amplicon of *gyr* B at 1.020-bp, lane 1, 2, 3, 4, and 5 are amplicon from clinical isolates; M: 100-bp ladder; K+: positive control is amplicon from *Mycobacterium tuberculosis* H37Rv; negative control: suspension of reaction mixture without DNA template.

These strains distance correlated with Nocardia, and no correlated to Gram positive Staphylococcus aureus and Gram bacteria negative bacteria Klebsiella pneumonia and Shigella sp were indicated as the outgroup (Figure 2). In these 764 bases sequenced found a single nucleotide polymorphism (SNP) of isolate P4 at base no. 14 A - to - T, no. 169 G - to - T, no. 423 A - to - T, no. 446 G - to - T, no. 449 A - to - T; isolate P5 at base no. 28 C - to - A, no. 32 A - to- C; and isolate P6 no. 480 G - to - C (Figure 2). The 1.020-bp region position in the whole genome, flanked with sequence of the target by next setting primers which target to almost the whole genome.

The strains PS1, PS2, PS3 isolates from patients that collected at 2013 revealed, in around the 1054 bases a part of the target sequenced of almost the whole gene of *gyrB*, the result of alignment analysis and phylogeny tree similar with the strains from TB patients in 2010, were as followed: three strains isolates from patients almost identically with *Mycobacterium tuberculosis* H37Rv and other members of MTBC; distance correlated with MOTT; and no correlated to Gram positive and Gram negative bacteria, except *Corynebacterium diphtheriae* have the same sequence gene region closed correlated to MTBC. These study also found SNPs in sequence gyrB gene of isolates PS1 at base no. 192 G – to - C, no. 436 C – to - G, no. 466 C – to - A, no. 866 G – to - C; and PS3 base no. 404 A – to – C, no. 410 A – to - C, no. 852 A – to - G. Isolate PS1 and PS2 also found two bases different with strain H37Rv at no. 417-418 AT – to – CC (Figure 2).

Figure 2 indicates sequence alignment of gyrB gene Mycobacterium tuberculosis clinical isolates from sputum of pulmonary TB patients in Indonesia at 2010 and 2013 (Genetix WIN V. 10 program), Mycobacterium tuberculosis H37Rv, other MTBC, MOTT, Corynebacterium diphtheriae, Staphylo coccus aureus, and Gram negative bacteria.

Figure 3 Phylogeny tree of gyrB Mycobacterium tuberculosis clinical isolates of pulmonary TB patients in Indonesia at 2010 and 2013, *M. tuberculosis* H37Rv, MTBC, MOTT, Gram positive and negative bacteria (Genetix WIN V.10 program).

This phylogeny tree of gyrB Mycobacterium tuberculosis from clinical isolates have revealed the closed relation among the MTBC strains, on the other hands need more investigate the other DNA region for differentiate to Corynebacterium spp.

DISCUSSION

All of the 41 (100%) Mycobacterium tuberculosis clinical isolates from pulmonary TB patients in some area in Indonesia were positive for the target gene at 1.020-bp gyrB. Primer MTUB-f and MTUB-r were used for amplification of the 1.020-bp fragment of the gyrB gene Mycobacterium tuberculosis similar to investigation Chimara et al., 2004 and Niemann et al., 2000. The DNA region of gyrB gene, 1.020-bp fragments is amplified with species-specific primers MTUBf and MTUBr which do not generate amplicons from other species of mycobacteria.^{7,14}

In this study have showed the similar result of alignment analysis in the sequence 1.020 fragment and a part of almost the whole genome, that all clinical isolates *Mycobacterium tuberculosis* showed almost identically with H37Rv wild type and members of MTBC, and there are a little number of SNP; however close related to *Corynebacterium diphtheriae*, that could be need to investigate the other region of DNA fragment analyzed in *gyrB* gene regions. The nucleotide sequence of polymorphism in *gyrB* gene represents a unique marker that facilitates the differentiation of the MTBC.^{4,8}

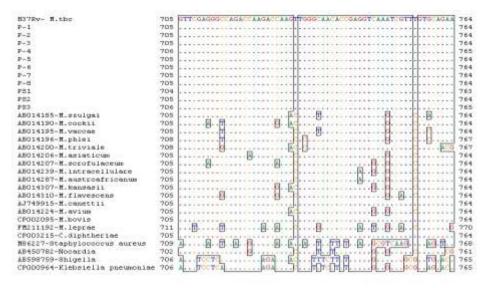
Convenient system for bacterial identification using species-specific *gyrB* gene sequences, because of *gyrB* gene rarely transmitted horizontally, is distributed ubiquitously among bacterial species, its molecular evolution rate is higher than 16S rRNA, and the

NJ7Ry- M.tbc	1 TCCACCCGGCTCGAAGTCGAG-ATCAAGCGCGACGGGTACGAGTGGTCHCAGGTTTATGA 5	9
P-1	1	
P-2	1	
P-3 P-4	1 1 1	
P-5	1	
P-6	1	
P-7	1	
P-8	1	
P51 P52	1	
PS3	1	
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AB014190-M.cookii	1	
AB014195-M.vaccae AB014196-M.phle1	1	
AB014196-A.phiei AB014200-M.triviale	1	
AB014206-M.asiaticum	1 . 0	
AB014207-M.scrofulaceum	1	
AB014239-M.intracellulare		
AB014287-M.austroafricanum AB014307-M.kansasii		
AB014310-M.flavescens	1	
AJ749915-M.canettii	1	
AB014224-M.avium	1	
CP002095-M.boviz FM211192-M.leprae	1	
CP003215-C.diphtheriae	1	
M66227-Staphylococcus aureus	1 -TGTTTTACA, GCTGG. TAA TCG GTGGC ATAC. AA. TATCTG. TGG. T. AC 5	
AB450782-Nocardia	1	
AB598759-Shigella	1CGTTCTG, AC, C, GGCG, TAAATTT, ACGAT, ACTCCTAT, AAG, STCCG SI	
CP000964-Klebsiella pneumoniae	1Carcerolaeleedeederaaanii Eallaanilaereeraalaaasas	·
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AB014195-M.vaccae		19
AB014196-M.phlei		19
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AB014239-M.intracellulare		19
AB014287-M.austroafricanum		19
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AJ749915-N.canettii		19
AB014224-M.avium		19
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M66227-Staphylococcus aureus		19
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AB598759-Shigella		10
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H37Rv- M.tbc	120 SCGGTTCTGGGCCGACCCCGCTGTTTTCGAA-ACCACGGAATACGACTTCGAAACCGTCG 1	78
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P-3 P-4		78
P-5	120	78
P-6		78
P-7 P-8		78 78
P31		77
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PS3		79
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AB014196-M.phlei		78
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FM211192-M.leprae		78
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CPOOO964-Klebsiella pneumoniae	111 TTATCCAGC. CGAT. A. AAAG, CCACAAAC, G, TTTAT, TGC GTG, ACCGCAG, CGC 1	70
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37Rv- M.thc	179	CCGCCGG TGCAAGAGATGGCGTTCCTCAACAAGGGGCTGACCATCAACCTGACCGACG
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-7	179	
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81	178	
*82	179	
83	180	
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B014239-N.intracellulare	179	R R
B014287-M.austroafricanum	179	N
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137Rv- H.tbc	239	IGAGGGTGACCCAAGACGAGGTCGTCGACGAAGTGGTCAGCGACGTCGCCGAGGCGCC
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B014200-M.triviale	239 0	СС
B014206-H.esiaticum	239	
B014207-H.scrofulaceum		
B014239-H.intracellulare	239	
B014287-M.austroafricanus	A115 1 1 1	
B014307-N.kansasii	239	
B014310-M.flavescens	239	
J749915-H.conettii	239	
B014224-N.avium	239 -	······································
P002095-M.bovis	239	
H211192-M.leprae	239	<u>E</u> _+ EEEEEEE _
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86227-Staphylococcus aureus	240 T	T. CH.M KA TI, TATI. AN TAT AA . T. A. AG AG GTA TAGA TTG TT
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References to 20	1993	
137Rv- M.the	297 -	GAAGTCOSCARDTDAACBCOCCAGCGAA TCCACTGCACCOCACAARDTTAAGA
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B014195-H.vaccae	297 -	
B014196-M.phlei B014200-M.triviale	297 -	
B014200-R.triviale B014206-H.asiaticum	297 -	
B014205-R.asiaticum B014207-M.scrofulaceum	297 -	
B014239-M.intracellulare	297 -	d
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B014287-H.austroafricanus	297 -	
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LBO14287-M. Kansasi LBO14310-M. Kansasi LBO14310-K. flavescens J749915-M. canettii LBO14224-M. avium P002095-M. bovis M211192-M. Lepras P003215-C. diphtherias D66227-Staphylococcus aureus	297 - 297 - 297 - 297 - 299 T 299 T 300 T 297 - 291 A	

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P-5	350	· · · · · · · · · · · · · · · · · · ·	
P-6	350		
P-7	350		
P=8	350		
P31	349		
P92	350		
P93	351		
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AB014196-M.phlei	353		G
AB014200-M.triviale	350		
AB014206-M.asiaticum	350		G
AB014207-H.sccofulaceum	350		B
AB014239-M.intracellulare	350		G
M014207-H.austroafricanum	350	The second se	
AB014307-H.kansas11	350	G	
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AB014224-M.evium	350	H. H	
CP002095-E.bovis	350		
M211192-M.leprae	356		
CPOD3215-C.diphtheriam	350		
M86227-Staphylococcus aureus	360	AGA T	TTAT. B BAS. AT.
AB450782-Nocardia	347	R. R. M. C. C. C. C. Marter	
kB598759-Shigella	351	AGALTA	R. T.B MAG. M.
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P-2	410	***************************************	
P=3	410		*****************
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P-5	410		
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AB014196-M.phle1	413	······································	TGAG
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AB014206~E.asiaticum	410	- F.F ACAG	
AB014207-H.sccofulaceum	410	- H.H	
AB014239-M.intracellulare	410	A.H	B
AB014287-E.austroafricanum	410		Gad.
AB014307-E.kansasii	410		A
AB014310-H.flavescens	410		F
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ABO14224-H.avium 2PO02095-H.bovis FN21192-H.lepras 2PO03215-C.diphtherlas HS6227-Staphylococcus aureus AB450782-Nocardia	416 410 420 407		ТО
ABO14224-M.avium POD2095-M.bovis FX11192-M.leprae CPO03215-C.diphtheriae M86227-Staphylococcus aureus AB450782-Nocardis AB598759-Shigelia	416 410 420 407 411		
ABO14224-M.avium POD2095-M.bovis FX11192-M.leprae CPO03215-C.diphtheriae M86227-Staphylococcus aureus AB450782-Nocardis AB598759-Shigelia	416 410 420 407 411		ТО
ABO14224-M.avium PO02095-M.bovis PO02095-M.bovis P003215-C.diphtheriae M86227-Staphylococcus aureus AB50782-Nocardia AB598759-Shigella P000964-Klebsiella pneumoniae	416 410 420 407 411		
ABO14224-M.avium PO02095-M.hovis YM211192-M.leprae PO03215-C.diphtherlae M86227-Staphylococcus aureus AB450782-Nocardia AB598759-Shigella PO00964-Klebsiella pneumoniae M378y- M.tbo -1	416 410 407 411 411 470 470		
ABO14224-M.avium PO02095-M.bovis PO03215-C.diphtherias PO03215-C.diphtherias M85227-Staphylococcus aureus M8598759-Shigella PO0964-Klebsiella pneusonias M378v- M.tbo P-1 -2	416 410 407 411 411 470 470 470		
AB014224-H.avium POD2095-H.bovis FX211192-H.leprae CP003215-C.diphtheriae M86227-Staphylococcus aureus AB598759-Shigella CP000964-Klebsiella pneusoniae 8378v- M.tbo P-1 P-2 F-3	\$16 \$10 \$20 \$07 \$11 \$11 \$11 \$70 \$70 \$70 \$70 \$70		
ABO14224-M.avium PO02095-M.bovis PO03215-C.diphtheriae B06227-Staphylococcus aureus AB450782-Nocardia AB598759-Shigella PO00964-Klebsiella pneusoniae B378v- M.tbo P-1 -2 -3 -4	416 410 420 407 411 411 470 470 470 470 470 470		
ABO14224-M.avium PO02095-M.bovis PO02095-M.bovis PO02215-C.diphtheriae 886227-Staphylococcus aureus M850782-Nocardia M8598759-Shigella PO00964-Klebsiella pneusoniae 8378v- M.tbo P-1 -2 -3 -4 -5	616 610 620 607 611 611 670 670 670 670 670		TI
AB014224-H.avium PPOD2095-H.bovis PPOD2095-H.bovis PPO1192-H.leprae 2PO03215-C.diphtheriae 886227-Staphylococcus aureus AB598759-Shigella 2P000964-Klebsiella pneusoniae 8378v- M.tbo P-1 F-2 F-3 F-4 F-5 F-6	616 610 620 607 611 611 670 670 670 670 670 670 670		
ABO14224-M.avium PO02095-M.bovis PO03215-C.diphtheriae B06227-Staphylococcus aureus AB450782-Nocardia AB598759-Shigella P000964-Klebsiella pneusoniae B375v- M.tbo P-1 -2 -3 -4 -5 -6 -7	616 610 620 607 611 611 611 670 670 670 670 670 670 670 670 670		
AB014224-M.avium P002095-M.bovis P003215-C.diphtheriae R06227-Staphylococcus aureus AB450782-Nocardia AB598759-Shigella CP000964-Klebsiella pneusoniae R37Rv- N.tbo P-1 -2 -3 P-4 -5 F-6 -7	\$16 \$20 \$07 \$11 \$11 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70		
AB014224-M.avium PPOD2095-M.bovis PPOD2095-M.bovis PPU1192-M.leprae 2P003215-C.diphtheriae 886227-Staphylococcus aureus AB598759-Shigella 2P000964-Klebsiella pneusoniae E37Rv- M.tbo P-1 F-2 F-3 F-4 F-5 F-6 P-7 P-6 E31	\$16 \$20 \$07 \$11 \$11 \$11 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70		
AB014224-M.avium PO02095-M.bovis PO02095-M.bovis M86227-Staphylococcus aureus M86227-Staphylococcus aureus M859759-Shigella PO00964-Klebsiella pneumoniae 8375V-M.tbo -1 -2 -3 -4 -5 -6 -7 -7 -8 82	\$16 \$20 \$07 \$11 \$10 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$7		AGAT. ATT. A. R. A.
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ABO14224-M.avium POD2095-M.bovis POD3215-C.diphtheriae SP003215-C.diphtheriae B85227-Staphylococcus aureus H850782-Nocardia H8598759-Shigella POD0964-Klebsiella pneusoniae B378v- N.tbo P-1 -2 -3 -4 -5 -6 27-7 -8 282 283	\$16 \$20 \$07 \$11 \$10 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$7		
ABO14224-M.avium POD2095-M.bovis POD3215-C.diphtheriae S05227-Staphylococcus aureus HS50725-Nocardia HS598759-Shigella POD0964-Klebsiella pneumoniae E37FW-M.tho -1 -2 -3 -4 -5 -6 -7 -8 23 HS014185-M.stulgai	\$16 \$10 \$20 \$07 \$11 \$11 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70		
ABO14224-M.avium PO02095-M.bovis PO02095-M.bovis M86227-Staphylococcus aureus M86227-Staphylococcus aureus M859759-Shigella PO00964-Kiebsiella pneumoniae 8378v- M.tbo -1 -2 -3 -4 -5 -6 -7 -8 82 83 M014185-M.srulgai M014190-M.cookii	\$16 \$10 \$20 \$11 \$11 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70		
ABO14224-M.avium POD2095-M.bovis POD3215-C.diphtheriae S22-Staphylococcus aureus Ma50282-Nocardia Ma598759-Shigella PO00964-Klebsiella pneusoniae S378v- M.tbo P-1 -2 -3 -4 -5 -6 27 -7 -8 283 MBO14185-M.stulgai MBO14195-M.vaccas	\$16 \$20 \$20 \$11 \$11 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70 \$70		
ABO14224-M.avium POD2095-M.bovis POD3215-C.diphtheriae S0527-Staphylococcus aureus HS50725-Nocardia HS598759-Shigella POD0964-Klebsiella pneumoniae S37FW- M.tho -1 -2 -3 -4 -5 -6 -7 -6 23 HS014185-M.stulgai HS014195-M.vaccas HS014195-M.vaccas HS014195-M.phei	416 410 4207 411 411 470 470 470 470 470 470 470 470 470 470		
H014224-H.avium PO02095-H.bovis PO02095-H.bovis P002215-C.diphtheriae M6227-Staphylococcus aureus M6527-Staphylococcus aureus M659759-Shigella P000964-Klebsiella pneusoniae 8375W- M.tbo P-1 -2 -3 -4 -5 -5 -6 -7 -6 -7 -6 -7 -6 -7 -8 -8 -9 -8 -9 -9 -8 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9	416 410 407 407 411 411 470 470 470 470 470 470 470 470 470 470		
H014224-H.avium P002095-H.bovis P003215-C.diphtheriae B0227-Staphylococcus aureus H050782-Nocardia H050782-Nocardia H050782-Nocardia B598759-Shigella P000964-Klebsiella pneusoniae B378v-N.tbo -1 -2 -3 -4 -5 -6 -7 -6 -7 -8 -8 -8 -8 -9 -8 -9 -8 -9 -8 -9 -8 -9 -8 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9	416 410 420 411 411 411 470 470 470 470 470 470 470 470 470 470		
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ABO14224-M.avium PO02095-M.bovis PO02095-M.bovis PO02215-C.diphtheriae M86227-Staphylococcus aureus M85978-Nocardia M859759-Shigella PO00964-Klebsiella pneumoniae M87870-M.tbo P-1 -2 -3 -4 -5 -6 -7 -6 2-7 2-8 M014185-M.stulgai M8014195-M.vaccas M8014195-M.vaccas M8014195-M.vaccas M8014195-M.vaccas M8014207-M.sisticum M8014207-M.sisticum M8014207-M.sisticum	416 410 420 407 411 411 411 470 470 470 470 470 470 470 470 470 470		
ABO14224-M.avium POD2095-M.bovis POD2095-M.bovis POD3215-C.diphtheriae B522-Staphylococcus aureus H850782-Nocardia H8598759-Shigella POD0964-Klebsiella pneusoniae B37FW- M.tbo -1 -2 -3 -4 -5 -6 -7 -6 23 23 24 10014185-M.srulgai H8014195-M.vaccas H8014195-M.vaccas H8014195-M.vaccas H8014195-M.srila H8014200-M.srila H801420-M.srila H801420-M.srila H801420-M.srila H801420-M.srila H801420-M.srila H801420-M.srila H801420-M.srila H801420-M.srila H801427-M.austoafricasum	416 410 420 407 411 470 470 470 470 470 470 470 470 470 470		
ABO14224-M.avium POD2095-M.bovis POD3215-C.diphtheriae S05227-Staphylococcus aureus HS50725-Shigella POD0964-Klebsiella pneumoniae S375V-M.tho -1 -2 -3 -4 -5 -6 -7 -5 -6 -7 -5 -6 23 HD14185-M.srulgai HD14195-M.vactae HB014196-M.cookii HB014196-M.shiitum HB014200-M.triviale HB014220-M.strofulacum HB014229-M.intracelulare HB014239-M.intracelulare HB014239-M.intracelulare HB014239-M.intracelulare	416 410 420 407 411 411 470 470 470 470 470 470 470 470 470 470		
AHO14224-H.avium PPOD2095-H.bovis PPOD2095-H.bovis HE211192-H.lepres PPO3215-C.diphtherise H86227-Staphylococcus aureus H859789-Shigella PPO09964-Klebsiella pneusonise H87FW- N.tbo P-1 -2 -3 -3 -4 -5 -6 -7 P-6 P-1 P-7 P-6 P51 H8014195-H.szulgai H8014195-H.vaccas H8014195-H.vaccas H8014195-H.vaccas H8014196-H.sisticum H8014207-H.sisticum H8014207-H.storofilaceum H8014207-H.storofilaceum H8014207-H.storofilaceum H8014207-H.storofilaceum H8014207-H.storofilaceum	416 410 420 407 411 411 470 470 470 470 470 470 470 470 470 470		
AHO14224-H.avium PPOD2095-H.bovis PPOD2095-H.bovis PPO121192-H.lepres 2PO03215-C.diphtheriae R86227-Staphylococcus aureus R8627-Staphylococcus aureus R8759-Shigella 2PO00964-Klebsiella pneusoniae E375V-N.tbo P-1 F-3 F-4 F-3 F-4 F-5 F-6 P-7 P-7 P-8 P31 P82 P33 AHO14185-H.szulgai AHO14195-H.vaccas AHO14195-H.vaccas AHO14196-H.phiei AHO14196-H.phiei AHO14207-H.ariticum AHO14207-H.ariticum AHO14237-H.austroafricanum AHO14307-H.kansail AHO14307-H.kansail	416 410 407 411 411 411 411 470 470 470 470 470 470 470 470 470 470		
AHO14224-H.avium CPOD2095-H.bovis PPOD215-C.diphtheriae R86227-Staphylococcus aureus AH50782-Nooardia AH50782-Nooardia AH50782-Nooardia CPOD0964-Klebsiella pneumoniae B375V-N.tho P-1 P-2 F-3 F-4 F-5 F-6 P-7 P-7 P-9 P31 P32 P33 AH014185-H.szulgai AH014195-H.vaccas AH014200-H.triviale AH01420-H.triviale AH01420-H.siaticum AH01428-H.sutroafricanum AH01428-H.sutroafricanum AH01428-H.sutroafricanum AH01428-H.tivecens AJ749915-E.conettii AH01424-H.sylum	416 410 420 420 411 411 411 470 470 470 470 470 470 470 470 470 470		
AHO14224-H.avium PPOD2095-H.bovis PPOD2095-H.bovis HE211192-H.lepree SPOD2215-C.dipbtherise HE450782-Nocardia AH598759-Shigella CPO00964-Klebsiells pneusonise HE37FW- N.tbo P-1 2 3 3 4 5 5 6 P-7 P-6 P-1 HE014195-H.szulgai AHO14195-H.vaccas AHO14195-H.vaccas AHO14195-H.vaccas AHO14195-H.vaccas AHO14195-H.sisticum AHO1420-H.si	416 410 407 411 411 470 470 470 470 470 470 470 470 470 470		
ARO14224-H. avium CPOD2095-H. hovis PR01192-H. lepres CPO03215-C. diphtherias M86227-Staphylococcus aureus AB598759-Shigella CPO00964-Riebsiella pneusonias E37Ev- M.tbo P-1 F-3 F-4 F-5 F-6 F-7 P-7 P-7 P-8 P31 P82 P93 ARO14195-H.srulgai AB014196-H.phisi AB014196-H.phisi AB014200-H.triviale AB01420-H.triviale AB01420-H.triviale AB014237-H.austoafricasum AB014237-H.austoafricasum AB014237-H.austoafricasum AB014237-H.austoafricasum AB014237-H.austoafricasum AB014237-H.austoafricasum AB014310-H.flavescens AJ749915-H.constil AB014224-H.avium CF002095-H.bovis	416 410 407 411 470 470 470 470 470 470 470 470 470 470		
AHO14224-H.avium CPOD2095-H.bovis PPOD215-C.diphtherise R86227-Staphylococcus aureus R455782-Nocardia R455782-Nocardia R559759-Shigella CPOD0964-Klebsiella pneumonise B375V-N.tbo P-1 P-2 F-3 P-4 P-5 P-6 P-7 P-6 P51 P52 P53 R5014185-H.srulgai AHO14195-H.vaccas AHO14195-H.vaccas AHO14195-H.sinticum AHO1420-H.triviale AHO1420-H.triviale AHO1420-H.sintracellulare AHO14287-H.austroafricanum AHO14310-H.Tlavescens AJ749915-E.conettii AHO1430-H.spist H01432-H.ayium CPO02295-H.bovis F1211192-H.leprae	416 410 407 411 411 411 470 470 470 470 470 470 470 470 470 470		
AHO14224-H.avium PPOD2095-H.bovis PPOD2095-H.bovis PPOD215-C.diphtheriae B6227-Staphylococcus aureus H850782-Nocardia H850782-Nocardia H850782-Shigella PPOD964-Klebsiella pneusoniae B7Fw- N.tbo P-1 F-2 F-3 F-4 F-5 F-6 P-7 F-7 F-7 F-7 F-8 H014185-H.stulgai H014190-H.cookii H014190-H.cookii H014206-H.asiaticum H8014206-H.asiaticum H8014207-H.astroafricanum H8014207-H.astroafricanum H8014207-H.austroafricanum H8014207-H.sturesens H8014287-H.austroafricanum H801427-H.austroafricanum H801427-H.conetiil H801422-H.leptae CF00205-H.bovis FM21192-F.leptae CF00215-C.diphtheriae M6027-Staphylococcus aureus	416 410 407 411 411 411 410 470 470 470 470 470 470 470 470 470 47		
ABO14224-M.avium POD2095-M.bovis POD3215-C.diphtheriae S0227-Staphylococcus aureus H8527-Staphylococcus aureus H852782-Nocardia H8598759-Shigella POD0964-Klebsiella pneusoniae S7FW- M.tbo -1 -2 -3 -4 -5 -6 -7 -6 23 23 H0014185-M.srulgai H8014196-M.shiel H8014196-M.shiel H8014196-M.shiel H801420-M.triviale H801426-M.shiel H801426-M.shiel H801427-M.austoafricasum H801427-M.austoafricasum H801430-M.triviale H801427-M.austoafricasum H801430-M.flavescens H801430-M.flavescens H801430-M.flavescens H801430-M.flavescens H801427-M.austoafricasum H801430-M.flavescens H901430-M.flavescens H901430-M.flavescens H901430-M.flavescens H901430-M.flavescens H9014224-M.avium PO02055-M.bovis FF003215-C.diphtherise H850742-Nocardis	416 410 407 411 470 470 470 470 470 470 470 470 470 470		
AD14224-H.awium CPOD2095-H.bovia FM211192-H.leprae CPOD215-C.diphtheriae M86227-Staphylococcus aureus AB598759-Shigelia CPOD964-Elebsielia pneusoniae E37Ev- M.tbo P-1 F-2 F-3 F-4 F-5 F-6 P-7 P-7 P-6 P53 AB014185-H.stulgai AB014195-H.vaccas AB014195-H.vaccas AB014195-H.stulgai AB014207-H.austroafilareum AB014207-H.stroafilareum AB014207-H.stroafilareum AB014207-H.stroafilareum AB014207-H.stroafilareum AB014287-H.austroafilareum AB014287-H.austroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014287-H.stroafilareum AB014224-H.stroafilareum AB014224-H.stroafilareum AB014224-H.stroafilareum AB014224-H.stroafilareum AB01424-H.stroafil	416 410 407 411 411 411 410 470 470 470 470 470 470 470 470 470 47		

837Rv- E.tbo	526	ACCATCANCACCACGAGGGGGGCACCCACGAROAGGGCTTCCGCAGCGCGCTGACGTCG
P-1	526	
P-2	526	
P-3	526	
P-4 P-5	527	
P-5 P-6	526	
8-7	526	
P-0	526	
P31	525	
P92	526	
PS3	527	
AB014185-N.szulgai AB01419D-N.cookii	526	m. Frank a s
AB014195-N.vaccae	526	Taxa.
AB014196-N.phlei	529	
AB01420D-M.triviale	529	Ingenerative Bernstein generative Berger Anne Sterre Berne
AB014206-N.asiaticum	526	0.0
AB014207-N.scrofulaceum	526 526	
AB014239-N.intracellulare AB014287-N.austroafricanum	526	
AB014307-N.kansasii	526	
AB014310-M.flavescens	526	
AJ749915-N.canetti1	52.6	
AB014224-M.evium	526	
CP002095-N.bovis	526	
FM211192-N. leprae	532	······································
CPOO3215-C.diphtheriae N86227-Staphylococcus aureus	526 536	
AB450782-Nocardia	523	
AB598759-Shigelia	527	M Free ad En. R. R. R. Fre. M
CP000964-Klebsiella pneumoniae	537	W
H37Rv- E.tbc	586	STOUTGAA AAGTA G AAGGA G AAG TA TGAAGGA AAGHA AA T A
P-1	586	
P-2	586	······································
P-3	586	······································
P-4 P-5	587	······································
P-5 P-6	586	H
P-0 P-7	586	
P-0	586	μ
P31	585	
PS2	586	
P93	587	······································
AB014185-M.sculgai	586	······································
kB014190-M.cookii	586	·····································
AB014195-N.vaccae	586	
AB014196-N.phlei AB014200-N.triviale	589	
AB014206-N.asiaticum	586	
AB014207-N.scrofulaceum	586	
AB014239-M.intracellulare	586	
kB014287-N.austroafricanum	586	H.D
AB014307-H.kansasii	586	B
AB01431D-N.flavescens	586	B
AJ749915-N.canett11 AB014324-N.avium	586	
CP002095-N.bovis	586	р
FM211192-N.leprae	592	
CP003215-C.diphtheriae	586	
NS6227-Staphylococcus aureus	596	
AB450782-Nocardia	583	
AB598759-Shigella CP000964-Elebsiella pneumoniae	587 587	ACCO
Croooso4-kiensiella pheumoniae	201	world for hearing and a found for the foundation of the form of a
HJ7Rv- M.tbc	645	GOTGACGATAT COSAAGGCCTGGCCG TOTGATCT GOTGAAGGTCAGCGAACCGCA 7
P-1 P-2	645 645	
P-3	645	
P = 4	646	
P-5	645	
P-6	645	······································
P-7	645	
P-6 P51		
P 21 1	645	
	644	
PS2	644 645	3
	644	
PS2 PS3	644 645 646	
PS2 PS3 AB014185-N.srulgai	644 645 646 645 645 645	
P52 P53 AB014185-N.srulgai AB014190-N.cookii AB014195-N.vaccae AB014196-N.phlei	644 645 646 645 645 645 648	
P52 P33 AB014185-N.szulgai AB014190-N.cookii AB014195-N.vaccae AB014196-N.phlei AB014300-N.triviale	644 645 646 645 645 645 648 648	
P52 P53 AB014185-M.srulgai AB014190-M.cookii AB014195-M.vaccae AB014200-M.triviale AB014200-M.triviale AB014206-M.asiaticum	644 645 645 645 645 648 648 648	
P52 P53 AB014185-M.srulgai AB014190-M.cookii AB014195-M.vaccas AB014196-M.phlei AB014200-M.triviale AB014206-M.stistrum AB014207-M.scrofulaceum	644 645 646 645 645 645 648 648 648 645	
P52 P53 AB014185-N.srulgai AB014195-N.vaccas AB014195-N.vaccas AB014196-N.phisi AB014300-N.stiviale AB014206-N.stiviale AB014207-N.scrofulaceum AB014239-N.intracellulare	644 645 646 645 645 645 648 648 645 645 645	
P52 P53 AB014185-M.srulgai AB014190-M.cookii AB014195-M.vaccas AB014196-M.phlei AB014200-M.triviale AB014206-M.stistrum AB014207-M.scrofulaceum	644 645 646 645 645 645 648 648 648 645	
P52 P33 ABO14185-M.srulgai ABO14195-M.vaccae ABO14195-N.vaccae ABO14196-N.vaccae ABO14200-N.triviale ABO14200-N.asiaticum ABO14207-M.acrofilaceum ABO14207-M.acrofilaceum ABO14207-M.austroafricanum	644 645 645 645 645 648 648 648 645 645 645 645	
PS2 PS3 ABO14185-M.srulgai ABO14190-M.cookii ABO14196-M.vaccme ABO14306-M.valet ABO14300-M.trivimle ABO14207-M.scrofulaceum ABO14237-M.scrofulaceum ABO14239-M.intracellulare ABO14239-M.austroafricanum ABO14237-M.austroafricanum	644 645 645 645 645 645 645 645 645 645	
P52 P33 AB014195-M.srulgai AB014195-M.vaccas AB014195-M.vaccas AB014306-M.shlei AB014306-M.sciaticum AB014307-M.scrofulaccum AB014237-M.scrofulaccum AB014237-M.sciaticum AB014237-M.sustroafricanum AB014237-M.sustroafricanum AB014310-M.flavezcans AJ749915-M.canettii AB014324-M.avium	644 645 645 645 645 645 645 645 645 645	
P52 P33 AB014195-N.srulgai AB014195-N.vaccae AB014195-N.vaccae AB014206-N.phei AB014206-N.sristrum AB014207-N.scrofulaceum AB014207-N.scrofulaceum AB014239-N.intracellulare AB014237-N.austroafricanum AB014207-N.kansmaii AB014310-N.flavescans AJ749915-N.canettii AB01424-N.avium CP002095-N.hovis	644 645 645 645 645 645 645 645 645 645	
P52 P33 AB014195-M.srulgai AB014195-M.vaccae AB014195-M.vaccae AB014200-M.stiviale AB014200-M.stiviale AB014207-M.scrofulaceum AB014207-M.scrofulaceum AB014287-M.austroafricanum AB014287-M.austroafricanum AB014300-M.flavecens AB014300-M.flavecens AJ749915-M.canettii AB014224-M.avium CP002095-M.bovis FM211192-M.leprae	644 645 645 645 645 645 645 645 645 645	
P52 P33 AB014195-M.srulgai AB014195-M.scokii AB014195-M.vaccme AB014306-M.shlei AB014206-M.sciaticum AB014207-M.scrofulaceum AB014207-M.scrofulaceum AB014239-M.intracellulare AB014307-M.sciaticanum AB01428-M.austroafricanum AB014310-M.flavezens AJ749915-M.canettii AB014310-M.flavezens AJ749915-M.canettii AB014224-M.avium CP0032155-C.diphtheriae	644 645	
P52 P33 ABO14195-N.srulgai ABO14195-N.vaccae ABO14195-N.vaccae ABO14206-N.phei ABO14206-N.sristroum ABO14207-N.scrofulaceum ABO14207-N.scrofulaceum ABO14207-N.scrofulaceum ABO14237-N.austroafricanum ABO1427-N.austroafricanum ABO1427-N.austroafricanum ABO1427-N.scroetii ABO14224-N.svium ABO14224-N.svium CF0002055-N.bovis FM21192-N.leprae CP003215-C.diphtheriae	644 645 645 645 646 648 648 6485 6485 6485 6445 645	
P52 P33 AB014195-M.srulgai AB014195-M.scokii AB014195-M.vaccme AB014306-M.shlei AB014206-M.sciaticum AB014207-M.scrofulaceum AB014207-M.scrofulaceum AB014239-M.intracellulare AB014307-M.sciaticanum AB01428-M.austroafricanum AB014310-M.flavezens AJ749915-M.canettii AB014310-M.flavezens AJ749915-M.canettii AB014224-M.avium CP0032155-C.diphtheriae	644 645	



2E

Figure 2A,B,C,D,E

Sequence alignment of gyrB gene Mycobacterium tuberculosis clinical isolates from sputum of pulmonary TB patients in Indonesia at 2010 and 2013 (Genetix WIN V. 10 program), Mycobacterium tuberculosis H37Rv, other MTBC, MOTT, Corynebacterium diphtheriae, Staphylococcus aureus, and Gram negative bacteria.

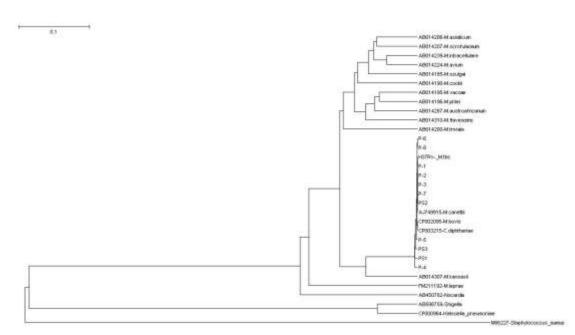


Figure 3

Phylogeny tree of gyrB Mycobacterium tuberculosis clinical isolates of pulmonary TB patients in Indonesia at 2010 and 2013, M. tuberculosis H37Rv, MTBC, MOTT, Gram positive and negative bacteria (Genetix WIN V.10 program).

essential roles of the gyrB product in DNA replication and transcription functions against the introduction of foreign gyrB. The gyrB sequences have high correlation between the the phylogenetic distance and the total genome DNA-DNA homology analyzed by hybridization.^{17,21} DNA topoisomerase are essential for DNA replication, transcription, recombination, repair and control the level of

supercoiling by cleaving and resealing the phosphordiester bond of DNA. They are classified into type I and type II according to enzymatic properties. The bacterial DNA gyrase is a type II topoisomerase that is capable of introducing negative supercoilling into a relaxed closed circular DNA molecule. DNA gyrase consists of two subunit proteinactive form enzymes in the hetero-quaternary

complex structure of A2B2, A protein (GyrA) and B protein (Gyr B) that coding by gyrA and gyrB gene, only in prokaryotes.^{16,17,19} In M. tuberculosis presence only single topoisomerase I and DNA gyrase as a type II DNA topoisomerase.^{19,21,23} Expression of virulence genes depend on topological status of the genome, the topoisomerase as sensor of influence supercoils the specific gene expression.¹⁹ The high validity gyrB of sequences as taxonomic marker was evaluated mainly: the rate of their base substitutions and the consistency of the results of gyrB-based analysis. Protein-coding genes evolve faster than rRNA genes because synonymous substitutions mainly at the third positions of codons in the protein-coding genes are permitted without causing any changes the amino acid sequences. The average base-substitution rate of 16S rRNA genes was 1% per 50 million years, while gyrB was estimated 0.7 - 0.8% per one million years, therefore some species with identical 16S rDNA sequences can be differentiated using their gyrB sequences.¹⁷ The two genes encoding DNA gyrase in Mycobacterium tuberculosis are present next to each other in the genome, with gyrB of gyrA, primary transcript is upstream dicistronic with multiple promoters that appear to fine-tune transcription, the gyr genes in Mycobacterium tuberculosis as in other species are subject to autoregulation, albeit with slower kinetics, probably reflecting the metabolism of organism.^{16,19,21,24} T slower The most probable translational start codon for M. tuberculosis GyrB is GTG (Val) which results in translation of a protein of 674 amino acids (74 kDa) at position 5240-5242 in M. tuberculosis genome sequence.16

Mycobacterium tuberculosis complex (MTBC) are the causative agents of tuberculosis in humans and animals, genetic close relationship, but differ in epidemiology pathogenicity, geographic range, host preferences, and in importance for tuberculosis disease in human. MTBC includes *Mycobacterium* tuberculosis, M. bovis subsp.bovis, M. bovis subsp. caprae, M. bovis BCG vaccine strain, *M. africanum (subtype I and II), M. microti, M. canettii, M. pinnipedii.*^{6,7,9,26} Routine differentiation is still base on phenotypic characteristics are time-consuming. Species belonging to MTBC cannot be differenteated by small subunit rRNA (16S rRNA) or internal trancribed spacer (ITS) 16-23S rRNA sequencing.⁵ Niemann et al. 2000 showed the result of DNA sequencing on gene region 1.020 bases of gyrB *Mycobacterium* gene tuberculosis and Mycobacterium africanum subtype II have an gyrB sequence that facilities identical discrimination from the other species.⁸ All

members of MTBC are identical sequence gene, closely related gene with *gyrB* discriminatory regions in 1.020 bases. Base on these revealed, DNA sequence of species-specific region on 1.020-bp of *gyrB* gene *Mycobacterium tuberculosis* could be sole as the primer design for nucleic acid amplification that purpose to detect and identificate MTBC.

The gyrB could be useful for differentiation of MTBC and MOTT. The gyrB-based phylogeny was better than ITS-based phylogeny. The gyrB sequence of MTBC *M. tuberculosis*, *M. bovis*, *M.africanum*, *M. microti* found substitution at four sites, at 675 in *M. microti*, *M. bovis* at 756, at 1410 of *M. bovis*, *M. tuberculosis* at 1450, these substitutions can be regarded as the result of naturally occurring divergent evolution. The quantitation analysis gyrB-based method more useful than 16S rDNA-based method because the copy number of gyrB is single, while that of 16S rDNA is variable.^{9,17}

Based on sequenced genome of MTBC and outside the MTBC (M. leprae, M. ulcerans, M. avium, M. paratuberculosis, M. marinum, and fast-growing *M. smegmatis*, the most distant of sequenced mycobacterial genomes the are minimally related by 60% DNA/DNA homology, and comparative genomic analysis has shown that gene loss is a significant part of the ongoing evolution of the slow-growing mycobacterial pathogens. Single nucleotide polymorphisms (SNPs) can result a silent amino acid substitution which the protein sequence in remains unchanged, synonymous or can alter the protein coding sequence (non synonymous) and act as a substrate for evolutionary selection. The identification of SNP markers for study of evolution, pathogenesis, and epidemiology in clinical M. tuberculosis and M. bovis, the ratio of SNPs type within a genome can act as a molecular clock, the high ratio of non synonymous to synonymous mutations across coding sequences suggests a recent divergence of M. tuberculosis and M. bovis. The prominent role of genomic deletion relative to M. tuberculosis i.e. *M. bovis* contains 66.037 bp less than *M*. tuberculosis H37Rv, genomic deletion can be used to reconstruct phylogeny trees. Genomic behave unidirectional deletion as event polymorphism which represent one time events in the evolution, can serve as robust markers of clonal organisms for determining phylogeny classification. MTBC present relatively little genomic diversity, based on SNPs similar with large-sequence polymorphism, genomic flexibility exist within MTBC for specific host adaptation. Genomic deletions seem to several regions of difference (RD) as a genetic instability at a locus.²⁰ Namouchi at 2012 reported the nucleotide substitution is a major mechanism for the

emergence of *M. tuberculosis* pathogenesis, MTBC genome exhibit significant regional variation in the density of SNPs, the region of high SNPs may harbor rapidly evolving genes which positively selected for adaptation to human environment, the extremely conserved genes essential for survival.26 The other advantages of gyrB as a marker differentiation, there were only a little evidence showed that are mutations related to quinolone resistance. 10,12,13,22,25

Gutierrez *et al.*, 2005, The highly successful human pathogen M. tuberculosis has the extremely low level of genetic variation, incongruence among gene phylogenies as mosaic gene sequences, despite its apparent homogeneity its genome appears to be a composite assembly resulting from horizontal gene transfer events predating clonal expansion, with synonymous nucleotide variation in housekeeping genes.15 Members of MTBC are the most successful human pathogens, M. tuberculosis, M. bovis, M. africanum, M. microti, M. pinnipedii, and species M. caprae, these members display different phenotypic characteristics and specific mammalian host ranges, they represent one of the most extreme genetic homogeneity with 0.01%-0.03% synonymous about nucleotide variation, and no significant trace of genetic exchange among them, it is believed the members are clonal progeny of a single successful ancestor that occurred 20.000 to 35.000 y ago. The six housekeeping genes, katG, gyrB, gyrA, rpoB, hsp65, sodA, and sequence of 16S rRNA revealed a smooth strain M. canettii and other members of MTBC form a single species defined by a compact phylogenetic clade.

Goh KS *et al.*, 2006, MTBC has been expanded additional members such as *M.canettii*, *M. pinnipedii*, *M. caprae*, dassie bacillus. *M. africanum*, *M. canettii* infect only human, *M. tuberculosis* infect human may also occasionally infect domestic and wild animals upon exposure. Host range *M. bovis* and *M. caprae* is very broad, causing disease among a wide range of feral, domestic mammals, and human. Analysis of gyrB and hsp65 targets could be used for differentiation of the species within MTBC.¹⁴

CONCLUSION

The nucleotide sequence of *gyrB* gene *Mycobacterium tuberculosis* clinical isolates from sputum of TB patients in Indonesia that collected at 2010 and 2013 revealed almost identically to *Mycobacterium tuberculosis* H37Rv and MTBC; all of 41/ 41 (100%) clinical isolates positive PCR with target 1.020-bp as the specific and conserved gene region.

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