Isolation and Characterization of Partial Sequence of merA Gene from Mercury Resistant Bacterium Klebsiella pneumonia Isolated from Sario River Estuary Manado

by Billy Kepel 1

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Research Article

Isolation and Characterization of Partial Sequence of *merA* Gene from Mercury Resistant Bacterium *Klebsiella pneumoniae* Isolated from Sario River Estuary Manado

[1]Fatimawali, [1]Billy Kepel, [2]Irawan Yusuf, [3]Fatmawaty Badaruddin, [2]Rosdiana Natsir and [3]Debbie Retnoningrum
[1]Faculty of Medicine, Sam Ratulangi University Manado Jl. Kampus Unsrat Bahu Manado 95115,
[2]Faculty of Medicine, Hasanuddin University Makassar Jl. Perintis Kemerdekaan Makassar,
[3]School of Farmacy, Bandung Technology Institute Jl. Ganesa 10 Bandung, Indonesia

Abstract: The most common bacterial mercury resistance mechanism is based on the reduction of Hg^{2+} to Hg^0, which is dependent on the mercuric reductase enzyme (*merA*) activity. The aims of this research were to isolate and characterize *merA* gene fragment of mercury resistant bacteria *Klebsiella pneumoniae* isolate A1.1.1. The gene fragment was amplified by PCR using previously designed primer pairs. Plasmid DNAs were used as template. The result showed that the partial sequence of *merA* gene has been found on plasmid DNA of mercury resistant bacterium *Klebsiella pneumoniae* isolates A1.1.1. The nucleotide sequence of the *merA* gene consists of 285 base pairs (bp) which encodes deduced 94 amino acids of mercury reductase *merA* protein. The *merA* protein sequence of isolate A1.1.1 has 99% similarity with some strains of *Klebsiella pneumoniae* deposited in Gen Bank. There is a gene mutation that causes the deduced amino acid threonine was replaced by serine at position 524 (Thr→Ser) in the *merA* protein of *Klebsiella pneumonia* as the accession number: AAR91471.1.

Keywords: *Klebsiella pneumoniae*, *merA* gene, *merA* protein, mercury resistance bacteria

INTRODUCTION

Mercury is a toxic compound that is widely distributed in the global environment and can accumulate in the food chain (Jan *et al*., 2009). Mercury poisoning has become a problem because of the pollution of mercury in the global environment. Mercury pollution continuously increases from time to time as a result of human activities such as the growth of electronics industry, the increasing use of antimicrobial agents, vaccines, amalgam, cosmetics and the higher activity of gold mines using mercury to extract gold (Jan *et al*., 2009; Schelert *et al*., 2004).

Mercuryis accumulated in soil and water as mercury ions (Hg^{2+}) that can be converted into more toxic methyl mercury by microbial activity. Various conventional techniques have been used to dispose toxic metals including preparation and chemical separation, oxidation-reduction reactions, ion exchange, reverse osmosis, filtration, adsorption using activated carbon, electrochemical and evaporation. However, those techniques were considered ineffective, especially for metal concentrations less than 100 mg/L and also quite expensive and their supporting chemicals become secondary pollutants (Habashi, 1978). Therefore the use of microorganisms to remove heavy metal contamination from mining and industrial wastes should be considered (Keramati *et al*., 2011). Mercury chloride ($HgCl_2$) is often used for research because it is easily soluble but toxic (Schelert *et al*., 2004). Microbial detoxification of mercury occurs by transforming Hg^{2+} to volatile metallic mercury (Hg^0). *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Citrobacteria*, *Klebsiella* and *Rhodococcus* are often used in microbial bioremediation for mercury (Adeniji, 2004).

Detoxification of mercury by mercury-resistant bacteria can occur due to the presence of mercury resistance genes located in *mer* operon unique to each bacterium (Silver and Phung, 1996). Mercury resistance genes are often found in plasmids or transposons (Ravel *et al*., 2000; Nascimento and Chartone-Souza, 2003) and in chromosome (Wang *et al*., 1988). The mercury detoxification is mediated by intracellular protein, mercury reductase (*merA*). Mercury ion is transported from outside the cell by a mercury transporter, merP or merC (Iohara *et al*., 2001; Sasaki *et al*., 2005), which is an extracellular protein that binds to mercury ions and merT, which is an inner membrane protein that transports mercury ions into the cells. Inside the cell, Hg^{2+} is bound through the process of ligand exchange reactions to the active site of flavine disulfide oxido reductase of mercury reductase *merA* (Ravel *et al*., 2000).

Corresponding Author: Fatimawali, Faculty of Medicine, Sam Ratulangi University Manado Jl. Kampus Unsrat Bahu Manado 95115, Indonesia, Tel.: +62-431825502; Fax: +62-431853715

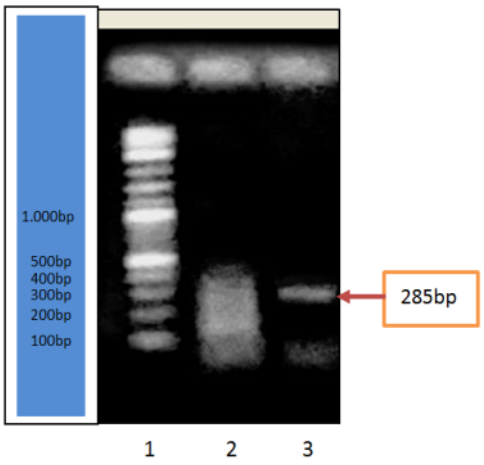

Fig. 1: PCR Product of *merA* gene fragment from *K. pneumoniae* isolate A1.1.1. Lane1. DNA marker; Lane 2.PCR product from *K. pneumoniae* colony pellets; Lane 3. PCR product from plasmid DNA

Mercury reductase catalyzes the reduction of Hg^{2+} to volatile and slightly reactive Hg^0 (Nascimento and Chartone-Souza, 2003). Narrow spectrum mercury-resistant bacteria only have protein *merA*. Broad-spectrum mercury resistant bacteria have *merA* and merB, a lyase organo mercury. The later catalyzes the cleavage of mercury-carbon bond to produce organic compounds and Hg^{2+} (Barkay *et al*., 2003; Barkay and Wagner-Döbler, 2005). There are many gold mining in North Sulawesi use mercury to extract gold from rock or ore and mercury waste is discharged into the environment, causing the surrounding water contaminated by mercury. Mercury contaminated-environment is a suitable source for the growth of mercury resistant bacteria. In our previous study, we isolated mercury resistant bacterium isolate A1.1.1, identified as *Klebsiella pneumoniae* from Sario River estuary. It showed a high mercury reduction activity, i.e., 75, 92 and 99.4% in 1, 12 and 24 h of incubation, respectively in nutrient broth (Fatimawali *et al*., 2011).

This study was aimed to isolate and characterize a *merA* gene fragment from isolate A1.1.1, as a molecular marker for mercury-resistant bacteria. The results of this research can be used as a basis for further study in mercury detoxification process in mercury waste waters.

MATERIALS AND METHODS

DNA was isolated using Plasmid DNA Isolation Kits (Promega, Madison, USA). The partial *merA* gene fragment was amplified using a primer pair previously designed by Ni Chadhain *et al*. (2006) using plasmid DNA as template. The nucleotide sequences of primers were 3'TCCGCAAGTNGCVACBGTNGG5' for A1s-nF and 5'-ACCATCGTAAGRTARGGRAAVA-3' for A5-nR. PCR was done according to previous work with modification (Ni Chadhain *et al*., 2006) using 1.5 mM $MgCl_2$ for 35 cycles and annealing temperature at 54°C. PCR products were analyzed using 1.5% agarose gel electrophoresis.

DNA sequencing was performed at Macrogen Korea. Nucleotide sequence of the *merA* gene fragment and the deduced amino acid sequences were analyzed using online BLAST program ClustalW2 http://blast.ncbi.nlm.nih.gov/Blast.cgi.,http://web.expasy.org/translate/and http://web.expasy.org/.

RESULTS

Amplified PCR products from plasmid, shown in Fig. 1, were analyzed using 1.5% agarose gel electrophoresis. Fragments of *merA* gene with size 285 bp exist in DNA plasmid. Ni Chadhain *et al*. (2006), which uses the same primer obtained *merA* gene in genomic DNA of bacterial isolated from marine sediment. Bacteria developed mercury resistance mechanisms depending on the group of genes that are located in the merOperon that can be contained in a plasmid or chromosome (Barkay *et al*., 2003; Essa *et al*., 2003).

5 *merA* gene sequencing and blast results: Sequencing was performed to determine the nucleotide sequence of the *merA* gene of *Klebsiella pneumoniae* isolate A1.1.1., as shown in Fig. 2.

To study the similarity of nucleotides sequence from *merA* gene of *Klebsiella pneumoniae* isolate A1.1.1 with *merA* gene of *Klebsiella pneumoniae* deposited in GenBank, blast analyzes was conducted by online at http://blast.ncbi.nlm.nih.gov/Blast.cgi.Blast result shows that *merA* gene of isolate A1.1.1 has 93% similarities with *merA* gene of *Klebsiella pneumoniae* deposited in GenBank. To study nucleotides differences/similarity, blast results were aligned using Clustal 2.1 multiple sequence alignment program. Alignment result was shown in Fig. 3.

TTCCGCAAGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCGCACCACGATGGCATC
GAGACCGACAGTCGCACGCTGACACTCGACAACGTTCCGCGAGCGCTTGCCAACTT
CGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGTAGCGGACGGCTCATCG
GCGTGCAGGCGGTGGCCCCGGAAGCGGGCGAACTGATCCAGACGGCGGTGCTCGC
CATCCGCAACCGCATGTCGGTGCAGGAACTGGCCGACCAGTTGTTCCCCTACCTGA
CAATGGT

Fig. 2: Sequensingresult of *merA* gene of *Klebsiella pneumonia* isolate A1.1.1

```
Klebsiella_pneumoniae_strain_I    CCGCAGGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCACATCACGACGG
Klebsiella_pneumoniae_strain_M    CCGCAGGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCACATCACGACGG
Klebsiella_pneumoniae_strain_N    CCGCAGGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCACATCACGACGG
Klebsiella_pneumoniae_plasmid_    CCGCAGGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCACATCACGACGG
Klebsiella_pneumoniae_plasmid_    CCGCAGGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCACATCACGACGG
Isolat_A111                       CCGCAAGTCGCCACCGTGGGCTACAGCGAGGCGGAAGCGCACCACGATGG
                                  ***** ******************************** ** ***** **

Klebsiella_pneumoniae_strain_     IGATCGAGACCGACAGTCGCCTGCTAACACTGGATAACGTGCCGCGTGCGC
Klebsiella_pneumoniae_strain_     MGATCGAGACCGACAGTCGCCTGCTAACACTGGATAACGTGCCGCGTGCGC
Klebsiella_pneumoniae_strain_     NGATCGAGACCGACAGTCGCCTGCTAACACTGGATAACGTGCCGCGTGCGC
Klebsiella_pneumoniae_plasmid_    GATCGAGACCGACAGTCGCCTGCTAACACTGGATAACGTGCCGCGTGCGC
Klebsiella_pneumoniae_plasmid_    GATCGAGACCGACAGTCGCCTGCTAACACTGGATAACGTGCCGCGTGCGC
Isolat_A111                       CATCGAGACCGACAGTCGCACGCTGACACTCGACAACGTTCCGCGAGCGC
                                  ******************  *** ****** ** ***** ***** ****

Klebsiella_pneumoniae_strain_I    TTGCCAACTTCGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGT
Klebsiella_pneumoniae_strain_M    TTGCCAACTTCGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGT
Klebsiella_pneumoniae_strain_N    TTGCCAACTTCGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGT
Klebsiella_pneumoniae_plasmid_    TTGCCAACTTCGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGT
Klebsiella_pneumoniae_plasmid_    TTGCCAACTTCGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGT
Isolat_A111                       TTGCCAACTTCGACACACGCGGCTTCATCAAGCTGGTCATCGAGGAAGGT
                                  **************************************************

Klebsiella_pneumoniae_strain_I    AGCGGACGGCTCATCGGCGTGCAAGCGGTGGCCCCGGAAGCGGGTGAACT
Klebsiella_pneumoniae_strain_M    AGCGGACGGCTCATCGGCGTGCAAGCGGTGGCCCCGGAAGCGGGTGAACT
Klebsiella_pneumoniae_strain_N    AGCGGACGGCTCATCGGCGTGCAAGCGGTGGCCCCGGAAGCGGGTGAACT
Klebsiella_pneumoniae_plasmid_    AGCGGACGGCTCATCGGCGTGCAAGCGGTGGCCCCGGAAGCGGGTGAACT
Klebsiella_pneumoniae_plasmid_    AGCGGACGGCTCATCGGCGTGCAAGCGGTGGCCCCGGAAGCGGGTGAACT
Isolat_A111                       AGCGGACGGCTCATCGGCGTGCAGGCGGTGGCCCCGGAAGCGGGCGAACT
                                  ********************** ******************** *****

Klebsiella_pneumoniae_strain_I    GATCCAGACGGCGGTGCTCGCCATTCGCAACCGTATGACCGTGCAGGAAC
Klebsiella_pneumoniae_strain_M    GATCCAGACGGCGGTGCTCGCCATTCGCAACCGTATGACCGTGCAGGAAC
Klebsiella_pneumoniae_strain_N    GATCCAGACGGCGGTGCTCGCCATTCGCAACCGTATGACCGTGCAGGAAC
Klebsiella_pneumoniae_plasmid_    GATCCAGACGGCGGTGCTCGCCATTCGCAACCGTATGACCGTGCAGGAAC
Klebsiella_pneumoniae_plasmid_    GATCCAGACGGCGGTGCTCGCCATTCGCAACCGTATGACCGTGCAGGAAC
Isolat_A111                       GATCCAGACGGCGGTGCTCGCCATCCGCAACCGCATGTCGGTGCAGGAAC
                                  ************************ ******** *** * **********

Klebsiella_pneumoniae_strain_I    TGGCCGACCAATTGTTCCCCTACCTGACCATGGT
Klebsiella_pneumoniae_strain_M    TGGCCGACCAATTGTTCCCCTACCTGACCATGGT
Klebsiella_pneumoniae_strain_N    TGGCCGACCAATTGTTCCCCTACCTGACCATGGT
Klebsiella_pneumoniae_plasmid_    TGGCCGACCAATTGTTCCCCTACCTGACCATGGT
Klebsiella_pneumoniae_plasmid_    TGGCCGACCAATTGTTCCCCTACCTGACCATGGT
Isolat_A111                       TGGCCGACCAGTTGTTCCCCTACCTGACAATGGT
                                  ********** ***************** *****
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Fig. 3: Alignment of *merA* gene of isolate A1.1.1 and *Klebsiella pneumonia* deposited in GenBank

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MerA_A111                       PQVATVGYSEAEAHHDGIETDSRTLTLDNVPRALANFDTRGFIKLVIEEG
MerA_Klebsiella_pneumoniae_1    PQVATVGYSEAEAHHDGIETDSRTLTLDNVPRALANFDTRGFIKLVIEEG
MerA_Klebsiella_pneumoniae_3    PQVATVGYSEAEAHHDGIETDSRLLTLDNVPRALANFDTRGFIKLVIEEG
MerA_Klebsiella_pneumoniae_2    PQVATVGYSEAEAHHDGIKTDSRTLTLDNVPRALANFDTRGFIKLVVEEG
MerA_Klebsiella_pneumoniae_4    PQVATVGYSEAEAHHDGIKTDSRTLTLDNVPRALANFDTRGFIKLVVEEG

MerA_A111                       SGRLIGVQAVAPEAGELIQTAVLAIRNRMSVQELADQLFPYLTM
MerA_Klebsiella_pneumoniae_1    SGRLIGVQVVAPEAGELIQTAVLAIRNRMTVQELADQLFPYLTM
MerA_Klebsiella_pneumoniae_3    SGRLIGVQAVAPEAGELIQTAVLAIRNRMTVQELADQLFPYLTM
MerA_Klebsiella_pneumoniae_2    SGRLIGVQAVAPEAGELIQTAALAIRNRMTVQELADQLFPYLTM
MerA_Klebsiella_pneumoniae_4    SGRLIGVQAVAPEAGELIQTAALAIRNRMTVQELADQLFPYLTM
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Fig. 4: Alignment results of amino acid sequences of merA protein of *Klebsiella pneumoniae* isolate A1.1.1. With the other *Klebsiella pneumonia* merA proteins deposited in GenBank

*MerA*gene isolate A1.1.1 of *Klebsiella pneumoniae* has only 93% similarity with those *merA* of other *Klebsiella pneumoniae* deposited in GenBank due to many nucleotide bases differences. It indicates that there are number of gene mutations that shown in alignment result (Fig. 3). The mutations consist of transition ofpurinetopyrimidine,namely G6 into A6, A39 into G39, T42 into C42, C48 into T48, T71 into C71, A75 into G75, T84 into C84, A174 into G174, T195 into C195, T225 into C225, T234 into C234 and A261 into G261. Besides that, there are trans-version mutation (purineto pyrimidine or otherwise), namely G81 into C81, G90 into T90, T96 into A96, A237 into T237, C239 into G239and C279 into A279. Number of mutations affects the translation process, so it will result in different deduced amino acid and in turn it will affect the protein structures.

To study the effect of mutation in translation process, protein of *MerA* isolate A1.1.1 were blasted to *MerA* proteinof *Klebsiella pneumoniae* in GenBank. Alignment was further conducted using Clustal 2.1 multiple sequence alignment. *MerA* protein deduced from the *merA* geneisolate A1.1.1 has 99% similarity with some strains *Klebsiella pneumoniae* deposited in GenBank, as shown in Fig. 4.

There is one amino acid difference of *merA* protein of isolate A.1.1.1 to other *Klebsiella pneumonia merA* protein in Gen Bank. The amino acid is threonine at position 524, which is replaced with serine (Thr→Ser) in the *merA* protein of *Klebsiella pneumoniae* (accession number: AAR91471.1.)

DISCUSSION

The *merA* gene of *Klebsiella pneumoniae* isolates A1.1.1 exists in the plasmid. This is in contrast to the result of Ni Chadhain *et al*. (2006), while using the same primers, they found *merA* gene in genomic DNA of bacterial isolated from marine sediment. These differences can occur due to mercury resistance mechanisms developed by any bacterium. Depending on the group of genes that are located in the mer Operon, the mer genes can be located in a plasmid or chromosome (Barkay *et al*., 2003; Adriana *et al*., 2008). *MerA* genes previously discovered by Essa *et al*. (2003), were located in plasmid of mercury-resistant bacterial cultures.

Alignment results of *merA* gene (Fig. 3) and merA protein of isolate A1.1.1 (Fig. 4) show that most of the mutations do not affect translation products. It is because the most of replaceable bases results in the same amino acid, for example CAG and CAA encode for glutamine, GCA and GCG encode for alanine. CAT and CAC, both encode for histidine, GAC and GAT encodefor asparagine, GGG and GGC encodefor glicine, CTG and CTA encodefor leucine, CTC and CTG encode for leucine and GTG and GTT encode for valin. In contrast with ACC, which encode threonine, is replaced with TCG, which encode for serin. This mutation resultsin different protein product of *merA*. Threonine (Thr = T) is a polar sidecha in amino acid, because of containing hydroxyl groups that can form strong hydrogen bonds with other amino acid sidecha in group containing atoms O, N and S, where the strength of the bond depends on the pH of the environment. Serine (Ser = S) which share the similar property as threonine, has R group of-CHOH, while threonine has R group of- $COHCH_3$.Bothaminoacids, threonine and serine, are not located in active sites, therefore are not involved in the binding process and the reduction of mercury by *merA*. Therefore, the change of threonine by serine on *merA* in amino acid chain does not result in structural change, or in mercury detoxification activity. The active site in volved in the reduction process of mercury contain cysteine C207, C212, C628 and C629, which facilitates the binding of Hg^{2+}on *merA* of Y605 and K613, which is described in the crystal structure model of *merA* of *Bacillussp* RC607 (Barkay *et al*., 2003).

The heart of mer on the mechanism of resistance is mercury reductase homodimer (*merA*), which reduces Hg^{2+} to Hg^0 and use the cofactor flavina deninedi nucleotide and electrons from NADPH (Barkay *et al*., 2003). Catalysis by mer Airport of a sustainable suppresses of nucleotide-disulfideoxido reductase doma in which contains two active cysteine's C207and C212 (Simbahan *et al*., 2005). Mercury reductase *merA* is an enzyme that catalyzes changes of thiol-Hg^{2+} into volatileHg^0. NADPH is used as the electron source (Furukawa and Tonomura, 1972) and is located in the cytoplasm (Summers and Sugarman, 1974). High reduction power of *Klebsiella pneumoniae* isolate A1.1.1 from Fatimawali *et al*. (2018) reduces 99.4% of $HgCl_2$ in 24 h. This is related to the presence of mercury reductase *merA* gene found in this research. *MerA* gene of isolate A1.1.1isplanned to be isolated and cloned to obtain *merA* enzyme to be used in detoxification of inorganic Mercury.

Identification and molecular analysis of mercury-resistant bacteria is still widely performed since the use of mercury is still widely used and exploited. *MerA* enzyme produced by mercury-resistant bacteria can be used in the remediation process or detoxification of mercury. Kargar *et al*. (2012) analyzed the molecular basis of mercury-resistant bacteria from Kor River Iran. They isolated 12.3 kb plasmid from *Pseudomonas* sp., *Serratia* sp. and *Escherichia coli*, containing merOperon.

CONCLUSION

The partial sequence of *merA* gene was found on DNA plasmid of mercury resistant bacterium *Klebsiella pneumoniae* is olate A1.1.1. The nucleotide sequence of the *merA* gene consist of285 base pairs (bp) which encodes for94deduced amino acids of mercury reductase *merA* protein. The *merA* protein sequence has

99% similarity with some strains of *Klebsiella pneumonia* deposited in GenBank. There is a nucleic acid mutation that causes the deduced amino acid threonine to be replaced by serine at position 524 (Thr→Ser) in the *merA* protein of *Klebsiella pneumonia* (accession number: AAR91471.1).

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