LAMPIRAN

LAMPIRAN 1

Jenis-Jenis Mutasi Gen *Mycobacterium tuberculosis* Terhadap Penyebab Resistansi Isoniazid (Studi Pustaka)

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Abstrak

Pengendalian tuberkulosis di dunia saat ini menghadapi tantangan yang ditimbulkan oleh penyebaran secara global *strain Mycobacterium tuberculosis* yang resistan terhadap isoniazid yang merupakan obat lini pertama dalam penanggulangan penyakit tuberkulois. Hal ini terjadi akibat mutasi pada gen target kerja obat. Tujuan penelitian ini adalah mengetahui jenis-jenis mutasi gen, perubahan asam amino, dan substitusi/perubahan kodon pada *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid. Bidang kajian penelitian ini adalah bakteriologi dan biologi molekuler. Jenis penelitian ini adalah studi pustaka menggunakan artikel ilmiah yang dipublikasikan secara nasional dan internasional sebagai objek kajian. Hasil studi pustaka berdasarkan 15 artikel ilmiah terdapat 2 artikel melaporkan mutasi gen *katG*, *inhA*, dan *ahpC*, 9 artikel melaporkan mutasi gen *katG* dan gen *inhA*, 3 artikel melaporkan mutasi gen *katG*, serta 1 artikel melaporkan mutasi gen *inhA* pada *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid. Perubahan asam amino serin pada gen *katG* paling banyak dilaporkan yaitu perubahan menjadi threoinin (Ser315Thr), asparagin (Ser315aAsn), isoleusin (Ser315Ile), glisin (Ser315Gly), arginin (Ser315Arg), triptophan (Ser315Trp), dan kodon stop (Ser575Stop). Substitusi/perubahan kodon paling banyak ditemukan pada AGC (Serin) menjadi ACC (Threonin) kodon 315 pada gen *katG* sebesar 67%, serta sitosin menjadi timin kodon 15 pada gen *inhA* sebesar 60%.

Kata Kunci : Mutasi Gen, *Mycobacterium tuberculosis*, Resistansi Isoniazid

Types of Mutations of *Mycobacterium tuberculosis* Genes Against that Causes of Isoniazid Resistance (Literature Study)

Abstract

Tuberculosis control in the world today faces challenges posed by the global spread of the resistant strain isoniazid *Mycobacterium tuberculosis*, a first-line drug in tackling tuberculosis. This occurs because of mutations in the target gene of the drug's work. The purpose of this study was to find out the types of gene mutations, amino acid changes, and codon replacement/changes in Mycobacterium tuberculosis against the causes of isoniazid resistance. The field of study of this research is bacteriology and molecular biology. This type of research is a literature study using scientific articles published nationally and internationally as objects of study. The results of the library study are based on 15 scientific articles there are 2 articles reporting mutations of *katG*, *inhA*, and *ahpC* genes, 9 articles reporting mutations of *katG* genes and *inhA* genes, 3 articles reporting *katG* gene mutations, and 1 article reporting *inhA* gene mutations in *Mycobacterium tuberculosis* against the causes of isoniazid resistance. The most common changes in amino acids in the *katG* gene are threonin (Ser315Thr), asparagin (Ser315Asn), isoleusin (Ser315Ile), glycine (Ser315Gly), arginine (Ser315Arg), tryptophan (Ser315Trp), and codon stop (Ser575Stop). Substitution/ change of codon is most commonly found in AGC (Serin) to ACC (Threonin) codon 315 in *katG* gene by 67%, and cytocin to tymin codon 15 in *inhA* gene by 60%.

Keywords: Gene Mutation, *Mycobacterium tuberculosis*, Isoniazid Resistance

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PENDAHULUAN

Tuberkulosis merupakan masalah kesehatan yang sangat penting dan serius di seluruh dunia serta merupakan penyakit yang menyebabkan kedaruratan global (*Global Emergency*). Hal ini dikarenakan kurang lebih sepertiga penduduk dunia terinfeksi Mycobacterium tuberculosis. Secara global, diperkirakan 10 juta jiwa menderita tuberkulosis tahun 2019 dengan tingkat kematian sekitar 1,2 juta jiwa pada strain dengan HIV-negatif dan sekitar 208.000 jiwa kematian pada strain dengan HIV-positif (WHO, 2020).

Indonesia menjadi negara dengan prevalensi tuberkulosis tertinggi kedua di dunia setelah India yaitu sebesar 8,5% (WHO, 2020). Kasus tuberkulosis di Indonesia diperkirakan mencapai 845 ribu kasus, sebanyak 543 ribu kasus melapor dan 35% lainnya tidak melapor/tidak terdiagnosa. Angka kematian akibat tuberkulosis tercatat 11 ribu jiwa (Ditjen P2P, 2020).

Pengendalian tuberkulosis di dunia saat ini menghadapi tantangan yang ditimbulkan oleh penyebaran secara global strain *Mycobacterium tuberculosis* yang resistan terhadap obat anti tuberkulosis. Hal ini menyebabkan terjadinya penyebaran *multi-drug resistant tuberculosis* (MDR-TB) di dunia (Falzon *et al*., 2011). Berdasarkan *Global Tuberculosis Report* 2020, di seluruh dunia diperkirakan terdapat 465.000 kasus *rifampicin-resistant tuberculosis* dimana 78% diantaranya mengalami *multi-drug resistant tuberculosis*. Angka kematian akibat *multi-drug resistant tuberculosis* diperkirakan mencapai 182.000 jiwa di seluruh dunia. Tiga negara dengan persebaran terbesar secara global yaitu India (27%), China (14%) dan Rusia (8%) (WHO, 2020). Indonesia turut menyumbang ±9.875 jiwa ternotifikasi kasus tuberkulosis resistan rifampisin (Ditjen P2P, 2020).

Penggunaan obat anti tuberkulosis yang berulang dan panjangnya waktu terapi menyebabkan kepatuhan pasien rendah. Akibatnya dapat terjadi strain resistan obat (Smith *et al*., 2013). *Multi-drug resistant tuberculosis* (MDR-TB) ialah strain yang resistan terhadap Isoniazid dan Rifampisin dengan atau tanpa resistan terhadap obat lain (Kemenkes RI, 2012).

Mycobacterium tuberculosis memiliki kemampuan untuk mengembangkan resistansi secara alamiah terhadap berbagai antibiotik. Resistansi dapatan (*acquired*) yang terjadi pada MDR-TB umumnya disebabkan oleh karena adanya sejumlah mutasi pada sejumlah gen yang mengkode sensitivitas *Mycobacterium tuberculosis* terhadap obat anti tuberkulosis (OAT) (Irianti dkk., 2016).

Mycobacterium tuberculosis mengembangkan mekanisme resistansi yang berbeda dengan bakteri lain pada umumnya. Pada prokariot, resistansi umumnya disebabkan karena adanya transfer materi genetik, baik melalui plasmid, transposon dan lain-lain. Pada *Mycobacterium tuberculosis*, resistansi dipicu oleh adanya mutasi yang terjadi secara spontan pada gen kromosomal. Resistansi hanya akan menguntungkan bakteri pada saat terpapar dengan obat target. Pada paparan obat anti tuberkulosis (OAT) yang tidak adekuat, bakteri yang sensitif akan mati dan mutan akan berkembang biak dengan pesat tanpa adanya persaingan yang berarti dalam hal nutrisi (Eduardo *et al*., 2011).

Isoniazid merupakan obat anti tuberkulosis dan salah satu komponen kunci pada terapi lini pertama untuk penyakit aktif. Isoniazid pertama kali disintesis pada tahun 1912 dan aktivitas anti-TB baru dilaporkan pada tahun 1952. Isoniazid adalah prodrug yang mengikat dan menghambat *InhA* yaitu enzim yang terlibat dalam biosintesis asam mikolat. Selain itu isoniazid akan diaktivasi oleh enzim katalase peroksidase (*katG*) yang dikode oleh gen *katG l*alu akan membentuk produk berupa adduct. Adduct ini bersifat toksik serta mempengaruhi target intraseluler seperti biosintesis asam mikolat yang merupakan komponen penting pada dinding sel bakteri (Irianti dkk., 2016).

Mutasi pada gen *katG* menyebabkan produk isoniazid kurang dalam pembentukan INH-NAD yang diperlukan untuk aktivitas mikroba INH, selanjutnya mutasi pada gen *inhA* menghasilkan penurunan afinitas ikatan inhA ke NADH sehingga terjadi penghambatan sintesis asam mikolat, serta mutasi pada gen *ahpC* merupakan kompensasi karena hilangnya aktivitas katalase-peroksidase, ketiga mutasi gen tersebut menyebabkan *Mycobacterium tuberculosis* resistan terhadap obat anti tuberkulosis yaitu isoniazid.

Meskipun berbagai gen terlibat di dalam *Mycobacterium tuberculosis* sebagai

penyebab resistansi terhadap INH, ada data yang mendukung bahwa mutasi yang sering difokuskan terutama pada *katG, inhA,* dan *ahpC* daerah regulator yaitu mutasi pada gen *katG* sebesar 66,3%, gen *inhA* (19%) dan gen *ahpC* (5,4%) (Seifert *et al*., 2015). Selain itu, penelitian di China mengungkapkan ada frekuensi mutasi yang tinggi pada gen *katG*, gen *inhA*, dan gen *ahpC* daerah regulator dalam isolat resistan isoniaizid dengan frekuensi masing-masing 86,2%, 19,6%, dan 18,6% terhadap 188 isolat sampel yang diperiksa (Liu *et al*., 2018).

Penelitian Allo dan Mursalim (2020) melaporkan bahwa dari 13 sampel, didapatkan empat (31%) sampel sputum penderita tuberkulosis yang baru memulai pengobatan, resistan terhadap isoniazid (Allo & Mursalim, 2020). Di India sebagai negara dengan jumlah kasus resistansi tuberkulosis terbesar di dunia, penelitian Charan *et al* (2020) menyebutkan bahwa mutasi paling banyak terhadap resistansi isoniazid terjadi pada gen *katG* sebesar 65,1% serta mutasi pada gen *inhA* sebesar 28,1%, terdapat pula mutasi pada kedua gen *katG* dan *inhA* sebesar 6,7% (Charan *et al*., 2020).

Dengan mengetahui penyebab tersering terjadinya resistansi terhadap isoniazid, kita dapat menentukan primer gen yang potensial menjadi marker genetik untuk pemeriksaan tuberkulosis yang resistan terhadap isoniazid (Siregar, 2015). Berdasarkan uraian di atas maka dilakukan penelitian studi pustaka tentang jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid.

METODE

Jenis Penelitian yang digunakan adalah studi pustaka dengan mengkaji artikel, jurnal ilmiah dan buku terkait penelitian tentang jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid.

Waktu penelitian studi pustaka ini dilaksanakan pada bulan Maret sampai Juni 2021. Adapun batasan dari literatur yang digunakan adalah artikel dari jurnal ilmiah yang dipublikasikan secara nasional dan internasional dalam 10 tahun terakhir, yaitu antara 2011-2020 yang memuat sumber data yang dibutuhkan secara detail, terutama mengenai jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid.

Sumber data yang menjadi bahan penelitian ini yaitu sumber data primer berupa artikel ilmiah, serta sumber data sekunder berupa buku dan bahan bacaan lainnya yang terkait dengan topik penelitian. Pencarian literatur dilakukan dengan menggunakan data terpilih dari *database Google Scholar, PubMED* dan *Research Gate*.

Teknik analisis data yang digunakan dalam studi pustaka ini berupa metode analisis isi (*Content Analysis*). Kemudian peneliti mengolah data-data dari artikel ilmiah yang sudah dikumpulkan hingga ditemukan hasil yang relevan sesuai dengan topik penelitian, yaitu tentang jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid. Pada tahap ini setelah hasil analisis data dilakukan, kemudian akan dibahas lebih rinci yang selanjutnya dapat ditarik kesimpulan terhadap hasil kajian.

HASIL

Berdasarkan hasil pengumpulan data tentang jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid, yang didapatkan melalui artikel, jurnal dan penelusuran internet dari database *Google Scholar*, *PubMED* dan *Research Gate*, diperoleh 14 jurnal internasional dan 1 jurnal nasional yang dapat dikaji secara studi pustaka. Peneliti telah melakukan meta analisis isi dari masing-masing artikel yang didapatkan, dan berdasarkan hasil pengkajian pada 15 artikel tersebut menunjukkan bahwa dari keseluruhan 15 artikel ilmiah (100%) menyatakan adanya mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid.

Berdasarkan hasil dari 15 artikel ilmiah yang telah dikaji terdapat 2 artikel melaporkan adanya mutasi pada 3 gen yaitu gen *katG*, gen *inhA*, dan gen *ahpC*, 9 artikel melaporkan mutasi pada 2 gen yaitu gen *katG* dan gen *inhA*, 3 artikel hanya melaporkan mutasi pada gen *katG*, serta 1 artikel hanya melaporkan mutasi gen *inhA* pada *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid. Mutasi gen dapat dibagi yaitu, mutasi salah satu gen pada setiap isolat (mutasi tunggal), mutasi dua gen pada setiap isolat (mutasi ganda), mutasi lebih dari dua gen pada setiap isolat (mutasi gabungan).

PEMBAHASAN

1. Jenis-Jenis Mutasi Gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid

Mycobacterium tuberculosis memiliki kemampuan untuk mengembangkan resistansi secara alamiah terhadap berbagai antibiotik. Resistansi dapatan (*acquired*) yang terjadi pada strain resistan umumnya disebabkan oleh karena adanya sejumlah mutasi pada sejumlah gen yang mengkode sensitivitas *Mycobacterium tuberculosis* terhadap obat anti tuberkulosis (OAT) (Irianti dkk., 2016). Dari artikel yang dikaji didapatkan mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid sebagai berikut:

a. Mutasi gen *katG*

Penelitian Lugou Liu (2018) terdapat frekuensi mutasi tertinggi pada gen *katG* baik mutasi tunggal, mutasi ganda, maupun mutasi gabungan sebesar 86,2 %. Frekuensi tinggi mutasi *katG* juga turut dilaporkan oleh Click, E tahun 2020, terdapat 313 (70%) mutasi *katG* dari 447 sampel. Hal ini juga sejalan dengan Diande, S *et al* (2019) sebesar 94,5% dan Isakova, J *et al* (2018) sebesar 91,2% turut melaporkan frekuensi tinggi terhadap mutasi *katG*.

Mutasi tersebut menyebabkan produk isoniazid kurang dalam pembentukkan INH-NAD yang diperlukan untuk aktivitas antimikroba INH. Kemampuan *katG* lebih efisien dari enzim mutan dalam upaya perubahan INH (*prodrug*) ke bentuk asam isonikotinat (INH teraktivasi). Sehingga, mutan Ser315Thr merupakan katalase-peroksidase kompeten dengan kemampuan metabolisme INH yang berkurang. Oleh karena itu, subtitusi asam amino pada posisi 315 muncul untuk menghilangkan keseimbangan antara kebutuhan pengaturan aktivitas katalase-peroksidase aktif dalam upaya detoksifikasi radikal antibakteri dari hospes dan pengurangan perubahan produg ke bentuk INH aktif, suatu proses yang akan membunuh bakteri secara normal (Irianti dkk., 2016).

b. Mutasi gen *inhA*

Selain mutasi pada gen *katG*, penelitian Erdenegerel Narmandakh *et al* (2020), adanya mutasi gen *inhA* dengan frekuensi tinggi sebagai penyebab resistansi isoniazid sebesar 294/409 (71,9%). Mutasi C-15T merupakan mutasi yang paling banyak dilaporkan pada penelitian ini sebesar 98% dari total isolat yang mengalami mutasi pada gen *inhA*. Mutasi promoter tersebut menghasilkan ekspresi berlebih *inhA* dan memicu resistansi INH (Narmandakh *et al.*, 2020). Selain itu, Pauline Lempens (2018) melaporkan adanya subtitusi Ser94Ala pada mutasi *inhA*. Hal ini menghasilkan penurunan afinitas ikatan *inhA* ke NADH sehingga terjadi penghambatan sintesis asam mikolat (Lempens *et al*., 2018).

Mutasi spesifik *inhA* atau ekspresi berlebih *inhA* menghasilkan organisme dengan peningkatan KHM (Kadar Hambat Minimum) terhadap isoniazid (INH). Kadar hambat minimum menjadi 5 kali lebih tinggi dari KHM untuk wild type. Sekitar 70-80% resistansi INH pada isolat klinis *Mycobacterium tuberculosis* dapat dianggap sebagai akibat mutasi gen *katG* dan *inhA* (Irianti dkk., 2016). Studi akhir-akhir ini menemukan bahwa mutasi pada daerah regulator *inhA* bersama dengan mutasi daerah *coding* dari *inhA* menghasilkan resistansi isoniazid tingkat tinggi (KHM > 1µg/mL) (Machado *et al*., 2013).

c. Mutasi gen *ahpC*

Mutasi pada gen *ahpC* juga turut dilaporkan oleh Jainagul Isakova (2018) yaitu perubahan pada *ahpC* G-9A dan C-12T. Tingkat resistansi yang tinggi pada *ahpC* akan dikaitkan dengan substitusi asam amino non-315 ini. Asam amino tersebut kemungkinan besar menempati posisi kunci *katG*, karena mutasi ini dikaitkan dengan hilangnya fungsi *katG* yang lebih besar dan memerlukan ekspresi berlebih *ahpC* untuk mikobakteri untuk menahan tekanan konsentrasi INH yang tinggi (Isakova *et al*., 2018).

Selain itu, terjadinya mutasi ini sering dikaitkan dengan kekambuhan atau kegagalan pengobatan. Frekuensi yang lebih tinggi mutasi *ahpC-oxyR* hadir dalam isolasi dari pasien MDR-TB yang kambuh dibandingkan dengan isolasi dari kasus baru (Liu *et al*., 2018).

2. Perubahan Asam Amino pada Gen *Mycobacterium tuberculosis* yang Bermutasi Terhadap Penyebab Resistansi Isoniazid

Mutasi gen pada dasarnya merupakan mutasi titik (*point mutation*). Pada mutasi ini terjadi perubahan kimiawi pada satu atau beberapa pasangan basa dalam satu gen tunggal yang menyebabkan perubahan sifat individu tanpa perubahan

jumlah dan susunan kromosomnya. Peristiwa yang terjadi pada mutasi gen adalah perubahan urutan-urutan DNA atau lebih tepatnya mutasi titik merupakan perubahan pada basa N dari DNA atau RNA. Peristiwa perubahan genetik seperti itu disebut dengan mutasi gen karena hanya terjadi di dalam gen (Warmadewi, 2017).

Berdasarkan artikel yang telah dikaji terdapat perubahan asam amino pada gen *Mycobacterium tuberculosis* yang bermutasi terhadap penyebab resistansi isoniazid. Penelitian Isakova, J *et al* tahun 2018 melaporkan terdapat 88% perubahan asam amino serin menjadi threoin (Ser315Thr) pada gen *katG* yang bermutasi. Perubahan asam amino ini menjadi yang tersering dan paling banyak ditemukan pada jurnal yang dikaji. Munir, A *et al* (2019)., Liu, L *et al* (2018)., Ahmad, B *et al* (2017) melaporkan adanya perubahan asam amino lainnya pada gen k*atG* yaitu, serin menjadi asparagin (Ser315Asn), serin menjadi isoleusin (Ser315Ile), serin menjadi arginin (Ser315Arg), serin menjadi glysine (Ser315Gly), serta arginine menjadi leusin (Arg463Leu).

Pada gen *inhA*, penelitian Lempens, P *et al* (2018) terdapat perubahan asam amino pada isoleusin menjadi threonin (Ile194Thr, Ile74Thr, Ile21Thr), dan serin menjadi alanin (Ser94Ala) pada gen *inhA* yang bermutasi. Selain itu, penelitian Liu, L *et al* (2018) terdapat perubahan asam amino pada gen *ahpC* yang bermutasi yaitu, glutamic menjadi aspartic (Glu189Asp), serta serin menjadi alanin (Ser94Ala).

3. Subtitusi atau Perubahan Kodon pada Mutasi Gen *Mycobacterium tuberculosis* Resistan Isoniazid

Berdasarkan artikel ilmiah yang dikaji, subtitusi/perubahan AGC (Serin) menjadi ACC (Threonin) kodon 315 pada gen *katG* menjadi yang banyak dilaporkan pada studi pustaka ini. Selain itu, penelitian Kusdianingrum, D dkk tahun 2014 melaporkan mutasi pada gen *inhA* terjadi akibat perubahan basa nukleutida cytosin menjadi timin (C-15T) merupakan yang tersering terlaporkan pada mutasi gen *inhA*, mutasi tersebut terjadi pada kodon 15, 12, 57, dan 81 (C-15T, C-12T, C-57T, C-81T). Pada mutasi gen *ahpC* perubahan terjadi pada guanin menjadi alanin (G-9A) dan perubahan sitosin menjadi timin (C-12T) pada kodon 9 dan 12.

Deteksi dini strain *Mycobacterium tuberculosis* resistan obat adalah kunci untuk pengobatan pasien yang cepat dan efektif serta pengurangan penularan yang berkelanjutan. Pemerintah Indonesia melalui Permenkes No. 67 tahun 2016 mengatur penggunaan TCM dengan Xpert MTB/RIF untuk pemeriksaan diagnosis tuberkulosis dan tuberkulosis resistan obat (PMK RI No. 67/2016: VI: B(2), 2016). Namun metode tersebut hanya terbatas pada deteksi pasien resistan obat rifampisin saja tidak dengan isoniazid, dimana keduanya merupakan obat lini pertama dalam penanggulangan tuberkulosis. Untuk itu perlu dilakukan evaluasi terhadap metode pemeriksaan resistan obat di Indonesia agar dapat mendiagnosis isolat resistan isoniazid, hal ini dianggap penting karena adanya frekuensi yang tinggi terhadap isolat monoresistan isoniazid terhadap isolat MDR-TB. Dengan adanya metode deteksi tepat yang dapat mendeteksi isolat resistan isoniazid dan rifampisin, maka pengobatan akan sesuai dengan kondisi pasien dan penyebaran strain resistan obat dapat ditanggulangi.

Faktor risiko yang terbukti berpengaruh pada kejadian resistan obat adalah motivasi yang rendah pada pasien dalam melakukan pengobatan, ketidakteraturan dalam meminum obat, pelayanan yang kurang memuaskan dari penyelenggara fasilitas kesehatan, serta kurangnya pengetahuan penderita tentang penyakitnya dan bagaimana mengobatinya (Sarwani Dewi dkk., 2012). Hal ini menyebabkan *Mycobacterium tuberculosis* memiliki kemampuan mengembangkan resistansi secara alamiah terhadap berbagai antibiotik. Resistansi dapatan (*acquired*) umumnya terjadi karena adanya sejumlah mutasi pada sejumlah gen yang mengkode sensitivitas *Mycobacterium tuberculosis* terhadap obat anti tuberkulosis (OAT) (Irianti dkk., 2016).

Mutasi gen pada *Mycobacterium tuberculosis* yang terkait dengan resistansi obat mengakibatkan perubahan fenotipe akibat perubahan interaksi obat-bakteri, termasuk stabilitas protein dan/atau perubahan struktural yang menggangu mekanisme kerja obat. Pemahaman rinci tentang mekanisme mutasi yang resistan terhadap obat dapat membantu dalam mendesain obat yang baru dan lebih baik dari yang sudah ada, pemilihan target obat yang lebih baik dan bahkan identifikasi target obat

baru. Hal ini dapat dilakukan dengan *Whole Genome Sequencing* (WGS) (Munir *et al*., 2019).

Whole Genome Sequencing merupakan proses menentukan urutan DNA lengkap dari suatu genom organisme pada satu waktu. Cara ini menawarkan potensi untuk medeteksi mutasi genetik yang terkait dengan resistansi fenotipik pada titik waktu yang lebih awal daripada metode berbasis kultur sehingga dapat memahami dan mengatasi resistansi obat. Berdasarkan studi pustaka yang dilakukan, terdapat 13 artikel yang merupakan artikel dari luar neger, dimana 2 artikel lainnya yang berasal dari Indonesia hanya melaporkan terbatas pada salah satu gen saja dan jumlah isolat yang diperiksa relatif sedikit. Selain itu, jumlah penelitian terkait mutasi gen *Mycobacterium tuberculosis* terhadap resistansi isoniazid tergolong sedikit dan dianggap belum merepresentasikan jumlah penduduk dan wilayah Indonesia yang besar serta kasus TB di Indonesia yang merupakan terbesar ke 2 di dunia.

Untuk itu perlu dilakukan analisis molekuler lebih lengkap dan menyeluruh pada *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid secara *Whole Genom Sequencing* (WGS) di wilayah Indonesia. Dengan hal tersebut diharapkan dapat memahami epidemiologi dan menghasilkan daftar lengkap mutasi sebagai penyebab resistansi terhadap obat anti tuberkulosis. Selain itu, dapat mengetahui karakteristik fenotip dan persebaran mutasi gen *Mycobacterium tuberculosis* di Indonesia sehingga strategi dalam penggulangan tuberkulosis dapat lebih tepat dan efektif.

Simpulan

Berdasarkan hasil studi pustaka pada 15 artikel penelitian mengenai jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid dapat disimpulkan sebagai berikut:

1. Diperoleh 2 artikel melaporkan adanya mutasi pada gen *katG*, *inhA*, dan *ahpC*, 9 artikel melaporkan adanya mutasi pada gen *katG* dan gen *inhA*, 3 artikel melaporkan adanya mutasi pada gen *katG*, serta 1 artikel melaporkan adanya mutasi pada gen *inhA* pada *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid.
2. Perubahan asam amino serin pada gen *katG* menjadi yang paling banyak dilaporkan. Terdapat 7 perubahan asam amino yang berasal dari serin yaitu perubahan serin menjadi threonin (Ser315Thr) dilaporkan pada 10 artikel, 4 artikel melaporkan perubahan serin menjadi asparagin (Ser315aAsn), serta perubahan serin menjadi isoleusin (Ser315Ile), glisin (Ser315Gly), arginin (Ser315Arg), triptophan (Ser315Trp), dan kodon stop (Ser575Stop) dilaporkan masing-masing pada 1 artikel.
3. Substitusi/perubahan AGC (Serin) menjadi ACC (Threonin) kodon 351 pada gen *katG* dilaporkan pada 10 artikel sebesar 67%, serta perubahan basa nitrogen cytocin menjadi timin kodon 15 pada gen *inhA* dilaporkan pada 9 artikel sebesar 60%. Kedua perubahan tersebut menjadi yang paling banyak dilaporkan mengenai mutasi gen *Mycobacterium tuberculosis* sebagai penyebab resistansi terhadap isoniazid.

Saran

Berdasarkan hasil studi pustaka yang dilakukan, disarankan sebagai berikut:

1. Agar dilakukan *Whole Genome Sequencing* terhadap isolat klinis *Mycobacterium tuberculosis* di Indonesia sehingga dapat diketahui karakteristik dan analisis molekulernya terhadap persebaran strain di Indonesia.
2. Agar dilakukan penelitian lebih lanjut tentang jenis-jenis mutasi gen *Mycobacterium tuberculosis* terhadap penyebab resistansi isoniazid secara lebih lengkap terhadap mutasi lain yang belum diketahui.
3. Agar dilakukan evaluasi terhadap metode pemeriksaan resistan obat di Indonesia dengan menggunakan metode pemeriksaan yang dapat mendeteksi adanya isolat resistan terhadap isoniazid dan rifampisin sekaligus seperti metode MTBDR*plus*.
4. Perlu adanya kesadaran masyarakat dengan terus menyebarkan informasi dalam penanggulangan *strain* tuberkulosis resistan obat dengan cara patuh meminum obat, menyelesaikan pengobatan sampai tuntas serta menjaga diri terhadap penyebaran strain resistan obat.

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LAMPIRAN 2

TAHAPAN ANALISIS MOLEKULER MENENTUKAN MUTASI GEN *Mycobacterium tuberculosis* TERHADAP PENYEBAB RESISTANSI ISONIAZID

JURNAL : (Liu *et al*., 2018)

A. Pegumpulan isolat klinis

Untuk memperoleh keragaman jenis mutan yang tinggi dengan mutasi gen yang terkait dengan resistensi INH, M. tuberculosis isolat dari survei resistansi obat nasional yang baru-baru ini dilakukan di China dipilih untuk analisis. Koleksi 201 isolat diperoleh secara acak dari survei nasional China tentang prevalensi TB yang resistan terhadap obat yang dilakukan pada tahun 2007, yang meliputi 168 isolat MDR-TB, 20 isolat resisten tunggal INH, dan 13 isolat yang rentan terhadap obat. Isolat ini diperoleh dari pasien TB di 72 wilayah perwakilan. Dengan demikian, polimorfisme tinggi dari mutasi terkait resistansi.

Klon tunggal untuk setiap isolat dibudidayakan di media Löwenstein-Jensen. Kultur dikumpulkan, dan pengenceran serial 10 kali lipat disiapkan. Pengenceran ini kemudian dibiakkan dan dipanen untuk mengekstrak DNA genom. Penelitian ini telah disetujui oleh Komite Peninjau Etik dari Institut Biologi Patogen, Akademi Ilmu Kedokteran Cina & Perguruan Tinggi Kedokteran Peking Union.

B. Pengujian kerentanan obat dan penentuan strain resistan tunggal MDR dan INH

Resistansi obat dari setiap isolat ditentukan dengan menggunakan metode konsentrasi absolut di media Löwenstein-Jensen. Enam kunci obat anti-tuberkulosis (isoniazid, 0,2 mg / L; streptomisin, 4 mg / L; rifampisin, 40 mg / L; etambutol, 2 mg / L; o fl oksasin, 2 mg / L; dan kanamycin, 40 mg / L) dari Sigma Aldrich dipilih untuk pengujian kerentanan obat menggunakan konsentrasi berdasarkan pedoman WHO.

Strain MDR ditentukan untuk menunjukkan resistansi terhadap INH dan rifampisin. Isolat monoresistan INH telah diidentifikasi sebagai mereka yang hanya tahan terhadap INH dan rentan terhadap lima obat lain. Konfirmasi fenotip lebih lanjut dengan pembuatan masing-masing jenis isolat dilakukan dengan menggunakan BACTEC MGIT 960 System (BD Diagnostic Systems).

Penentuan konsentrasi hambat minimum (KHM) dilakukan pada mikroplat 96 sumur dengan menggunakan metode kolorimetri 34 . Isolat diperlakukan dengan 10 konsentrasi INH, mulai dari 0,05 hingga 102,4mg / L, yang dibuat dengan pengenceran dua kali lipat dalam kaldu 7H9. Hasil MIC dibaca sebagai konsentrasi INH terendah yang mencegah terjadinya perubahan warna pada masing-masing isolat.

Isolat dibagi menjadi empat kelompok menurut derajat resistensi INH sebagai berikut: 1) rentan (MIC <0,01mg / L); 2) resistensi tingkat rendah (LR; 0.1mg / L. ≤ MIC ≤ 0.4mg / L); 3) resistensi tingkat menengah (MR; 0.8mg / L. ≤ MIC ≤ 3.2mg / L); dan 4) resistensi tingkat tinggi (HR; MIC ≥ 6.4mg / L).

C. Identifikasi mutasi terkait resistensi INH menggunakan *Next-Generation Sequencing* (NGS)

DNA genom dari masing-masing isolat diekstraksi menggunakan Kit Wizard Genomic DNA Purifikasi (Promega, Co., Madison, USA). Perpustakaan pengurutan dibangun dan diurutkan dengan Truseq ® Kit DNA Nano (Illumina, Inc., San Diego, CA, USA). Penilaian kualitas data sekuensing dilakukan dengan menggunakan NGS QC Toolkit dengan cutoff Q20, dan panjang baca minimum 101 pasang basa digunakan untuk pemetaan selanjutnya. Pembacaan yang valid dipetakan ke urutan genom referensi *Mycobacterium tuberculosis* H37Rv (aksesi GenBank NC_000962) menggunakan Burrows - Algoritma Wheeler seperti yang diterapkan dalam paket perangkat lunak BWA.

Untuk semua isolat, cakupan genom referensi> 99% dengan kedalaman minimal 10x dan skor kualitas konsensus 50 menggunakan SAMtools. Mutasi pada setiap gen terkait resistensi INH diidentifikasi dengan menyelaraskan bacaan yang sesuai dengan urutan referensi (*Mycobacterium tuberculosis* H37Rv). Pembacaan urutan telah dikirimkan ke arsip baca urutan NCBI (SRA) di bawah aksesi PRJNA268900.

(Hsu et al., 2020)

(Lempens et al., 2018)

(Ahmad et al., 2017)

(Liu et al., 2018)

(Huo et al., 2019)

(Munir et al., 2019) (Diandé et al., 2019)

(Narmandakh et al., 2020)

(Click et al., 2020)

(Hsu et al., 2020)

(Charan et al., 2020)

(Kusdianingrum et al., 2014)

(Tseng et al., 2013) (Isakova et al., 2018) (Purkan et al., 2018)(Heidary et al., 2020)

LAMPIRAN 3

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Pembimbing Utama : Nurminha, S.Pd., M.Sc

No.	Hari/Tanggal	Materi	Keterangan	Paraf
1.	Rabu, 16 Desember 2020	Penjelasan Penulisan Skripsi	Perbaikan	
2.	Senin, 11 Januari 2021	BAB I	Perbaikan	
3.	Kamis, 21 Januari 2021	BAB I, II, dan III	Perbaikan	
4.	Selasa, 02 Februari 2021	BAB II dan III	Perbaikan	
5.	Senin, 08 Februari 2021	BAB I dan Daftar Pustaka	Perbaikan	
6.	Senin, 15 Februari 2021	BAB I dan II	Perbaikan	
7.	Senin, 22 Februari 2021	BAB II dan Penulisan	Perbaikan	
8.	Jum'at, 05 Maret 2021	BAB I, II, III	ACC Seminar Proposal	
9.	Senin, 10 Mei 2021	BAB I, II, III	ACC Revisi Seminar Proposal	
10.	Selasa, 18 Mei 2021	BAB IV dan V	Perbaikan	
11.	Jum'at, 04 Juni 2021	BAB IV dan V	Perbaikan	
12.	Selasa, 15 Juni 2021	BAB IV dan V	Perbaikan	
13.	Kamis, 24 Juni 2021	Skripsi Perbaikan	ACC Seminar Hasil	
14.	Rabu, 21 Juli 2021	Skripsi Perbaikan Seminar Hasil	ACC Cetak	

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LAMPIRAN 4

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No.	Hari/Tanggal	Materi	Keterangan	Paraf
1.	Senin, 08 Februari 2021	BAB I dan Kerangka Teori	Perbaikan	
2.	Rabu, 10 Februari 2021	BAB I, II, III	ACC Seminar Proposal	
3.	Selasa, 22 Juni 2021	BAB IV dan V	Perbaikan	
4.	Rabu, 23 Juni 2021	Skripsi	ACC Seminar Hasil	

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LAMPIRAN 5

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EPIDEMIOLOGY AND SURVEILLANCE

Genetic Mutations Associated with Isoniazid Resistance in *Mycobacterium tuberculosis* in Mongolia

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ABSTRACT Globally, mutations in the *katG* gene account for the majority of isoniazid-resistant strains of *Mycobacterium tuberculosis*. Buyankhishig et al. analyzed a limited number of *Mycobacterium tuberculosis* strains in Mongolia and found that isoniazid resistance was mainly attributable to *inhA* mutations (B. Buyankhishig, T. Oyuntuya, B. Tserelmaa, J. Sarantuya, et al., Int J Mycobacteriol 1:40–44, 2012, https://doi.org/10.1016/j.ijmyco.2012.01.007). The GenoType MTBDR*plus* assay was performed for isolates collected in the First National Tuberculosis Prevalence Survey and the Third Anti-Tuberculosis Drug Resistance Survey to investigate genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis* in Mongolia. Of the 409 isoniazid-resistant isolates detected by the GenoType MTBDR*plus* assay, 127 (31.1%) were resistant to rifampin, 294 (71.9%) had *inhA* mutations without *katG* mutations, 113 (27.6%) had *katG* mutations without *inhA* mutations, and 2 (0.5%) had mutations in both the *inhA* and *katG* genes. Of the 115 strains with any *katG* mutation, 114 (99.1%) had mutations in codon 315 (S315T). Of the 296 strains with any *inhA* mutation, 290 (98.0%) had a C15T mutation. The proportions of isoniazid-resistant strains with *katG* mutations were 25.3% among new cases and 36.2% among retreatment cases ($P = 0.03$) and 17.0% among rifampin-susceptible strains and 52.8% among rifampin-resistant strains ($P < 0.01$). Rifampin resistance was significantly associated with the *katG* mutation (adjusted odds ratio, 5.36; 95% confidence interval [CI], 3.3 to 8.67, $P < 0.001$). Mutations in *inhA* predominated in isoniazid-resistant tuberculosis in Mongolia. However, the proportion of *katG* mutations in isolates from previously treated cases was higher than in those from new cases, and the proportion in cases with rifampin resistance was higher than in cases without rifampin resistance.

KEYWORDS *Mycobacterium tuberculosis*, drug resistance, *inhA*, *katG*, mutation

Multidrug-resistant tuberculosis (MDR-TB), defined as a disease due to *Mycobacterium tuberculosis* that is resistant to at least both rifampin (RIF) and isoniazid (INH), is a major public health problem. Globally, in 2017, there were an estimated 460,000 incident cases of MDR-TB. MDR-TB is difficult to treat. The proportion of MDR-TB with treatment success is approximately 55% globally (1). Acquisition of INH resistance is a critical step in the development of MDR-TB (2). Studies have reported that genetic mutations that confer resistance to INH usually arise before mutations that confer resistance to RIF (3, 4). The global average rate of INH resistance without concurrent RIF

Citation Narmandakh E, Tumenbayar O, Borolzoi T, Erkhembayar B, Boldoo T, Dambaa N, Burneebaatar B, Nymadawa N, Mitarai S, Jav S, Chiang C-Y. 2020. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis* in Mongolia. Antimicrob Agents Chemother 64:e00537-20. https://doi.org/10.1128/AAC.00537-20.

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Received 19 March 2020
Accepted 15 April 2020
Accepted manuscript posted online 20 April 2020
Published 23 June 2020

LAMPIRAN 6

frontiers in Microbiology

ORIGINAL RESEARCH
published: 15 July 2020
doi: 10.3389/fmicb.2020.01644

Two Novel *katG* Mutations Conferring Isoniazid Resistance in *Mycobacterium tuberculosis*

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Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, is among the top 10 leading causes of death worldwide. The treatment course for TB is challenging; it requires antibiotic administration for at least 6 months, and bacterial drug resistance makes treatment even more difficult. Understanding the mechanisms of resistance is important for improving treatment. To investigate new mechanisms of isoniazid (INH) resistance, we obtained three INH-resistant (INH-R) *M. tuberculosis* clinical isolates collected by the Taiwan Centers for Disease Control (TCDC) and sequenced genes known to harbor INH resistance-conferring mutations. Then, the relationship between the mutations and INH resistance of these three INH-R isolates was investigated. Sequencing of the INH-R isolates identified three novel *katG* mutations resulting in R146P, W341R, and L398P KatG proteins, respectively. To investigate the correlation between the observed INH-R phenotypes of the clinical isolates and these *katG* mutations, wild-type *katG* from H37Rv was expressed on a plasmid (pMN437-*katG*) in the isolates, and their susceptibilities to INH were determined. The plasmid expressing H37Rv *katG* restored INH susceptibility in the two INH-R isolates encoding the W341R KatG and L398P KatG proteins. In contrast, no phenotypic change was observed in the KatG R146P isolate harboring pMN437-*katG*. H37Rv isogenic mutant with W341R KatG or L398P KatG was further generated. Both showed resistant to INH. In conclusion, W341R KatG and L398P KatG conferred resistance to INH in *M. tuberculosis*, whereas R146P KatG did not affect the INH susceptibility of *M. tuberculosis*.

Keywords: ***Mycobacterium tuberculosis*, drug resistance, isoniazid, mutation, *katG***

OPEN ACCESS

Edited by:
Giorgia Mori,
The University of Queensland, Australia

Reviewed by:
Diana Machado,
New University of Lisbon, Portugal
Daniela Fernandes Ramos,
Federal University of Rio Grande, Brazil

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Specialty section:
This article was submitted to Antimicrobials, Resistance and Chemotherapy, a section of the journal Frontiers in Microbiology

Received: *29 April 2020*
Accepted: *24 June 2020*
Published: *15 July 2020*

Citation:
Hsu L-Y, Lai L-Y, Hsieh P-F, Lin T-L, Lin W-H, Tasi H-Y, Lee W-T, Jou R and Wang J-T (2020) Two Novel katG Mutations Conferring Isoniazid Resistance in Mycobacterium tuberculosis. Front. Microbiol. 11:1644. doi: 10.3389/fmicb.2020.01644

INTRODUCTION

According to the Global Tuberculosis Report published by the World Health Organization (WHO), tuberculosis (TB), the airborne infectious disease caused by *Mycobacterium tuberculosis* (Cambau and Drancourt, 2014), is one of the top 10 causes of death worldwide, and thus remains a major global public health problem (WHO, 2019). The emergence of drug-resistant TB has made the need for improvements in diagnostic accuracy and successful treatment even more urgent, as both are major challenges in TB control and key causes of its high mortality rate (Nguyen et al., 2019).

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Original Article

Pattern of *InhA* and *KatG* mutations in isoniazid monoresistant *Mycobacterium tuberculosis* isolates

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ABSTRACT

Aims and Objectives: The aim of the study is to detect the pattern of genetic mutation, i.e., *Inh*A or *Kat*G or both (*Inh*A and *kat*G) in isoniazid (INH) monoresistant mycobacteria and to correlate with the pattern in multidrug-resistant (MDR) isolates. **Materials and Methods:** In this study, a quantitative research approach was used. The research design was descriptive observational study. The study was conducted at the Department of Respiratory Medicine, JLN Medical College, Ajmer, Rajasthan, and Intermediate Referral Laboratory, State TB Demonstration Centre, Ajmer. A total of 298 samples found to have resistant strains of *Mycobacterium tuberculosis* were enrolled with purposive sampling. **Results:** The mean age of patients was 40.27 ± 13.82 years. There were 250 (83.9%) males, while 48 (16.1%) were females. One hundred ninety-two (64.4%) were resistant for INH only, while the rest were resistant to both INH as well as rifampicin (MDR-tuberculosis). The most common mutation in INH monoresistance was *kat*G (125; 65.1%) as compared to *inh*A (54; 28.1%) and both *inh*A and *kat*G (13; 6.7%). Among *kat*G mutations, the most common gene pattern was the absence of WT (S315T) and the presence of MUT1 (S315T1). **Conclusion:** Knowledge about mutation patterns of different INH resistant strains is important in the present era where there is a provision of separate regimens for INH monoresistant TB. Since these mutations are very closely related to high- or low-degree resistance to INH, the therapeutic regimens cannot be generalized.

KEY WORDS: Gene pattern, *Inh*A, isoniazid resistance, *Kat*G, multidrug-resistant tuberculosis, mutations

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Submitted: 12-May-2019 **Revised:** 29-Sep-2019 **Accepted:** 06-Dec-2019 **Published:** 04-May-2020

INTRODUCTION

Drug resistance in tuberculosis (TB) is a major public health challenge in developing countries, and the emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains has become a major obstacle in the management of TB.[1] Isoniazid (INH) is one of the most potent antimycobacterial agents available for the treatment of TB and has both bactericidal and sterilizing actions. It inhibits mycolic acid biosynthesis of *M. tuberculosis*. However, the resistance to INH is most common among all first-line anti-TB drugs.[2] As far as monoresistance is concerned, resistance to INH (7.2%) exceeds other first-line anti-TB drugs (6.85% for streptomycin, 1.6% for ethambutol, and 4.6% for rifampicin).[3]

Access this article online

Quick Response Code:

Website: www.lungindia.com

DOI: 10.4103/lungindia.lungindia_204_19

How to cite this article: Charan AS, Gupta N, Dixit R, Arora P, Patni T, Antony K, *et al*. Pattern of *InhA* and *KatG* mutations in isoniazid monoresistant *Mycobacterium tuberculosis* isolates. Lung India 2020;37:227-31.

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The Journal of Infectious Diseases

MAJOR ARTICLE

Isoniazid and Rifampin-Resistance Mutations Associated With Resistance to Second-Line Drugs and With Sputum Culture Conversion

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Background. Mutations in the genes *inhA*, *katG*, and *rpoB* confer resistance to anti-tuberculosis (TB) drugs isoniazid and rifampin. We questioned whether specific mutations in these genes were associated with different clinical and microbiological characteristics.

Methods. In a multicountry prospective cohort study of multidrug-resistant TB, we identified *inhA*, *katG*, and *rpoB* mutations in sputum isolates using the Hain MTBDR*plus* line probe assay. For specific mutations, we performed bivariate analysis to determine relative risk of baseline or acquired resistance to other TB drugs. We compared time to sputum culture conversion (TSCC) using Kaplan-Meier curves and stratified Cox regression.

Results. In total, 447 participants enrolled from January 2005 to December 2008 from 7 countries were included. Relative to *rpoB* S531L, isolates with *rpoB* D516V had less cross-resistance to rifabutin, increased baseline resistance to other drugs, and increased acquired fluoroquinolone resistance. Relative to mutation of *katG* only, mutation of *inhA* promoter and *katG* was associated with baseline extensively drug resistant (XDR) TB, increased acquired fluoroquinolone resistance, and slower TSCC (125.5 vs 89.0 days).

Conclusions. Specific mutations in *inhA* and *katG* are associated with differences in resistance to other drugs and TSCC. Molecular testing may make it possible to tailor treatment and assess additional drug resistance risk according to specific mutation profile.

Keywords. culture conversion; drug resistance; isoniazid; rifampin; second-line drugs tuberculosis.

Drug-resistant tuberculosis (TB) is an increasing public health problem, with an estimated 457 560 new cases of multidrug-resistant TB ([MDR-TB] resistant to at least isoniazid and rifampin) in 2017 [1]. Drug-resistant TB is more difficult and expensive to treat than drug-sensitive TB disease leading to poor treatment outcomes and amplified drug resistance [2]. Determining risk factors for poor TB treatment outcomes would allow clinicians to focus on high-risk patients, tailoring treatment to optimize outcomes, and reduce costs [3].

Molecular assays for drug resistance test for common mutations are known to cause resistance. The Hain MTBDR*plus* assay detects common mutations in the *rpoB* gene causing rifampin resistance and in the *katG* gene and *inhA* promoter causing isoniazid resistance [4]. As a result, such tests not only provide diagnostic information but may aid in understanding the functional and clinical significance of drug resistance at the genetic level.

Different mutations can result in functional differences, leading to different levels of resistance or resistance to different drugs within or between drug classes. In addition, mutations could affect mycobacterial fitness and consequently clinical characteristics, including time to sputum culture conversion (TSCC). In other words, not all MDR-TB strains are the same biologically or behave the same clinically. For example, resistance to isoniazid can be due to mutations in *katG* or the promoter region of *inhA*, resulting in different mechanisms of resistance. Mutations in the *inhA* promoter also confer cross-resistance to ethionamide, which shares the same target as isoniazid (InhA), whereas mutations in *katG* do not [5]. Likewise, different mutations within a single gene could result in differing biological properties and clinical manifestations. Differences in frequencies of specific mutations in a single gene suggest mutations may differ biologically and may result from selective advantage of certain mutations relative to others [6].

The genes most commonly associated with resistance to isoniazid and rifampin encode proteins that have critical functions

Received 5 September 2019; editorial decision 21 January 2020; accepted 28 January 2020; published online January 31, 2020.

Presented in part: International Union Against TB and Lung Disease World Conference, December 2–6, 2015, Cape Town, South Africa.

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The Journal of Infectious Diseases® 2020;221:2072–82

DOI: 10.1093/infdis/jiaa042

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Arch Clin Infect Dis. 2020 February; 15(1):e81436. doi: 10.5812/archcid.81436.

Published online 2020 February 5. **Research Article**

Molecular Detection of Isoniazid and Rifampin Resistance in *Mycobacterium tuberculosis* Isolates from Lorestan Province, Iran from 2014 to 2017

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Received 2018 June 28; **Revised** 2019 December 21; **Accepted** 2019 December 29.

Abstract

Background: A rise in multidrug-resistant tuberculosis (MDR-TB), which is defined as the resistance to the two most effective first-line therapeutic drugs, Isoniazid (INH) and Rifampin (RIF), threatens global public health worldwide. Resistance of *Mycobacterium tuberculosis* to INH results from mutations in several genes most commonly in *katG* gene, and resistance to RIF is due to mutations in *rpoß* gene. Therefore, rapid diagnosis of MDR-TB is of high importance in controlling the disease progress and outcome. The accurate detection of the resistant TB strains can be accelerated by developing molecular tests.
Objectives: The aim of the present local study was to isolate MDR-TB from the patients who were the residents in the west of Iran and examination the frequency of MDR-TB between patients of Lorestan province for the first time and assess the mutations in the regions related to RIF/INH resistance via PCR and sequencing methods.
Methods: In this study, 106 isolates of *M. tuberculosis* were selected in health centers of Lorestan, Iran from 2014 to 2017. After culturing *M. tuberculosis* isolates on Löwenstein-Jensen medium, classical susceptibility testing proportional method against INH and RIF was performed. After DNA extraction, PCR and sequencing were used to detect mutations related to RIF and INH resistance.
Results: The results demonstrated 3.8%, 0.9%, and 0.9% frequency for INH + RIF, INH and RIF resistance, respectively. Importantly, 4 out of 6 isolates harbored mutations in codon290 of *katG* gene. Also, these isolates contained mutations in codon339 of *rpoß* gene. No mutation was observed in *inhA* gene of *M. tuberculosis* isolates.
Conclusions: The results suggest that molecular techniques can be used as a rapid method for the identification of drug resistance in clinical isolates of *M. tuberculosis*. DNA sequencing has a high sensitivity for the detection of resistance mutations to RIF and INH in MDR-TB cases. Also, the results showed that the most frequent resistance associated-mutations occurred in codon290 of *katG* and codon 339 *rpoß* gene segments.

Keywords: *Mycobacterium tuberculosis*, Isoniazid, Rifampin, Multidrug Resistance

1. Background

An increase in the global incidence of drug-resistant *Mycobacterium tuberculosis (MTB)* infection threatens appropriate TB prevention, diagnosis, treatment, and case management. Therefore, there is a critical need in the healthcare system for efficient approaches to rapidly identify drug-resistant cases of *MTB* (1). According to the world health organization (WHO), multi-drug-resistant tuberculosis (MDR-TB) is the strain that does not respond to at least isoniazid and rifampin, which are among the most powerful first-line anti-TB drugs (2). However, taking multiple drugs concomitantly can successfully treat patients and limit the growth of MDR strains (3). As it is well-known that random genetic mutations in specific genes encoding either the target of the drug, are involved in drug activation and confers resistance to *MTB* isolates (4, 5). Several mechanisms of *TB* drug resistance have been well characterized. Point mutations, deletions, or insertions have been described for all of the first-line drugs (isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin), and for the several second-line and newer drugs (5). Thus, the establishment of molecular assays allows the rapid detection of drug resistance in clinical *MTB* isolates (6).

Isoniazid (INH) is a prodrug and a catalase-peroxidase encoded by *katG* gene, associated with peroxidase activ-

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Research Article

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Occurrence of mutations associated with rifampicin and isoniazid resistant in *Mycobacterium tuberculosis* isolates from patients in Burkina Faso

Abstract

Genetic mutations are responsible for the high rate of resistance observed in the treatment of tuberculosis. This study aimed at determining the occurrence of mutations associated with rifampicin (RIF) and isoniazid (INH) resistance of *Mycobacterium tuberculosis* complex (MTBC) isolates. MTBC strains isolated by culture from 110 TB patients diagnosed with resistant to rifampicin (RR-TB) by Xpert MTB/RIF were studied. The isolates were obtained from the National Tuberculosis Reference Laboratory in Ouagadougou. They were identified culturally using Antigenic method (SD Bioline TB Ag MPT64). Polymerase Chain Reaction, PCR (*DRplus*) was used to detect the occurrence of mutations in the genes associated with resistance *katG and inhA* promoter for INH, and *rpoB* for RIF. Out of 103 isolates with RIF resistant, mutations were detected in 87(84.5%) of gene *rpoB* while no mutation was found in 16(15.5%) of the gene of the isolates even though the wild probes had disappeared. Single mutations were found in the codons D516V (41.7%) and H526Y (17.5%) while combined mutations (single and double) were mostly detected in the codons D516 (51.5%), H526Y (20.4%), S531L (11.7%) and H526D (10.7%) respectively. Single mutations responsible for high-level isoniazid resistance, *katG* were observed in the codon S315T1 while the combined *inhA* and *katG* were detected in the codon C8T and S315T, 16 (14.5%) respectively. The highest mutation occurrence was observed with *rpoB*516, *rpoB*526 for RIF and *katG*315 for INH associated with resistance of MTBC isolates. There is a need to improve molecular assay kit diagnosis to curb the geographic specificity of the target genes needed to detect more possible mutations.

Keywords: *mycobacterium tuberculosis* strains, genes, mutations, resistance

Volume 3 Issue 3 - 2019

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Received: June 11, 2019 | **Published:** June 27, 2019

Introduction

The emergence of multidrug resistant (MDR) strains of MTBC has become one of the most critical issues for tuberculosis (TB) control programmes worldwide. It is a public health concern threatening global TB control programs. Its diagnosis has evolved in recent years following the development of new molecular techniques based on detection of mutations in MTBC genes by Polymerase Chain Reaction (PCR).[1–3]

The Genotype *MTBDRplus* is a commercially available molecular gene Line Probe Assay developed by Hain Life Science, (Nehren, Germany). It is performed on MTBC isolates or directly from clinical specimens. It able to identify the MTBC and detect the genetic mutations in the *rpoB* gene related to rifampicin resistance, the *katG*, *inhA* regulatory region and *inhA* genes related to isoniazid resistance. Its targets points are the 81-bp "hot spot" region of the *rpoB* gene of RIF, codon 315 of *katG* and *inhA* promoter regions of INH.[4–7]

The genetic basis of multidrug resistant MTB isolates has been widely studied worldwide and commonly believed to be caused by point mutations in important genes like *rpoB* and *katG*. Multiple studies carried out at different time periods in the same country/geographical setting have yielded variable incidence of specific *rpoB* mutation.[8,9]

In Burkina Faso, the fight against MDR-TB/rifampicin-resistant tuberculosis (RR-TB) has become a National concern. For this purpose, the technical platform of the National Reference Laboratory (NRL) for *Mycobacteria* in Ouagadougou was strengthened with Molecular tests such as Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) and Line Probe Assay (Hain Life Science GmbH, Nehren, Germany). However, other fourteen peripheral Laboratories in the Country have also been equipped with GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA). So, the guidelines of the National Tuberculosis Control Program recommend the use of *DRplus* and *DRsl* for all TB-patients confirmed RR-TB by Xpert test (Cepheid, Sunnyvale, CA, USA).

This study was to determine the occurrence of specific *rpoB, katG and inhA* gene promoters' mutations in rifampicin and isoniazid resistant *M. tuberculosis* isolates from TB-patients in Burkina Faso.

Materials and methods

Study area and laboratory analysis

We studied rifampicin resistant *M tuberculosis* strains isolated from 110 TB patients diagnosed with resistant to rifampicin (RR-TB) by Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) at the NRL in Ouagadougou. The patients were undergoing treatment at Centres for Diagnostic Tuberculosis (CDT) between 2014 and 2016 in the 13 Health regions of Burkina Faso. During this period, suspected MDR-TB patient's sputa were collected from the various CDTs in the Health regions of the Country and transported to the NRL in Ouagadougou where they were identified culturally using Antigenic method (SD Bioline TB Ag MPT64); thereafter, PCR (*DRplus)* confirmation. The patients' medical records were review to obtain relevant data on the age, sex, category of patients, HIV status, and region of origin.

Molecular analysis by genotype MTBDR plus 2.0

One hundred and ten (110) *Mycobacteria tuberculosis* isolates were

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SCIENTIFIC REPORTS

OPEN

Identification and Characterization of Genetic Determinants of Isoniazid and Rifampicin Resistance in *Mycobacterium tuberculosis* in Southern India

Received: 18 March 2019
Accepted: 28 June 2019
Published online: 16 July 2019

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Drug-resistant tuberculosis (TB), one of the leading causes of death worldwide, arises mainly from spontaneous mutations in the genome of *Mycobacterium tuberculosis*. There is an urgent need to understand the mechanisms by which the mutations confer resistance in order to identify new drug targets and to design new drugs. Previous studies have reported numerous mutations that confer resistance to anti-TB drugs, but there has been little systematic analysis to understand their genetic background and the potential impacts on the drug target stability and/or interactions. Here, we report the analysis of whole-genome sequence data for 98 clinical *M. tuberculosis* isolates from a city in southern India. The collection was screened for phenotypic resistance and sequenced to mine the genetic mutations conferring resistance to isoniazid and rifampicin. The most frequent mutation among isoniazid and rifampicin isolates was S315T in *katG* and S450L in *rpoB* respectively. The impacts of mutations on protein stability, protein-protein interactions and protein-ligand interactions were analysed using both statistical and machine-learning approaches. Drug-resistant mutations were predicted not only to target active sites in an orthosteric manner, but also to act through allosteric mechanisms arising from distant sites, sometimes at the protein-protein interface.

Tuberculosis (TB) caused an estimated 1.3 million deaths worldwide in 2016 (WHO Global Tuberculosis Report, 2017). The major challenge in the treatment of tuberculosis is the emergence of drug-resistant *Mycobacterium tuberculosis*[1]. The drugs available for tuberculosis treatment are categorised into first-line (isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol (EMB) and streptomycin (STR)) and second line (including fluoroquinolones, thioamides, cycloserine and the injectable aminoglycosides). Rising rates of multi-drug-resistant tuberculosis (MDR-TB, defined as resistance to INH and RIF), are of immense concern for TB control worldwide. Extended treatment is required with multiple drugs that have a higher rate of side effects but limited rate of treatment success (Gygli *et al.*[2]). India accounts for the highest burden of tuberculosis globally and also ranks top among the countries for MDR-TB cases (WHO Global Tuberculosis Report, 2017).

Drug resistance arises mainly from spontaneous mutations in the bacterial genome. Resistance to first-line anti-TB drugs has been linked to mutations in *katG*[3] and *inhA*[4] for INH resistance; *rpoB* for RIF resistance[5]; *embB*

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Huo et al. BMC Infectious Diseases (2019) 19:689
https://doi.org/10.1186/s12879-019-4333-4

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Change in prevalence and molecular characteristics of isoniazid-resistant tuberculosis over a 10-year period in China

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Abstract

Background: Isoniazid (INH) represents the cornerstone for the treatment of cases infected with *Mycobacterium tuberculosis* (MTB) strains. Several molecular mechanisms have been shown to be the major causes for INH resistance, while the dynamic change of mutations conferring INH resistance among MTB strains during the past decade is still unknown in China.

Methods: In this study, we carried out a comparative analysis of the INH minimal inhibitory concentration (MIC) distribution, and investigate the dynamic change of molecular characteristics among INH-resistant MTB strains between 2005 and 2015.

Results: The proportion of INH resistance (39.0%, 105/269) in 2015 was significantly higher than in 2005 (30.0%, 82/273; $P = 0.03$). Among 269 isolates collected in 2015, 76 (28.3%, 76/269) exhibited high-level INH-resistance (MIC≥32 mg/L), which was significantly higher than that in 2005 (20.5%, 56/273, $P = 0.04$). In addition, a significantly higher percentage of INH-resistant isolates carried *inhA* promoter mutations in 2015 (26.7%) versus that in 2005 (14.6%, $P = 0.04$), while no significant difference was observed in the rates of isolates containing *katG* mutations between 2005 (76.8%) and 2015 (70.5%, $P = 0.33$). Notably, the proportion of MTB isolates with *inhA* mutations (26.7%, 28/105) for patients who had previous exposure to protionamide (PTH) was higher than that for patients who had no previous exposure to PTH (21.4%, 6/28).

Conclusions: In conclusion, our results demonstrated that the proportion of INH-resistant MTB isolates significantly increased during the last decade, which was mainly attributed to an increase of high-level INH-resistant MTB. In addition, prior exposure to PTH may be associated with the increased frequency of INH-resistant tuberculosis strains with *inhA* mutations in China.

Keywords: Tuberculosis, Isoniazid, Drug resistance, inhA, Protionamide

Background

Tuberculosis (TB), is a serious global public health concern, with an incidence of 10.0 million new cases and 1.6 million deaths in 2017 [1]. Despite achieving great progress in curbing the TB epidemic during the last two decades, the efforts to control TB are threatened by the emergence of drug-resistant TB [2], especially multidrug-resistant TB (MDR-TB), defined as the strains resistant to at least isoniazid (INH) and rifampicin (RIF) [3, 4]. As one of the most potent anti-TB drug, isoniazid, together with rifampicin, represents the cornerstone for the treatment of cases infected with RIF-susceptible TB strains [5]. Prior clinical trials have shown that the TB cases with initial INH resistance is at high risk for poor clinical outcomes among the cases receiving the standard first-line therapy regimen, who are more likely to progress to MDR-TB [6, 7]. Hence, the early detection of isoniazid resistance is essential to allow clinicians to adjust

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Liu et al. *Emerging Microbes & Infections* (2018)7:183
DOI 10.1038/s41426-018-0184-0

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ARTICLE Open Access

The impact of combined gene mutations in *inhA* and *ahpC* genes on high levels of isoniazid resistance amongst *katG* non-315 in multidrug-resistant tuberculosis isolates from China

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Abstract

Whole-genome sequencing was used to analyze the profiles of isoniazid (INH) resistance-related mutations among 188 multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB) and mono-INH-resistant isolates collected in a recent Chinese national survey. Mutations were detected in 18 structural genes and two promoter regions in 96.8% of 188 resistant isolates. There were high mutation frequencies in *katG*, the *inhA* promoter, and *ahpC-oxyR* regulator regions in INH-resistant isolates with frequencies of 86.2%, 19.6%, and 18.6%, respectively. Moreover, a high diversity of mutations was identified as 102 mutants contained various types of single or combined gene mutations in the INH-resistant group of isolates. The cumulative frequencies of *katG* 315 or *inhA*-P/*inhA* mutations was 68.1% (128/188) for the INH-resistant isolates. Of these isolates, 46 isolates (24.5% of 188) exhibited a high level of resistance. A high level of resistance was also observed in 21 isolates (11.2% of 188) with single *ahpC-oxyR* mutations or a combination of *ahpC-oxyR* and *katG* non-315 mutations. The remaining 17 mutations occurred sporadically and emerged in isolates with combined *katG* mutations. Such development of INH resistance is likely due to an accumulation of mutations under the pressure of drug selection. Thus, these findings provided insights on the levels of INH resistance and its correlation with the combinatorial mutation effect resulting from less frequent genes (*inhA* and/or *ahpC*). Such knowledge of other genes (apart from *katG*) in high-level resistance will aid in developing better strategies for the diagnosis and management of TB.

Introduction

Tuberculosis (TB) is caused by infection with *Mycobacterium tuberculosis* and represents one of the greatest threats to human health worldwide, and it is associated with 1.4 million deaths annually (2016 WHO report). As a component of first-line TB drugs, isoniazid (INH) is used both for the treatment of active TB and as a preventive therapy for latent infections[1]. The emergence of INH-resistant *M. tuberculosis* strains readily leads to the development of multidrug-resistant TB (MDR-TB; *M.*

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Journal of Medicine and Life Vol. 11, Issue 2, April-June 2018, **pp.160-167**

Molecular Analysis of *katG* Encoding Catalase-Peroxidase from Clinical Isolate of Isoniazid-Resistant Mycobacterium tuberculosis

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Received: September 29th, 2017 – Accepted: April 18th, 2018

Abstract

Isoniazid (INH) is a drug for the treatment of tuberculosis in patients infected with Mycobacterium tuberculosis. The katG enzyme, or catalase-peroxidase, activates the pro-drug INH that is coded by the katG gene in M. tuberculosis. Mutations of the katG gene in M. tuberculosis are a major INH resistance mechanism. The M. tuberculosis clinical isolate R2 showed INH resistance at a high level of 10 μg/mL. However, the molecular basis for the resistance is unclear. The identification of a mutation in the katG gene of the clinical isolate R2 showed four mutations, i.e., C1061T, G1261 A, G1388T, G2161A, which correspond to the amino acid substitutions T354I, G421S, R463L, and V721M, respectively. The mutant katG gene, along with the wild-type were cloned, expressed and purified. The mutant enzyme showed 86.5% of catalase and 45% of peroxidase activities in comparison to the wild type. The substitutions of $T_{354}I$ and $G_{421}S$ in mutant katG R2 created significant instability in the adduct triad complex (Trp107-Tyr229-Met255), a part of the active site of the catalase-peroxidase enzyme in the model structure analysis. The events could be based on the high resistance of the clinical isolate R2 toward INH as the molecular basis.

Keywords: isoniazid, Mycobacterium tuberculosis, katG, catalase-peroxidase

Introduction

Isoniazid (isonicotinic acid hydrazide, INH) is a prodrug which forms a key part of the frontline chemotherapy used to treat tuberculosis (TB) in many countries. INH has been widely used to treat the TB disease caused by *Mycobacterium tuberculosis* since it is cost-effective and exhibits a high bactericidal effect [1,2]. INH has a minimal inhibitory concentration (MIC) to kill *M. tuberculosis* at a dosage between 0.02 – 0.2 μg/mL [3,4]. Apart from multidrug-resistant TB (MDR-TB), about 9.5% (8.1% in new and 14.0% in previously treated) of TB cases worldwide in 2017 were estimated to have isoniazid-resistant TB without MDR-TB. This is associated with an increased risk of treatment failure in patients who receive first-line regimens [1].

To function as an antitubercular agent, INH requires activation of the catalase-peroxidase enzyme encoded by the *M. tuberculosis* katG gene [4]. The INH is bound by catalase-peroxidase in its active site, then converted to an isonicotinoyl acyl radical through the use of a diazene intermediate [4]. The isonicotinoyl acyl radical interacts with the NADH electron donor in the active site of the enoyl ACP reductase (InhA) enzyme [5]. The NAD-INH complex is known as a potent inhibitor of InhA, the enzyme that has an important role in the biosynthesis of mycolic acid, the cell wall component in mycobacteria [5].

The catalase-peroxidase from *M. tuberculosis* (katG) is a homodimer protein with two subunits of 80 kDa. Each subunit has two dominant α-helix domains, which means that the domains originated from gene duplication. The N domain has a heme, an active site and a substrate binding site. While the C domain does not have those, its presence is needed to support the overall enzyme activity [6, 7, 8]. The catalytic activity of katG is mediated by some residues in the active site that resided around the heme group. The heme is surrounded by six residues which are Arg-104, Trp-107 and His-108 in the distal pocket, and His270, Trp321 and Asp381 in the proximal pocket. In the heme, the Trp107 residue is connected to Tyr229 and Met255 residues to form an adduct triad complex. The adduct triad is likely conserved in many catalase-peroxidase structures and it is involved in the catalase activity [9]. The binding of INH to katG takes place at the edges of the δ-meso heme. In the region, the residues of the distal pocket, i.e., Arg104, Trp107 and His108, are involved in the interactions with INH [9].

Mutations in katG that change catalase-peroxidase activities are generally associated with INH

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Isakova *et al. BMC Microbiology* (2018) 18:22
https://doi.org/10.1186/s12866-018-1168-x

BMC Microbiology

RESEARCH ARTICLE Open Access

Mutations of *rpoB, katG, inhA* and *ahp* genes in rifampicin and isoniazid-resistant *Mycobacterium tuberculosis* in Kyrgyz Republic

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Abstract

Background: The aim of this study was to identify mutations of *rpoB, katG, inhA* and *ahp*-genes associated *Mycobacterium tuberculosis* resistance to rifampicin (RIF) and isoniazid (INH) in Kyrgyz Republic. We studied 633 smear samples from the primary pulmonary tuberculosis (TB) patients. We verified *Mycobacterium tuberculosis* susceptibility to RIF and INH using culture method of absolute concentrations, and commercially available test named "TB-BIOCHIP" (Biochip-IMB, Moscow, Russian Federation).

Results: For RIF-resistance, TB-BIOCHIP's sensitivity and specificity were 88% and 97%, 84% and 95% for INH-resistance, and 90% and 97% for multi-drug resistance (MDR). In RIF-resistant strains, TB-BIOCHIP showed mutations in codons 531 (64.8%), 526 (17.3%), 516 (8.1%), 511 (5.4%), 533 (3.2%), 522 (0.6%) and 513 (0.6%) of *rpoB* gene. The most prevalent was Ser531 > Leu mutation (63.7%). 91.2% of mutations entailing resistance to INH were in *katG* gene, 7% in *inhA* gene, and 1.8% in *ahpC* gene. Ser315→Thr (88.6%) was the most prevalent mutation leading to resistance to INH.

Conclusions: In Kyrgyz Republic, the most prevalent mutation in RIF-resistant strains was Ser531 → Leu in *rpoB* gene, as opposed to Ser315 → Thr in *katG* gene in INH-resistant *Mycobacterium tuberculosis*. In Kyrgyz Republic, the major reservoir of MDR *Mycobacterium tuberculosis* were strains with combined mutations Ser531 → Leu in *rpoB* gene and Ser315 → Thr in *katG* gene. TB-BIOCHIP has shown moderate sensitivity with the advantage of obtaining results in only two days.

Keywords: *Mycobacterium tuberculosis, rpoB, katG, inhA, Ahp,* TB-BIOCHIP, Kyrgyz Republic

Background

Kyrgyzstan is a country in Central Asia with the total population of 6 million, whereas mountainous terrains with poor access to medical care occupy 90% of its territory. As reported by the WHO, Kyrgyzstan shows high incidence and mortality from TB, and the former peaked in 2001 with 167.8 cases per 100,000 population including penitentiary system patients. TB incidence in Kyrgyzstan that year was 16.8 times the worldwide threshold incidence of 10.0 per 100,000. The corresponding mortality rate that year was 27.0 per 100,000 including penitentiary system deaths. Since 2002, there was a consistent reduction in both incidence and mortality from TB in the country. By 2012, the incidence dropped by 37.8% to 104.3 per 100,000, whereas 68.1% reduction in mortality was registered, and the latter equaled 8.6 per 100,000, including penitentiary system patients. During the period of 2012–2016, both incidence and mortality continued to drop. In 2016, 93.1 new cases and 5.8 deaths per 100,000 were reported [1].

Parallel to the overall persistent reduction in TB incidence, the number of multidrug resistant (MDR) and

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Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations

Received: 8 September 2017
Accepted: 1 February 2018
Published online: 19 February 2018

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The majority of *Mycobacterium tuberculosis* isolates resistant to isoniazid harbour a mutation in *katG*. Since these mutations cause a wide range of minimum inhibitory concentrations (MICs), largely below the serum level reached with higher dosing (15 mg/L upon 15–20 mg/kg), the drug might still remain partly active in presence of a *katG* mutation. We therefore investigated which genetic mutations predict the level of phenotypic isoniazid resistance in clinical *M. tuberculosis* isolates. To this end, the association between known and unknown isoniazid resistance-conferring mutations in whole genome sequences, and the isoniazid MICs of 176 isolates was examined. We found mostly moderate-level resistance characterized by a mode of 6.4 mg/L for the very common *katG* Ser315Thr mutation, and always very high MICs (≥19.2 mg/L) for the combination of *katG* Ser315Thr and *inhA* c-15t. Contrary to common belief, isolates harbouring *inhA* c-15t alone, partly also showed moderate-level resistance, particularly when combined with *inhA* Ser94Ala. No overt association between low-confidence or unknown mutations, except in *katG*, and isoniazid resistance (level) was found. Except for the rare *katG* deletion, line probe assay is thus not sufficiently accurate to predict the level of isoniazid resistance for a single mutation in *katG* or *inhA*.

Effective control of tuberculosis (TB) is especially complicated among patients with multidrug-resistant TB (MDR-TB), which is characterized by resistance to at least isoniazid and rifampicin, the two most powerful drugs against TB, used in standard first-line treatment[1,2]. Preventing the activation of the pro-drug isoniazid, mutations in *katG* are the most frequent cause of isoniazid resistance[3,4]. Although more than 300 different *katG* mutations have been identified, mutations at codon 315 of the gene are most prevalent, with, on average, 64% of isoniazid-resistant clinical isolates worldwide carrying a *katG* 315 mutation[3,4]. Moreover, one particular amino acid substitution (serine to threonine) accounts for 95% of all *katG* 315 mutations[4]. Mutations in *katG* are associated with a wide range of moderate- to high-level isoniazid resistance, above the commonly tested concentrations of 0.2 and 1 mg/L in solid- and 0.1 and 0.4 mg/L in liquid medium[5]. The *katG* Ser315Thr mutation in particular is associated with minimum inhibitory concentrations (MICs) ranging from 2 to >10 mg/L[5], while (partial) deletion of the *katG* gene causes very high MICs (>25.6 mg/L)[6,7].

In addition to *katG* mutations, isoniazid resistance arises from mutations in the promoter region of *inhA*, which lead to overexpression of isoniazid's target InhA, requiring higher doses of the drug to achieve complete inhibition[3]. Mutations in the promoter region of *inhA* tend to result in low-level phenotypic resistance[5] and also confer resistance to the second-line drugs ethionamide and prothionamide[3]. The most prevalent *inhA* promoter region mutation is the c-15t mutation, which is present in, on average, 19% of isoniazid-resistant clinical isolates worldwide[4]. In addition to the two most frequent causes of isoniazid resistance, mutations in *katG* expression regulatory genes (e.g. the *furA-katG* intergenic region and *sigI*) and in the coding region of *inhA*, as well as mutations

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RESEARCH ARTICLE

Molecular characterisation of isoniazid resistant clinical isolates of Mycobacterium tuberculosis from Khyber Pakhtunkhwa, Pakistan

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Abstract

Objectives: To investigate the frequency of mutations in catalase-peroxidase and inhibin alpha genes in clinical isolates of isoniazid resistant Mycobacterium tuberculosis strains.

Methods: The study was conducted at Provincial Tuberculosis Reference Laboratory, Peshawar, Pakistan, from April 2015 to March 2016, and comprised sputum specimens obtained from patients of different ages. All the isolates were analysed for isoniazid resistance. Thirty resistant isolates were randomly selected for mutation analysis of the hotspot regions of catalase-peroxidase and inhibin alpha genes.

Results: Of the 163 positive isolates, 79(48.46%) were resistant to isoniazid. Among these, 21(70%) had mutation in catalase-peroxidase gene and 2(6.6%) had C15T mutation in inhibin alpha promoter region. Among the 21 catalase-peroxidase mutants, Ser315Thr mutation was detected in 15(71.4%) isolates. Gly316Ser mutation was detected in 3(14.2%) isolates. Ser315Arg mutation was identified in 2(9.5%) isolates. Double mutation of Ser303Trp and Lys274Arg was detected in 1(4.7%) isolate. Among the inhibin alpha promoter region mutations, 2(6.6%) of the thirty isolates had the most common C15T mutation in the promoter region.

Conclusion: One novel mutation at codon 303 in catalase-peroxidase gene was found in the study, and it could contribute to isoniazid resistance.

Keywords: inhA, katG, PCR, Sequencing. (JPMA 67: 1224; 2017)

Introduction

Mycobacterium tuberculosis (MTB) is the causative organism of the devastating tuberculosis (TB) disease. Tuberculosis is a global health problem and could be transmitted from one individual to another by droplet aerosol.[1] The disease is caused by a group of related bacterial species called mycobacterium tuberculosis complex (MTBC). These include mycobacterium (M.) africanum, M. microti, M. cannetii, M. bovis and M. tuberculosis. About 9.6 million people were infected with Mycobacterium tuberculosis infection and 1.5 million death cases were reported in 2014.[2] About 480,000 people developed multiple drug-resistant tuberculosis (MDR-TB) in 2014. Many new cases are arising rapidly in countries like China, Bangladesh, Pakistan, India and Indonesia, which are highly populated countries of the world. Pakistan ranked sixth amongst highest TB reported countries.[3] According to World Health Organisation's (WHO) estimates, 43 million people were saved through advancement in TB diagnosis techniques and treatments during the last fourteen years.[2]

Antimicrobial susceptibility testing is performed invitro to measure Mycobacterium tuberculosis growth response against antimicrobial agents. BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system is an automated instrument that has been reliably used for antimicrobial susceptibility testing. Antimicrobial resistance develops when microorganism acquire new mechanisms to overcome the effects of antimicrobial agents used for the treatment of various infections. Mycobacterium TB isolates develop resistance to the targeted drug as a result of modification in drug activating and target including genes and/or their promoter regions.[4] The first-line TB drugs are generally used against mycobacterium tuberculosis as first treatment option. These include isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin. The bacterium could develop resistance to these drugs. The resistant strains are called MDR-TB strains, i.e. tuberculosis strains with resistance to a minimum of two (isoniazid and rifampicin) of the first-line drugs.[5] To control these MDR-TB strains, second-line drugs which include capreomycin, kanamycin or amikacin are used; however, these drugs have more toxic effect and are more expensive. Mycobacterium tuberculosis strains resistant to both the first- and second-line drugs are known as totally drug-resistant TB strains or extremely drug-resistant TB(XDR TB) strains which need more advanced treatment options.[6]

Isoniazid (INH) is a first-line anti-tuberculosis antibiotic used for treatment of tuberculosis since its introduction in 1952.[7]

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Cakra Kimia (Indonesian E-Journal of Applied Chemistry)
Volume 2, Nomor 2, Oktober 2014

AMPLIFIKASI DAN IDENTIFIKASI MUTASI REGIO PROMOTER *inhA* PADA ISOLAT *Mycobacterium tuberculosis MULTIDRUG RESISTANCE* DENGAN TEKNIK *POLYMERASE CHAIN REACTION*

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ABSTRAK: Sekitar 8-20% isolate *M. tuberculosis* yang resisten terhadap isoniazid diketahui telah mengalami mutasi pada posisi regio promoter *inhA* [1]. Untuk memperoleh titik mutasi pada regio promoter, maka amplifikasi fragmen target perlu untuk dilakukan. Tujuan dilakukannya penelitian ini adalah untuk mengamplifikasi regio promoter *inhA*, mengetahui ada tidaknya mutasi dan jenis mutasi pada isolat 134 MDR-TB. Tahap isolasi DNA dilakukan menggunakan metode Boom yang telah dimodifikasi. Fragmen target diamplifikasi dengan teknik PCR menggunakan sepasang primer (*forward primer* 5' ACATACCTGCTGCGCAAT 3' dan *reverse primer* 5' CTCCGGTAACCAGGACT GAA 3'). Amplikon disekuensing secara satu arah menggunakan *forward primer*. Analisis homologi dilakukan menggunakan program *online* BLASTn, sementara identifikasi mutasi dilakukan menggunakan *software* MEGA4. Hasil penelitian menunjukkan bahwa analisis homologi isolate 134 terhadap *M. tuberculosis* H37Rv adalah sebesar 99%. Tahap analisis mutasi menemukan terjadinya perubahan sitosin menjadi timin (C→T) pada posisi -15 isolat 134 MDR-TB.

Kata kunci: *M. tuberculosis,* regio promoter *inhA,* PCR, homologi, mutasi

ABSTRACT: Approximately 8-20% *M. tuberculosis* isolates that are resistant to isoniazid habe been known to have a mutation in *inhA* promoter region [1]. To find the mutation in *inhA* promoter region, it is necessary to carry out the amplification of the target fragment. The purpose of this research were to amplify the *inhA* promoter region and to find out if there is a mutation and type of mutation at MDR-TB isolate. DNA isolation was done by a modified Boom method. Target fragment was amplified by a pair primer (forward primer 5' ACATACCTGCTGCGCAAT 3' and reverse primer 5' CTCCGGTAACCAGGACT GAA 3' using Polymerase Chain Reaction (PCR) technique. Amplicon was sequenced in one forward direction. Homology analysis was conducted by online BLASTn program, while the mutation was identified by MEGA4. The result of this research showed that homology analysis of 134 was homolog by 99% of *M. tuberculosis* H37Rv. The mutation of cytosine to thymine (C→T) was found occurring at position -15 of isolate 134 MDR-TB.

Keywords: *M. tuberculosis, inhA* promoter region, PCR, homology, mutation

1. PENDAHULUAN

Penyakit tuberkulosis (TB) merupakan masalah kesehatan di dunia. Penyakit ini menempati peringkat kedua penyebab kematian karena infeksi setelah HIV [2]. Di Provinsi Bali, khususnya pada tahun 2011 ditemukan sebanyak 1450 kasus TB paru dan 513 (35%) diantaranya ditemukan di Kota Denpasar [3]. Penanganan TB menghadapi tantangan baru dengan munculnya *Multidrug resistance* TB (MDR-TB). MDR-TB

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Journal of Microbiology, Immunology and Infection (2015) 48, 249–255

Available online at www.sciencedirect.com

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journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

The mutations of *katG* and *inhA* genes of isoniazid-resistant *Mycobacterium tuberculosis* isolates in Taiwan

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Received 29 April 2013; received in revised form 12 August 2013; accepted 29 August 2013
Available online 1 November 2013

KEYWORDS
inhA gene;
katG gene;
Mycobacterium tuberculosis

Background/purpose: The isoniazid (INH) resistance of *Mycobacterium tuberculosis* is caused by mutations in the *katG* and *inhA* genes encoding for catalase-peroxidase and inhA, respectively. Sequences of the *katG* and *inhA* gene of 70 isolates were analyzed to identify the mutations and to compare the mutations with their related susceptibilities.
Methods: Sequences of the *katG* and *inhA* genes and the resistance profiles were analyzed for the 70 *M. tuberculosis* isolates, collected from nine hospitals in Taiwan during the period from 1999 to 2011.
Results: Fifteen alleles were identified in the *katG* gene and two alleles were identified in the *inhA* gene. Among the 15 alleles identified in the *katG* gene, 14 alleles were found in isolates resistant to isoniazid, while only three alleles were found in isolates susceptible to isoniazid. The mutations of the *katG* gene and their frequencies of 41 INH-resistant isolates were Arg463Leu (51%), Ser315Thr (29%), Ser315Asn (9.8%), and other loci (22%). The sensitivity and specificity of the Ser315Thr mutation for the detection of INH-resistant isolates were 29% and 100%, respectively. The frequency of *inhA* gene mutation was low (2.44%) in the 41 INH-resistant isolates.
Conclusion: The diverse alleles of the *katG* gene associated with INH resistance are present in the *M. tuberculosis* isolates in Taiwan. These data may be applied to develop new probes for various alleles associated with INH resistance in order to increase the sensitivity for the

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http://dx.doi.org/10.1016/j.jmii.2013.08.018