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Current Issue

Vol. 7 No. 2 (2024)

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Editorial

Eyelid Infection (Blepharitis) Problem In The Elderly

Husnun Amalia; Megawati Yulia Wina Pratiwi, Ita Tazkiatul Izzati Mustopa

PDF

Original Article

The Relationship Between Spiritual Quotient And Stress Level Of S1 Students Of Medicine Faculty Of Muhammadiyah University Semarang Class Of 2021

Nurahmat Yanisa irfandi

PDF

Massive Tranfusion and Intensive Management after Hysterectomy in Placenta Accreta

Alfi Marita Trsitiarti, Eric Edwin Yuliantara

PDF

The Relationship Between Self-Esteem and Emotional Disorders in Adolescents at Senior High School

Rifat Adi Hendrianto, Erita Istriana

PDF

The Analysis of Work Fatigue and Proposed Improvement Using the Bourdon Wiersma Method and New Seven Tools

Vera Devani

PDF

Dietary Arrangements and Exercise Activities were Associated with Glycemic Control in Diabetes Patients at Grogol Petamburan Subdistrict Public Health Center

Sirly Hidhayanti, Kartini Kartini

PDF

The Effect of Preoperative Oral Glucose Administration on Blood Glucose Levels in Diabetes Mellitus Patients in General Anesthesia Surgery

Dhiny Yolanda Harahap, Achsanuddin Hanafie, Andriamuri Primaputra Lubis, Rina Amelia

PDF

Factors Affecting Stunted Status in Children Under-Two-Years at Karya Mulia Public Health Center 2023

Lala Namira Alifah Fahiratunnisa

PDF

The Effect of Mung Bean Sprout Extract (Phaseolus radiatus L.) on Catalase Levels in Male Wistar Rats Induced Paraquat Herbicide

Angga Pria Sundawa, Reza Adityas Trisnasdi , Annisa Nurul Hikmah

PDF

MATOS-CARVALHO INDEX AS A COMPARISON TO OTHER DISCRIMINANT INDEXES IN INITIAL BETA THALASSEMIA

Mulyadi Ong, Niken Satuti Nur Handayani, Mulyati, Tri Ratnaningsih, Nur Imma Harahap, Indra Lesmana

PDF

Impact of Cotrimoxazole on the Development of Chicken Embryo Neural Tube

Alifia Shafanaura Pamuji

PDF

DNase I Inactivation for Salivary DNA Preservation

Nurul Sulviani

PDF

Case Report

Eyelid Dermoid Cyst: A Case Report

Riani Witjaksana, Husnun Amalia, Nany Hairunisa, Erlani Kartadinata, Anggraeni Adhiwardan, Noviani Prasetyaningsih

PDF

Cholestatic Jaundice Due To Biliary Atresia With Cytomegalovirus And Malaria Infection: Blood Transfusion-Transmitted

Infection?

PDF

Review Article

Emerging Threats in the Age of Pandemics: A Focus on COVID-19 and the Novel Sub-Variant EG 5 ("Eris"): Review Article

Raghda Alsayed, Hamsa Thamer, Seenar Hameed, Mohammed Kadhom, Nany Hairunisa, Husnun Amalia, Yasmine Mashabi, Dina Ahmed, Sarah Mahdi, Amani Husain, Israa Salman, Emad Yousif

PDF

Microbiology Examination for Diagnosis of Mycobacterium other than Tuberculosis (MOTT) Infection

Arleen Devita, Ade Dharmawan

PDF

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⟵ Back to Submissions

509 / **Devita et al.** / Microbiology Examination for Diagnosis of Mycobacterium other than Tuberculosis (MOTT) Infection

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Round 1

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REVIEW ARTICLE

Microbiology Examination for Diagnosis of Mycobacterium other than Tuberculosis (MOTT) Infection

Pemeriksaan Penunjang Mikrobiologi untuk Diagnosis Infeksi *Mycobacterium Other Than Tuberculosis* (MOTT)

Arleen Devita[1], Ade Dharmawan[2]

[1]Microbiology Department, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia
[2]Microbiology Department, Faculty of Medicine dan Health Science, Universitas Kristen Krida Wacana, Jakarta, Indonesia

arleen.devita@trisakti.ac.id

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ABSTRACT

Mycobacterium other than tuberculosis (MOTT) is an environmental bacterium that can be an opportunistic pathogen. These bacteria are resistant to various types of disinfectants and antibiotics because they have the characteristics of thick cell wall peptidoglycan that are rich in lipids and mycolic acid. There are now over a hundred MOTT species, some of which are known to infect people with immune system disorders such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), people with a history of tuberculosis (TB), HIV infection, or diabetes mellitus, but can also infect individuals with good immune systems. This type of mycobacterium can also cause nosocomial infections because it can contaminate hospital water as well as medical devices such as bronchoscopes, endoscopes, and dialysis fluids. Infections in humans originate from environmental exposure and spread through ingestion or inhalation. The clinical manifestations of MOTT infection can be pulmonary and extrapulmonary infections, including skin, soft tissue, the gastrointestinal system, bones, and joints, and disseminated with symptoms that are difficult to distinguish from a Mycobacterium tuberculosis infection. Therefore, it is necessary to conduct supporting examinations, in particular microbiological examinations, to detect and identify the species of MOTT and then determine the appropriate therapeutic management. The types of microbiological examination that can be performed are microscopic examination with acid-fast staining, culture, identification with biochemical tests, molecular tests, and immunodiagnostic tests.

Keywords: MOTT; Mycobacterium tuberculosis; microbiological examination.

ABSTRAK

Mycobacterium other than tuberculosis (MOTT) merupakan bakteri lingkungan yang dapat menjadi patogen oportunistik. Bakteri ini resisten terhadap berbagai jenis disinfektan dan antibiotik karena memiliki karakteristik dinding sel tebal peptidoglikan yang kaya lipid dan asam mikolat. Saat ini terdapat lebih dari ratusan spesies MOTT, dengan beberapa di antaranya diketahui dapat menginfeksi orang dengan gangguan sistem kekebalan tubuh seperti penyakit paru obstruksi kronik (PPOK), *cystic fibrosis* (CF), orang dengan riwayat penyakit tuberkulosis (TB), infeksi HIV atau diabetes melitus, tetapi juga dapat menginfeksi orang dengan sistem kekebalan tubuh yang baik. Mycobacterium jenis ini juga dapat menjadi patogen penyebab infeksi nosokomial karena dapat mengontaminasi air di rumah sakit dan juga alat medis seperti bronkoskop, endoskop dan cairan dialisis. Infeksi pada

manusia berasal dari pajanan lingkungan dan menyebar melalui ingesti atau inhalasi. Manifestasi klinis infeksi MOTT dapat berupa infeksi pulmonal dan ekstrapulmonal antara lain kulit, jaringan lunak, sistem gastrointestinal, tulang dan sendi serta diseminata dengan gejala yang sulit dibedakan dengan infeksi *Mycobacterium tuberculosis*. Oleh karena itu, perlu pemeriksaan penunjang khususnya laboratorium mikrobiologi untuk mendeteksi dan mengidentifikasi spesies dari MOTT untuk menentukan tatalaksana terapi yang sesuai. Jenis pemeriksaan mikrobiologi yang dapat dilakukan adalah pemeriksaan mikroskopik dengan pewarnaan tahan asam, biakan, identifikasi dengan uji biokimia, uji molekular dan uji imunodiagnostik.

Kata Kunci: MOTT; *Mycobacterium tuberculosis;* pemeriksaan mikrobiologi.

INTRODUCTION

Mycobacterium is a gram-positive, rod-shaped bacterium from the Mycobacteriaceae family, measuring 0.2-0.6 × 1-10 µm, is aerobic, does not form spores, and cannot move.[1,2] This bacterium has a thick cell wall rich in lipids so its surface is hydrophobic. The cell wall structure requires a special stain, namely an acid-fast stain, to detect this bacteria. This also causes Mycobacterium to be resistant to various disinfectants and antibiotics.[3] The characteristic of Mycobacterium that differentiates it from other disease-causing bacteria is its slower growth time, ranging from 7 days, some even up to 12 weeks.[4]

Currently, there are more than 170 species of Mycobacterium with various distinctive virulence characteristics, with a third of them known to cause disease in humans and animals.[5-7] Based on epidemiology and their relationship to disease, there are 3 groups of Mycobacterium, namely Mycobacterium tuberculosis which causes tuberculosis, Mycobacterium leprae which causes leprosy and Mycobacterium other than tuberculosis (MOTT) or also known as atypical Mycobacterium and non-tuberculous Mycobacteria (NTM).[4,7]

Mycobacterium other than tuberculosis are bacteria in the surrounding environment such as soil, water, air, dust, plants, natural water sources, and drinking water including biofilms, wild animals, milk, and food products.[8-10] Water contamination in hospitals, and medical equipment, for example, bronchoscopes, endoscopes, and dialysis fluids can cause MOTT colonization and nosocomial infections.[11] So far, infection by Mycobacterium is widely known to be caused by Mycobacterium tuberculosis. Still, currently, MOTT can also cause disease with various clinical symptoms and is suspected to be the cause of iatrogenic infection so it has been determined to be a pathogen that causes nosocomial infections.[9,12] Several MOTT species have been known to be opportunistic pathogens. Of all infections by Mycobacterium, 0.5-30% are caused by MOTT.[13] As with infections in general, MOTT infections are also influenced by host factors and the pathogenicity of the causes which varies between species. Several risk factors that cause MOTT infection include conditions where the immune system is compromised (immunocompromised) such as chronic obstructive pulmonary disease (COPD), pneumoconiosis, bronchiectasis, history of TB, post-radiotherapy fibrosis, chronic pulmonary aspiration, cystic fibrosis (CF), HIV infection, alcoholism, malignancy and diabetes mellitus (DM).[14,15]

Mycobacterium other than tuberculosis can also cause disease in people with a good immune system (immunocompetent).[4,16] Transmission from human to human or animal to human generally does not occur unless caused by M. abscessus in CF patients, although animals can act as a MOTT reservoir. Infections in humans are thought to originate from environmental exposure with transmission via ingestion or inhalation.[10,11]

As with TB, infection with MOTT can have clinical manifestations in the form of pulmonary infections (such as pneumonia, lung abscess, and pleurisy) and extrapulmonary infections (lymphadenitis, skin, and soft tissue infections, meningitis, gastrointestinal infections, joint

infections, osteomyelitis, genital infections and infertility).[5,17] Based on the organs affected, clinical manifestations of MOTT infection are divided into 4 groups: chronic lung infections, lymphadenopathy, infections of the skin and soft tissues, and disseminated infections. The species of MOTT and the clinical disease it causes can be seen in Table 1.[10] Symptoms and clinical signs that arise in infection with MOTT are often difficult to distinguish clinically from Mycobacterium tuberculosis.[10,14,18]

Table 1. The clinical disease of some species MOTT[10]

Clinical	Species name
Lung disease	*Mycobacterium avium complex (MAC), M. kansasii, M. abscessus, M. xenopi, M. simiae, M. malmoense*
Limfadenitis *cervico-facial*	*M. scrofulaceum, M. avium, M. malmoense, M. lentiflavum, M. bohemicum*
Skin and soft tissue diseases	*M. ulcerans, M. marinum, M. abscessus, M. fortuitum, M. haemophilum, M. chelonae*
Bone and joint diseases	*Mycobacterium avium complex (MAC), M. kansasii, M. abscessus, M. xenopi, M. goodii, M. terrae*
Disseminated infections	*M. avium, M. intracellulare, M. haemophilum, M. genavense*

MICROBIOLOGICAL SUPPORTING EXAMINATION

Diagnosis of MOTT infection requires integrating clinical, radiological, and microbiological data.[19,20] Microbiological laboratory examination is one of the supporting examinations used to establish the diagnosis of MOTT infection. The examinations that can be performed are microscopic examination, culture, identification with biochemical tests, high-performance liquid chromatography (HPLC) methods, and molecular and immunodiagnostic tests.[10,14,21] To perform microbiological examination, good specimens are needed, so it is necessary to pay attention to the correct way to take specimens and it is important to avoid possible sources of contamination, especially from the environment. Specimens can be taken from almost all body parts according to the affected organs. MOTT infection in the lungs requires taking specimens from the respiratory tract in the form of sputum, bronchial aspirate, bronchoalveolar lavage (BAL), and lung biopsy. In contrast, for the diagnosis of extrapulmonary MOTT infection, specimens can be used in the form of tissue (lymph nodes, skin), wound aspirate, abscess, blood, body fluids (cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid, joint fluid).[2,4,9,14,20]

Sputum specimen collection from the respiratory tract is required in one set, consisting of at least three sputum samples taken in the morning on different days.[4,10] Bronchoscopy procedures for BAL or bronchial lavage specimen collection are performed only if there is suspicion of pulmonary MOTT infection in patients who cannot produce sputum spontaneously or by induction.[20] Tissue specimens are taken to confirm the diagnosis of lymphadenitis by MOTT, namely by fine needle aspiration or lymph node excision, while skin biopsy is the best specimen needed to confirm the diagnosis of skin infection by MOTT. The histopathological examination should also be performed on skin biopsies to determine the presence of granulomatous inflammation caused by Mycobacteria infection and is needed for difficult cases.[20]

Specimen collection and transportation to the laboratory

In taking specimens, several things must be considered. Patients should not gargle with tap water until sputum is taken. The use of water for taking BAL specimens or bronchial lavage should use sterile saline. Likewise, the bronchoscope used for the bronchoscopy procedure must also be sterile. For taking extrapulmonary specimens, the surgical instruments used must also be sterile and should not be cleaned with tap water.[10] Tissue specimens sent should be given a little sterile saline fluid to avoid drying and should not be given formalin when transferring the specimen to the

laboratory.[22] The specimens that have been taken must be placed in a sterile, leak-proof container and labeled, and should not be opened until they arrive at the laboratory. If there is a delay in delivering the specimen to the laboratory for more than 1 hour, the specimen should be stored at a temperature of 2-8°C. If possible, specimens should be taken before antibiotics are given.[10]

Decontamination process

The decontamination process must be carried out on sterile specimens after they arrive in the laboratory. The choice of disinfectant is important because MOTT is resistant to most disinfectants such as chlorine, benzalkonium chloride, cetylpyridinium chloride, quaternary ammonium compounds, phenol compounds, or glutaraldehyde-based disinfectants.[10,23] The decontamination process must be carried out on specimens taken from non-sterile locations. This must be done to minimize contamination and the growth of other organisms such as bacteria and fungi so that they can inhibit the growth of Mycobacterium. Tissue specimens must be ground with sterile saline carried out aseptically and then planted into Mycobacterium selective media. The decontamination method that is widely used is using 0.25% N-acetyl-L-cysteine sodium hydroxide and 1% NaOH (NALC-NaOH).20 In sputum specimens taken from CF patients, the decontamination process is continued using 5% oxalic acid to minimize contamination by gram-negative rod bacteria such as Pseudomonas aeruginosa.[24]

Microscopic examination

The staining methods used for MOTT detection are acid-fast staining such as Ziehl-Neelsen (ZN) staining, Kinyoun, and fluorochrome staining using auramine and rhodamine observed with a fluorescent microscope. The number of AFB seen in microscopic examination reflects the number of AFB in clinical specimens. Microscopic examination has limited sensitivity and it is difficult to distinguish between MOTT and Mycobacterium tuberculosis with this examination. In general, the sensitivity of microscopic staining ranges from 20% to 80%.[3] ZN and auramine staining have higher sensitivity than Kinyoun, but when compared to culture, the sensitivity is still lower.[21] Based on previous studies, the sensitivity of ZN staining was 70% with a specificity of 90%, while fluorochrome staining had a sensitivity of 90% with a specificity of 84%.[25]

In histopathological examination, the sensitivity of fluorochrome and ZN staining was also low. This is due to the use of formalin in the fixation process. To obtain positive results, the number of bacteria (detection limit) required is relatively large, namely 104-105 bacteria/ml of sputum, so it is only effective in patients who already show clinical symptoms.[22] Therefore, MOTT identification must still be determined by culture. Assessment of ZN and fluorochrome staining can be seen in Table 2.[3,26]

Table 2. Acid-resistant staining reporting[3,26]

Number of visible BTAs with ZN (1000x magnification)	Number of BTAs visible with fluorochrome staining (450x magnification)	Reporting
0	0	Invisible BTA
1-2/300 LP	1-2/70 LP	Duspicious, suggestion: repeat the test with a new specimen
1-9/100 LP	2-18/50 LP	1+
1-9/10 LP	4-36/10 LP	2+
1-9/LP	4-36/LP	3+
> 9/LP	> 36/LP	4+
LP : Field of view		

CULTURE

Culture is considered more effective than staining because it can detect Mycobacterium in small amounts (10-100 Mycobacterium/ml specimen).[22] Mycobacterium cultures can be grown on solid and liquid media. Solid media is considered to be able to identify accurately because it can observe colony morphology, growth rate, species categorization based on pigmentation, and the number of organisms growing. While liquid media can provide faster results and increase Mycobacterium recovery. The solid media used are media with egg ingredients such as Lowenstein-Jensen (LJ) agar or media with agar ingredients such as Middlebrook 7H10 and 7H11.[3] Media with this agar ingredient can also be used for sensitivity testing. The liquid media that is widely used is Middlebrook 7H9 with the Mycobacterium growth indicator tube (MGIT) system. This system detects bacterial growth with a fluorescence quenching-based oxygen sensor. In liquid media, antibiotics are added to suppress the growth of contaminating bacteria and fungi. The antibiotics added were polymyxin B 50 U/ml, amphotericin B 5 µg/ml, nalidixic acid 20 µg/ml, trimethoprim 5 µg/ml and azlocillin 10 µg/ml. Nutrients such as albumin, dextrose, and oleic acid were also added to the media to increase the rate of bacterial growth.[21]

The conventional method with solid media takes 6-8 weeks to detect bacterial growth and is the gold standard examination.[21] Meanwhile, culture with liquid media has high sensitivity because it can detect growth in 1-2 weeks. The sensitivity of MOTT culture increases by 15% when culture is carried out on solid media with liquid media. The results of the culture examination can be issued after 6 weeks if no growth is detected in liquid media and 8 weeks of incubation in solid media.[21]

The optimal incubation temperature for Mycobacterium culture is 28-37°C with a temperature variation of 27-45°C.[10,20] Exceptions for cultures performed from skin specimens, soft tissues, and joint fluids that require a lower optimal incubation temperature, so additional inoculation is needed on 1 set of media incubated at 28-30oC in addition to incubation at 35-37°C. Most MOTT grow within 2-3 weeks.[27] There is a group of MOTT that grows slowly (slow growers) which is within 8-12 weeks and a group of rapid growers which is within 7 days.[3]

In cases of MOTT isolated from patients who do not show disease progression or MOTT isolation is suspected due to environmental contamination, the microbiological diagnosis of MOTT is confirmed if there is more than one positive sputum culture and the same MOTT species (or subspecies in the case of M. abscessus) must be found in two or more sputum cultures. [20] The diagnosis can also be confirmed if one positive culture is found in bronchial lavage or BAL specimens, positive cultures in lungs, or transbronchial biopsies accompanied by a picture of granulomatous inflammation. [19]

MOTT IDENTIFICATION

Identification of MOTT to the species level is important to determine whether the isolate obtained is clinically significant in addition to determining the right therapy because of differences in antimicrobial sensitivity for each species.[20] So far, identification of Mycobacterium has used phenotype tests. Phenotype tests were used to look at growth rate, pigment formation, and biochemical tests.[21] Based on growth rate and pigment formation, Runyon classifies MOTT into 4 phenotype groups known as the Runyon classification which can be seen in Table 3.3 Categories I-III were classified as slow-growing MOTT.

Table 3. Runyon Classification[3]

Runyon Classification	Group Name	Description
I	Photochromogenic	MOTT colonies form pigments when exposed to light and take more than 7 days to grow on solid media
II	Skotochromogen	MOTT colonies form pigments in dark or light conditions and take more than 7 days to grow on solid media
III	Nonphotochromogens	MOTT colonies that do not form pigment and take more than 7 days to grow on solid media
IV	*Rapid growers*	MOTT colonies grow in less than 7 days on solid media

Biochemical Tests

Biochemical tests used to identify MOTT include niacin production test, nitrate reduction, and p-nitrobenzoic acid (PNB) test. The PNB test is an important test to differentiate MOTT from Mycobacterium tuberculosis. In this test, MOTT bacteria will grow on growth media containing PNB because they are resistant to PNB, while Mycobacterium tuberculosis will be inhibited in growth. Several studies have shown that identification using biochemical tests takes a long time, namely 7-28 days, thus slowing down the establishment of the diagnosis.[14] Biochemical tests require a complicated process and currently have been abandoned because they are not useful for definite species identification, especially with the emergence of new MOTT species.[10]

High-Performance Liquid Chromatography (HPLC) METHOD

Identification of MOTT can also be determined using the HPLC method.28 The HPLC method is a test that analyzes the number of carbon atoms in mycolic acid compounds found in the walls of MOTT species, but identification using this method has now been abandoned because it is not specific enough to identify MOTT species that are rapid growers.[10]

Molecular METHOD

Since the last 20 years after the molecular era emerged, many new species have been identified. Molecular tests are superior to conventional tests such as biochemical tests and HPLC which are currently abandoned for MOTT identification. Molecular tests are also used for the detection of MOTT that cannot be cultured or performed on patients with high suspicion of MOTT infection but negative culture results. 10 Currently, the most widely used MOTT identification is the molecular method because it can identify MOTT subspecies levels. There are several molecular tests for MOTT detection and identification, including line probe assay (LPA), DNA probes, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and DNA sequencing as well as matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.[14,21,28] The results of the examination using the molecular method can provide fast and accurate results in less than 24 hours.[21]

DNA Probe

The basis of this technique is the hybridization of a specific DNA probe with the 16s rRNA of Mycobacterium to form a stable DNA-RNA hybrid.[10] Probes are commercially available for rapid identification of several MOTT species. One example of a readily available DNA probe is Gen-Probe which can be used to detect Mycobacterium species such as M. tuberculosis complex, M. intracellulare, M. avium, M. kansasii, M. avium complex, M chelonae, M fortutium, and M. gordonae.[21] This test has the advantage that it can be performed directly on clinical samples so that the results can be obtained quickly, however, there is a possibility of cross-reaction between Mycobacterium species and is limited to identifying frequently isolated MOTT species only.[10,28]

Line Probe Assays (LPA)

The basis of this technique is the reverse hybridization of amplified DNA products with complementary probes. The DNA targets used are the 16S-23S rDNA spacer region and 23S rDNA.[14] This technique uses nitrocellulose DNA membrane strip technology to detect and identify the genus and species of Mycobacterium. The stages of this technique are amplification of DNA products with polymerase chain reactions (PCR), hybridization of DNA products on the strip, and detection and interpretation of results. The time required until detection is approximately 6 hours. There are currently 3 commercially available DNA strip tests, namely Inno-LiPA Mycobacteria, GenoType Mycobacterium common Mycobacteria (GenoType CM), and GenoType additional species (GenoType AS).[14]

The Inno-LiPA Mycobacteria kit is designed to identify 17 different species, namely M. tuberculosis complex, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium kansasii, Mycobacterium chelonae, Mycobacterium gordonae, Mycobacterium xenopi, Mycobacterium scrofulaceum, M. avium complex and can differentiate the three subgroups of M. chelonae and M. kansasii. The GenoType CM kit has probes to detect 15 Mycobacterium species, while the GenoType AS kit has an additional 16 MOTT species.[21] The advantage of this test is that it can be performed directly on liquid cultures of primary isolates without waiting for the results of cultures on solid media.[14] The LPA method is considered very precise, fast, and consistent with a sensitivity of 96%, however, there are shortcomings because it is limited to identifying only frequently isolated MOTT species.[28]

DNA Amplification, Sequencing Analysis and Whole *Genome Sequencing* (WGS)

PCR technique is a DNA amplification technique followed by amplicon sequence analysis. Several DNA targets can be used, including 65-kD heat shock protein (hsp65), 16s rRNA gene, 23s rRNA gene, rpoB gene, and internal transcribed spacer (ITS) DNA sequence.[10,16,20,21] Multi-locus sequencing technique is the choice because MOTT species are identified more precisely. Among the several DNA targets, the 16s rRNA gene is the most widely used because this gene is owned by all bacterial species and there are conserved and variable regions in it which make this gene an ideal target for taxonomic purposes up to the subspecies level.[20]

The amplified DNA fragments can be detected by various techniques based on probe hybridization, for example, PCR restriction fragment length polymorphism analysis (PRA). After the amplification process, it is continued with amplicon sequencing analysis of DNA fragments to identify Mycobacterium. The PRA technique currently widely used for MOTT identification is based on PCR of a 441-base pair sequence of the 65-kD hsp65 gene followed by restriction enzyme digestion. The DNA fragments are observed on an electrophoresis gel and the resulting pattern is used for species identification. The size of the restriction fragments is usually species-specific. Identification of the organism is done by comparing the resulting nucleotide sequence with an

existing reference sequence.[13] The problem is when the tested isolate does not match the available reference sequence database. The result will be reported as "closely related to a given species," depending on the sequence difference between the unknown isolate and the available database.

The sensitivity of this method in distinguishing between MOTT and Mycobacterium tuberculosis is 99.2%.[13] This PRA technique provides relatively fast results (1-2 days) and can identify MOTT species without a hybridization process and is not limited to the availability of specific probes.[29] DNA amplification examination followed by sequencing analysis is the most widely used examination today to detect MOTT species that cannot be grown in culture media.[30,31] The disadvantage is that new species that emerge may also have 16s rRNA gene sequences that are almost similar to existing ones. For example, the difference between M. szulgai and M. malmoense lies in only 2 nucleotides, but in fact, the two species are very different. In addition, there is no clear limit regarding the difference in nucleotide sequences in 1 strain to identify Mycobacterium. Whole genome sequencing (WGS) is the gold standard examination for identifying various MOTT species and is useful in determining the distribution of MOTT species based on geographic location to transmission in the event of outbreaks related to healthcare-associated infections (HAIs). WGS can also provide information on virulence factors and MOTT resistance to various antimicrobials. The drawback is that this test is expensive so it is not available for routine diagnostics in developing countries and requires expert personnel to perform it.[10,14]

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS)

There is one method used to identify and differentiate Mycobacterium species, especially new species, namely MALDI-TOF combined with mass spectrometry.[14] This technology is designed to produce a protein 'fingerprint' based on ions absorbed from the cell surface by measuring the ratio of mass to charge. The tool software will automatically analyze the data and produce a profile to be compared with a reference database of spectra in identifying all common MOTT species and some uncommon MOTT species. [28]

This method can identify MOTT directly from liquid and solid media, is easy to do, fast, has good reliability with the ability to identify as many as 160 MOTT species, and can differentiate to the subspecies level.[10,20,28] Another advantage of this technique is that it can reduce the risk of infection by Mycobacterium. The specificity of this method is 98.6% and the results of the examination can be obtained in just 1-2 hours.[14] The limitations of this technique are that it is not yet available in laboratories in Indonesia because of its expensive price, and the limited reference database. Like other techniques, this test cannot accurately identify closely related MOTT species.[10]

IMMUNOCHROMATOGRAPHY TEST

Rapid and accurate identification of Mycobacterium can help in the management of MOTT infection cases. An immunochromatography test with MPT64 TB antigen kit can help in the rapid detection and differentiation of MPT64 antigen in Mycobacterium tuberculosis and MOTT isolates. Its sensitivity is 99% with 100% specificity. Another advantage of this kit is that it is easy to perform and can be done directly on positive cultures.[19,21]

CONCLUSION

Mycobacterium other than tuberculosis (MOTT) is an environmental bacteria that can be found in soil and water and can cause disease in humans with various clinical manifestations. Clinically, the symptoms of the disease it causes resemble infection by Mycobacterium tuberculosis. Identification of the species level is needed to determine the appropriate therapy. Supporting examinations that can be used include microbiological examinations. Several microbiological

examinations that can be used to detect and/or identify MOTT species are microscopic examination, culture, identification with biochemical tests, high-performance liquid chromatography (HPLC) methods, and molecular and immunodiagnostic tests. Molecular techniques that are widely used include DNA probes, LPA, DNA amplification followed by sequencing analysis, WGS, and MALDI-TOF-MS.

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Microbiology examination for diagnosis of mycobacterium other than tuberculosis (MOTT) infection

by Arleen Devita

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REVIEW ARTICLE

Microbiology Examination for Diagnosis of Mycobacterium other than Tuberculosis (MOTT) Infection

Pemeriksaan Penunjang Mikrobiologi untuk Diagnosis Infeksi *Mycobacterium Other Than Tuberculosis* (MOTT)

ABSTRACT

Mycobacterium other than tuberculosis (MOTT) is an environmental bacterium that can be an opportunistic pathogen. These bacteria are resistant to various types of disinfectants and antibiotics because they have the characteristics of thick cell wall peptidoglycan that are rich in lipids and mycolic acid. There are now over a hundred MOTT species, some of which are known to infect people with immune system disorders such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), people with a history of tuberculosis (TB), HIV infection, or diabetes mellitus, but can also infect individuals with good immune systems. This type of mycobacterium can also cause nosocomial infections because it can contaminate hospital water as well as medical devices such as bronchoscopes, endoscopes, and dialysis fluids. Infections in humans originate from environmental exposure and spread through ingestion or inhalation. The clinical manifestations of MOTT infection can be pulmonary and extrapulmonary infections, including skin, soft tissue, the gastrointestinal system, bones, and joints, and disseminated with symptoms that are difficult to distinguish from a Mycobacterium tuberculosis infection. Therefore, it is necessary to conduct supporting examinations, in particular microbiological examinations, to detect and identify the species of MOTT and then determine the appropriate therapeutic management. The types of microbiological examination that can be performed are microscopic examination with acid-fast staining, culture, identification with biochemical tests, molecular tests, and immunodiagnostic tests.

Keywords: MOTT; Mycobacterium tuberculosis; microbiological examination.

ABSTRAK

Mycobacterium other than tuberculosis (MOTT) merupakan bakteri lingkungan yang dapat menjadi patogen oportunistik. Bakteri ini resisten terhadap berbagai jenis disinfektan dan antibiotik karena memiliki karakteristik dinding sel tebal peptidoglikan yang kaya lipid dan asam mikolat. Saat ini terdapat lebih dari ratusan spesies MOTT, dengan beberapa di antaranya diketahui dapat menginfeksi orang dengan gangguan sistem kekebalan tubuh seperti penyakit paru obstruksi kronik (PPOK), *cystic fibrosis* (CF), orang dengan riwayat penyakit tuberkulosis (TB), infeksi HIV atau diabetes melitus, tetapi juga dapat menginfeksi orang dengan sistem kekebalan tubuh yang baik. Mycobacterium jenis ini juga dapat menjadi patogen penyebab infeksi nosokomial karena dapat mengontaminasi air di rumah sakit dan juga alat medis seperti bronkoskop, endoskop dan cairan dialisis. Infeksi pada

manusia berasal dari pajanan lingkungan dan menyebar melalui ingesti atau inhalasi. Manifestasi klinis infeksi MOTT dapat berupa infeksi pulmonal dan ekstrapulmonal antara lain kulit, jaringan lunak, sistem gastrointestinal, tulang dan sendi serta diseminata dengan gejala yang sulit dibedakan dengan infeksi *Mycobacterium tuberculosis*. Oleh karena itu, perlu pemeriksaan penunjang khususnya laboratorium mikrobiologi untuk mendeteksi dan mengidentifikasi spesies dari MOTT untuk menentukan tatalaksana terapi yang sesuai. Jenis pemeriksaan mikrobiologi yang dapat dilakukan adalah pemeriksaan mikroskopik dengan pewarnaan tahan asam, biakan, identifikasi dengan uji biokimia, uji molekular dan uji imunodiagnostik.

Kata Kunci: MOTT; *Mycobacterium tuberculosis*; pemeriksaan mikrobiologi.

INTRODUCTION

Mycobacterium is a gram-positive, rod-shaped bacterium from the Mycobacteriaceae family, measuring 0.2-0.6 × 1-10 µm, is aerobic, does not form spores, and cannot move.[1,2] This bacterium has a thick cell wall rich in lipids so its surface is hydrophobic. The cell wall structure requires a special stain, namely an acid-fast stain, to detect this bacteria. This also causes Mycobacterium to be resistant to various disinfectants and antibiotics.[3] The characteristic of Mycobacterium that differentiates it from other disease-causing bacteria is its slower growth time, ranging from 7 days, some even up to 12 weeks.[4]

Currently, there are more than 170 species of Mycobacterium with various distinctive virulence characteristics, with a third of them known to cause disease in humans and animals.[5-7] Based on epidemiology and their relationship to disease, there are 3 groups of Mycobacterium, namely Mycobacterium tuberculosis which causes tuberculosis, Mycobacterium leprae which causes leprosy and Mycobacterium other than tuberculosis (MOTT) or also known as atypical Mycobacterium and non-tuberculous Mycobacteria (NTM).[4,7]

Mycobacterium other than tuberculosis are bacteria in the surrounding environment such as soil, water, air, dust, plants, natural water sources, and drinking water including biofilms, wild animals, milk, and food products.[8-10] Water contamination in hospitals, and medical equipment, for example, bronchoscopes, endoscopes, and dialysis fluids can cause MOTT colonization and nosocomial infections.[11] So far, infection by Mycobacterium is widely known to be caused by Mycobacterium tuberculosis. Still, currently, MOTT can also cause disease with various clinical symptoms and is suspected to be the cause of iatrogenic infection so it has been determined to be a pathogen that causes nosocomial infections.[9,12] Several MOTT species have been known to be opportunistic pathogens. Of all infections by Mycobacterium, 0.5-30% are caused by MOTT.[13] As with infections in general, MOTT infections are also influenced by host factors and the pathogenicity of the causes which varies between species. Several risk factors that cause MOTT infection include conditions where the immune system is compromised (immunocompromised) such as chronic obstructive pulmonary disease (COPD), pneumoconiosis, bronchiectasis, history of TB, post-radiotherapy fibrosis, chronic pulmonary aspiration, cystic fibrosis (CF), HIV infection, alcoholism, malignancy and diabetes mellitus (DM).[14,15]

Mycobacterium other than tuberculosis can also cause disease in people with a good immune system (immunocompetent).[4,16] Transmission from human to human or animal to human generally does not occur unless caused by M. abscessus in CF patients, although animals can act as a MOTT reservoir. Infections in humans are thought to originate from environmental exposure with transmission via ingestion or inhalation.[10,11]

As with TB, infection with MOTT can have clinical manifestations in the form of pulmonary infections (such as pneumonia, lung abscess, and pleurisy) and extrapulmonary infections (lymphadenitis, skin, and soft tissue infections, meningitis, gastrointestinal infections, joint

infections, osteomyelitis, genital infections and infertility).[5,17] Based on the organs affected, clinical manifestations of MOTT infection are divided into 4 groups: chronic lung infections, lymphadenopathy, infections of the skin and soft tissues, and disseminated infections. The species of MOTT and the clinical disease it causes can be seen in Table 1.[10] Symptoms and clinical signs that arise in infection with MOTT are often difficult to distinguish clinically from Mycobacterium tuberculosis.[10,14,18]

Table 1. The clinical disease of some species MOTT[10]

Clinical	Species name
Lung disease	*Mycobacterium avium complex (MAC), M. kansasii, M. abscessus, M. xenopi, M. simiae, M. malmoense*
Limfadenitis *cervico-facial*	*M. scrofulaceum, M. avium, M. malmoense, M. lentiflavum, M. bohemicum*
Skin and soft tissue diseases	*M. ulcerans, M. marinum, M. abscessus, M. fortuitum, M. haemophilum, M. chelonae*
Bone and joint diseases	*Mycobacterium avium complex (MAC), M. kansasii, M. abscessus, M. xenopi, M. goodii, M. terrae*
Disseminated infections	*M. avium, M. intracellulare, M. haemophilum, M. genavense*

MICROBIOLOGICAL SUPPORTING EXAMINATION

Diagnosis of MOTT infection requires integrating clinical, radiological, and microbiological data.[19,20] Microbiological laboratory examination is one of the supporting examinations used to establish the diagnosis of MOTT infection. The examinations that can be performed are microscopic examination, culture, identification with biochemical tests, high-performance liquid chromatography (HPLC) methods, and molecular and immunodiagnostic tests.[10,14,21] To perform microbiological examination, good specimens are needed, so it is necessary to pay attention to the correct way to take specimens and it is important to avoid possible sources of contamination, especially from the environment. Specimens can be taken from almost all body parts according to the affected organs. MOTT infection in the lungs requires taking specimens from the respiratory tract in the form of sputum, bronchial aspirate, bronchoalveolar lavage (BAL), and lung biopsy. In contrast, for the diagnosis of extrapulmonary MOTT infection, specimens can be used in the form of tissue (lymph nodes, skin), wound aspirate, abscess, blood, body fluids (cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid, joint fluid).[2,4,9,14,20]

Sputum specimen collection from the respiratory tract is required in one set, consisting of at least three sputum samples taken in the morning on different days.[4,10] Bronchoscopy procedures for BAL or bronchial lavage specimen collection are performed only if there is suspicion of pulmonary MOTT infection in patients who cannot produce sputum spontaneously or by induction.[20] Tissue specimens are taken to confirm the diagnosis of lymphadenitis by MOTT, namely by fine needle aspiration or lymph node excision, while skin biopsy is the best specimen needed to confirm the diagnosis of skin infection by MOTT. The histopathological examination should also be performed on skin biopsies to determine the presence of granulomatous inflammation caused by Mycobacteria infection and is needed for difficult cases.[20]

Specimen collection and transportation to the laboratory

In taking specimens, several things must be considered. Patients should not gargle with tap water until sputum is taken. The use of water for taking BAL specimens or bronchial lavage should use sterile saline. Likewise, the bronchoscope used for the bronchoscopy procedure must also be sterile. For taking extrapulmonary specimens, the surgical instruments used must also be sterile and should not be cleaned with tap water.[10] Tissue specimens sent should be given a little sterile saline fluid to avoid drying and should not be given formalin when transferring the specimen to the

laboratory.[22] The specimens that have been taken must be placed in a sterile, leak-proof container and labeled, and should not be opened until they arrive at the laboratory. If there is a delay in delivering the specimen to the laboratory for more than 1 hour, the specimen should be stored at a temperature of 2-8°C. If possible, specimens should be taken before antibiotics are given.[10]

Decontamination process

The decontamination process must be carried out on sterile specimens after they arrive in the laboratory. The choice of disinfectant is important because MOTT is resistant to most disinfectants such as chlorine, benzalkonium chloride, cetylpyridinium chloride, quaternary ammonium compounds, phenol compounds, or glutaraldehyde-based disinfectants.[10,23] The decontamination process must be carried out on specimens taken from non-sterile locations. This must be done to minimize contamination and the growth of other organisms such as bacteria and fungi so that they can inhibit the growth of Mycobacterium. Tissue specimens must be ground with sterile saline carried out aseptically and then planted into Mycobacterium selective media. The decontamination method that is widely used is using 0.25% N-acetyl-L-cysteine sodium hydroxide and 1% NaOH (NALC-NaOH).20 In sputum specimens taken from CF patients, the decontamination process is continued using 5% oxalic acid to minimize contamination by gram-negative rod bacteria such as Pseudomonas aeruginosa.[24]

Microscopic examination

The staining methods used for MOTT detection are acid-fast staining such as Ziehl-Neelsen (ZN) staining, Kinyoun, and fluorochrome staining using auramine and rhodamine observed with a fluorescent microscope. The number of AFB seen in microscopic examination reflects the number of AFB in clinical specimens. Microscopic examination has limited sensitivity and it is difficult to distinguish between MOTT and Mycobacterium tuberculosis with this examination. In general, the sensitivity of microscopic staining ranges from 20% to 80%.[3] ZN and auramine staining have higher sensitivity than Kinyoun, but when compared to culture, the sensitivity is still lower.[21] Based on previous studies, the sensitivity of ZN staining was 70% with a specificity of 90%, while fluorochrome staining had a sensitivity of 90% with a specificity of 84%.[25]

In histopathological examination, the sensitivity of fluorochrome and ZN staining was also low. This is due to the use of formalin in the fixation process. To obtain positive results, the number of bacteria (detection limit) required is relatively large, namely 104-105 bacteria/ml of sputum, so it is only effective in patients who already show clinical symptoms.[22] Therefore, MOTT identification must still be determined by culture. Assessment of ZN and fluorochrome staining can be seen in Table 2.[3,26]

Table 2. Acid-resistant staining reporting[3,26]

Number of visible BTAs with ZN (1000x magnification)	Number of BTAs visible with fluorochrome staining (450x magnification)	Reporting
0	0	Invisible BTA
1-2/300 LP	1-2/70 LP	Duspicious, suggestion: repeat the test with a new specimen
1-9/100 LP	2-18/50 LP	1+
1-9/10 LP	4-36/10 LP	2+
1-9/LP	4-36/LP	3+
> 9/LP	> 36/LP	4+
LP : Field of view		

CULTURE

Culture is considered more effective than staining because it can detect Mycobacterium in small amounts (10-100 Mycobacterium/ml specimen).[22] Mycobacterium cultures can be grown on solid and liquid media. Solid media is considered to be able to identify accurately because it can observe colony morphology, growth rate, species categorization based on pigmentation, and the number of organisms growing. While liquid media can provide faster results and increase Mycobacterium recovery. The solid media used are media with egg ingredients such as Lowenstein-Jensen (LJ) agar or media with agar ingredients such as Middlebrook 7H10 and 7H11.[3] Media with this agar ingredient can also be used for sensitivity testing. The liquid media that is widely used is Middlebrook 7H9 with the Mycobacterium growth indicator tube (MGIT) system. This system detects bacterial growth with a fluorescence quenching-based oxygen sensor. In liquid media, antibiotics are added to suppress the growth of contaminating bacteria and fungi. The antibiotics added were polymyxin B 50 U/ml, amphotericin B 5 µg/ml, nalidixic acid 20 µg/ml, trimethoprim 5 µg/ml and azlocillin 10 µg/ml. Nutrients such as albumin, dextrose, and oleic acid were also added to the media to increase the rate of bacterial growth.[21]

The conventional method with solid media takes 6-8 weeks to detect bacterial growth and is the gold standard examination.[21] Meanwhile, culture with liquid media has high sensitivity because it can detect growth in 1-2 weeks. The sensitivity of MOTT culture increases by 15% when culture is carried out on solid media with liquid media. The results of the culture examination can be issued after 6 weeks if no growth is detected in liquid media and 8 weeks of incubation in solid media.[21]

The optimal incubation temperature for Mycobacterium culture is 28-37°C with a temperature variation of 27-45°C.[10,20] Exceptions for cultures performed from skin specimens, soft tissues, and joint fluids that require a lower optimal incubation temperature, so additional inoculation is needed on 1 set of media incubated at 28-300C in addition to incubation at 35-37°C. Most MOTT grow within 2-3 weeks.[27] There is a group of MOTT that grows slowly (slow growers) which is within 8-12 weeks and a group of rapid growers which is within 7 days.[3]

In cases of MOTT isolated from patients who do not show disease progression or MOTT isolation is suspected due to environmental contamination, the microbiological diagnosis of MOTT is confirmed if there is more than one positive sputum culture and the same MOTT species (or subspecies in the case of M. abscessus) must be found in two or more sputum cultures. [20] The diagnosis can also be confirmed if one positive culture is found in bronchial lavage or BAL specimens, positive cultures in lungs, or transbronchial biopsies accompanied by a picture of granulomatous inflammation. [19]

MOTT IDENTIFICATION

Identification of MOTT to the species level is important to determine whether the isolate obtained is clinically significant in addition to determining the right therapy because of differences in antimicrobial sensitivity for each species.[20] So far, identification of Mycobacterium has used phenotype tests. Phenotype tests were used to look at growth rate, pigment formation, and biochemical tests.[21] Based on growth rate and pigment formation, Runyon classifies MOTT into 4 phenotype groups known as the Runyon classification which can be seen in Table 3.3 Categories I-III were classified as slow-growing MOTT.

Table 3. Runyon Classification[3]

Runyon Classification	Group Name	Description
I	Photochromogenic	MOTT colonies form pigments when exposed to light and take more than 7 days to grow on solid media
II	Skotochromogen	MOTT colonies form pigments in dark or light conditions and take more than 7 days to grow on solid media
III	Nonphotochromogens	MOTT colonies that do not form pigment and take more than 7 days to grow on solid media
IV	*Rapid growers*	MOTT colonies grow in less than 7 days on solid media

Biochemical Tests

Biochemical tests used to identify MOTT include niacin production test, nitrate reduction, and p-nitrobenzoic acid (PNB) test. The PNB test is an important test to differentiate MOTT from Mycobacterium tuberculosis. In this test, MOTT bacteria will grow on growth media containing PNB because they are resistant to PNB, while Mycobacterium tuberculosis will be inhibited in growth. Several studies have shown that identification using biochemical tests takes a long time, namely 7-28 days, thus slowing down the establishment of the diagnosis.[14] Biochemical tests require a complicated process and currently have been abandoned because they are not useful for definite species identification, especially with the emergence of new MOTT species.[10]

High-Performance Liquid Chromatography (HPLC) METHOD

Identification of MOTT can also be determined using the HPLC method.28 The HPLC method is a test that analyzes the number of carbon atoms in mycolic acid compounds found in the walls of MOTT species, but identification using this method has now been abandoned because it is not specific enough to identify MOTT species that are rapid growers.[10]

Molecular METHOD

Since the last 20 years after the molecular era emerged, many new species have been identified. Molecular tests are superior to conventional tests such as biochemical tests and HPLC which are currently abandoned for MOTT identification. Molecular tests are also used for the detection of MOTT that cannot be cultured or performed on patients with high suspicion of MOTT infection but negative culture results. 10 Currently, the most widely used MOTT identification is the molecular method because it can identify MOTT subspecies levels. There are several molecular tests for MOTT detection and identification, including line probe assay (LPA), DNA probes, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and DNA sequencing as well as matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.[14,21,28] The results of the examination using the molecular method can provide fast and accurate results in less than 24 hours.[21]

DNA Probe

The basis of this technique is the hybridization of a specific DNA probe with the 16s rRNA of Mycobacterium to form a stable DNA-RNA hybrid.[10] Probes are commercially available for rapid identification of several MOTT species. One example of a readily available DNA probe is Gen-Probe which can be used to detect Mycobacterium species such as M. tuberculosis complex, M. intracellulare, M. avium, M. kansasii, M. avium complex, M chelonae, M fortutium, and M. gordonae.[21] This test has the advantage that it can be performed directly on clinical samples so that the results can be obtained quickly, however, there is a possibility of cross-reaction between Mycobacterium species and is limited to identifying frequently isolated MOTT species only.[10,28]

Line Probe Assays (LPA)

The basis of this technique is the reverse hybridization of amplified DNA products with complementary probes. The DNA targets used are the 16S-23S rDNA spacer region and 23S rDNA.[14] This technique uses nitrocellulose DNA membrane strip technology to detect and identify the genus and species of Mycobacterium. The stages of this technique are amplification of DNA products with polymerase chain reactions (PCR), hybridization of DNA products on the strip, and detection and interpretation of results. The time required until detection is approximately 6 hours. There are currently 3 commercially available DNA strip tests, namely Inno-LiPA Mycobacteria, GenoType Mycobacterium common Mycobacteria (GenoType CM), and GenoType additional species (GenoType AS).[14]

The Inno-LiPA Mycobacteria kit is designed to identify 17 different species, namely M. tuberculosis complex, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium kansasii, Mycobacterium chelonae, Mycobacterium gordonae, Mycobacterium xenopi, Mycobacterium scrofulaceum, M. avium complex and can differentiate the three subgroups of M. chelonae and M. kansasii. The GenoType CM kit has probes to detect 15 Mycobacterium species, while the GenoType AS kit has an additional 16 MOTT species.[21] The advantage of this test is that it can be performed directly on liquid cultures of primary isolates without waiting for the results of cultures on solid media.[14] The LPA method is considered very precise, fast, and consistent with a sensitivity of 96%, however, there are shortcomings because it is limited to identifying only frequently isolated MOTT species.[28]

DNA Amplification, Sequencing Analysis and Whole *Genome Sequencing* (WGS)

PCR technique is a DNA amplification technique followed by amplicon sequence analysis. Several DNA targets can be used, including 65-kD heat shock protein (hsp65), 16s rRNA gene, 23s rRNA gene, rpoB gene, and internal transcribed spacer (ITS) DNA sequence.[10,16,20,21] Multi-locus sequencing technique is the choice because MOTT species are identified more precisely. Among the several DNA targets, the 16s rRNA gene is the most widely used because this gene is owned by all bacterial species and there are conserved and variable regions in it which make this gene an ideal target for taxonomic purposes up to the subspecies level.[20]

The amplified DNA fragments can be detected by various techniques based on probe hybridization, for example, PCR restriction fragment length polymorphism analysis (PRA). After the amplification process, it is continued with amplicon sequencing analysis of DNA fragments to identify Mycobacterium. The PRA technique currently widely used for MOTT identification is based on PCR of a 441-base pair sequence of the 65-kD hsp65 gene followed by restriction enzyme digestion. The DNA fragments are observed on an electrophoresis gel and the resulting pattern is used for species identification. The size of the restriction fragments is usually species-specific. Identification of the organism is done by comparing the resulting nucleotide sequence with an

existing reference sequence.[13] The problem is when the tested isolate does not match the available reference sequence database. The result will be reported as "closely related to a given species," depending on the sequence difference between the unknown isolate and the available database.

The sensitivity of this method in distinguishing between MOTT and Mycobacterium tuberculosis is 99.2%.[13] This PRA technique provides relatively fast results (1-2 days) and can identify MOTT species without a hybridization process and is not limited to the availability of specific probes.[29] DNA amplification examination followed by sequencing analysis is the most widely used examination today to detect MOTT species that cannot be grown in culture media.[30,31] The disadvantage is that new species that emerge may also have 16s rRNA gene sequences that are almost similar to existing ones. For example, the difference between M. szulgai and M. malmoense lies in only 2 nucleotides, but in fact, the two species are very different. In addition, there is no clear limit regarding the difference in nucleotide sequences in 1 strain to identify Mycobacterium. Whole genome sequencing (WGS) is the gold standard examination for identifying various MOTT species and is useful in determining the distribution of MOTT species based on geographic location to transmission in the event of outbreaks related to healthcare-associated infections (HAIs). WGS can also provide information on virulence factors and MOTT resistance to various antimicrobials. The drawback is that this test is expensive so it is not available for routine diagnostics in developing countries and requires expert personnel to perform it.[10,14]

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS)

There is one method used to identify and differentiate Mycobacterium species, especially new species, namely MALDI-TOF combined with mass spectrometry.[14] This technology is designed to produce a protein 'fingerprint' based on ions absorbed from the cell surface by measuring the ratio of mass to charge. The tool software will automatically analyze the data and produce a profile to be compared with a reference database of spectra in identifying all common MOTT species and some uncommon MOTT species. [28]

This method can identify MOTT directly from liquid and solid media, is easy to do, fast, has good reliability with the ability to identify as many as 160 MOTT species, and can differentiate to the subspecies level.[10,20,28] Another advantage of this technique is that it can reduce the risk of infection by Mycobacterium. The specificity of this method is 98.6% and the results of the examination can be obtained in just 1-2 hours.[14] The limitations of this technique are that it is not yet available in laboratories in Indonesia because of its expensive price, and the limited reference database. Like other techniques, this test cannot accurately identify closely related MOTT species.[10]

IMMUNOCHROMATOGRAPHY TEST

Rapid and accurate identification of Mycobacterium can help in the management of MOTT infection cases. An immunochromatography test with MPT64 TB antigen kit can help in the rapid detection and differentiation of MPT64 antigen in Mycobacterium tuberculosis and MOTT isolates. Its sensitivity is 99% with 100% specificity. Another advantage of this kit is that it is easy to perform and can be done directly on positive cultures.[19,21]

CONCLUSION

Mycobacterium other than tuberculosis (MOTT) is an environmental bacteria that can be found in soil and water and can cause disease in humans with various clinical manifestations. Clinically, the symptoms of the disease it causes resemble infection by Mycobacterium tuberculosis. Identification of the species level is needed to determine the appropriate therapy. Supporting examinations that can be used include microbiological examinations. Several microbiological

examinations that can be used to detect and/or identify MOTT species are microscopic examination, culture, identification with biochemical tests, high-performance liquid chromatography (HPLC) methods, and molecular and immunodiagnostic tests. Molecular techniques that are widely used include DNA probes, LPA, DNA amplification followed by sequencing analysis, WGS, and MALDI-TOF-MS.

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CONFLICT OF INTEREST

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