Working

Study of drug susceptibility of slowly growing nontuberculous mycobacteria (NTM).

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ABSTRACT

The research protocol on the topic:
Study of drug susceptibility of slowly growing nontuberculous mycobacteria (NTM) contains information on sample collection, processing diagnostic material, culture growth, primary differentiation of the isolated culture, and determination of mycobacterial species, drug susceptibility testing of slowly growing nontuberculous mycobacteria prevailing in Moscow region, MICs determination by Sensititre SlowMyco test.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

1 Samples of respiratory material in a volume of 10-25 ml collected in sterile polypropylene 50 ml tubes with a hermetically sealed cap (Corning, USA).

2 All samples used in a study were coded and lacked personal information about the patients

3 Diagnostic material processed with the NALC-method
https://legacy.bd.com/ds/technicalCenter/promotionalFlyers/ss-mycoprep.pdf

4 Centrifugation of the samples was carried out at an acceleration of 3000g for 20 minutes
00:20:00

5 Sample inoculated into liquid medium Middlebrook 7H9 in BACTECTMMGITTM960 system according to manufacturer's manual (Becton, Dickinson, USA)
http://www.bd.com/ds/technicalCenter/clsi/clsi-960pza.pdf

6 Primary differentiation of the isolated culture of acid-fast mycobacteria belonging to Mycobacterium tuberculosis complex from nontuberculous mycobacteria (NTM) performed according to their growth rate, colony morphology, Ziehl-Neelsen smear microscopy, and immunohromatographic 'BD MGIT TBC ID' (Becton, Dickinson, USA) test

http://www.bd.com/contentmanager/b_article.asp

7 Identification of the species of NTM culture was carried out by the Hain Lifescience test system and biochemical tests (niacin, nitrate reductase, semi-quantitative catalase, urease, arylsulfatase, Tween-80 hydrolysis, determination of thermostable catalase and potassium tellurite potency).

https://www.hain-lifescience.de/en/

8 Drug susceptibility testing of slowly growing MTM was done by Sensititre SlowMyco test system (TREK DIAGNOSTIC Systems Ltd., GB)
http://www.trekds.com/

9 Isolate suspensions were prepared from NTM culture and adjusted to 0.5 McFarland standard, and 100 µl of the suspension was transferred into a tube with a Mueller-Hinton nutrient broth until a final concentration of 1 × 105 -1 × 106 CFU/ml obtained.10. A 100 µl aliquot of the final inoculum was transferred into each well of the SlowMyco plate, and plates was covered with an adhesive seal.

10 A 100 µl aliquot of the final inoculum was transferred into each well of the SlowMyco plate, and plates were covered with an adhesive seal.
100 µl

11 The culture suspension incubated at 37 ° C in 5% CO2 for 7-14 days, depending on the growth of mycobacteria in drug-free control well.
37 °C

12 The MIC was recorded as the lowest antibiotic concentration that reduced the visible growth of mycobacteria in the well, and its determination was carried out with the Vizion TREK (DIAGNOSTIC Systems Ltd., GB)
http://www.trekds.com/

13 To interpret the results, we used the meanings of MICs indicating the resistance to the drug, as recommended in CLSI M24-A2 (2011). Ltd., GB)
https://global.ihs.com/doc_detail.cfm?document_name=CLSI%20M24&item_s_key=00591769