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ANTIMICROBIAL SUSCEPTIBILITY TESTING OF RAPIDLY GROWING MYCOBACTERIA BY MICRODILUTION - EXPERIENCE OF A TERTIARY CARE CENTER

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ABSTRACT

Purpose: The objective of the study was to perform antimicrobial susceptibility testing of rapidly growing mycobacteria (RGM) isolated from various clinically suspected cases of extrapulmonary tuberculosis, from January 2007 to April 2008, at a tertiary care centre in Mumbai.

Materials and Methods: The specimens were processed for microscopy and culture using the standard procedures. Minimum inhibitory concentrations (MIC) were determined by broth microdilution, using Sensititre CA MHBT. Susceptibility testing was also carried out on Mueller Hinton agar by the Kirby Bauer disc diffusion method.

Results: Of the 1062 specimens received for mycobacterial cultures, 104 (9.79%) grew mycobacteria. Of the mycobacterial isolates, six (5.76%) were rapid growers. *M. abscessus* and *M. chelonae* appeared to be resistant organisms, with *M. chelonae* showing intermediate resistance to amikacin and minocycline. However, all the six isolates showed sensitivity to vancomycin and gentamicin by the disc diffusion test. Also all three isolates of *M. abscessus* were sensitive to piperacillin and erythromycin. Further studies are required to test their sensitivity to these four antimicrobials by using the microbroth dilution test, before they can be prescribed to patients.

Conclusions: We wish to emphasize that reporting of rapidly growing mycobacteria from clinical settings, along with their sensitivity patterns, is an absolute need of the hour.

KEYWORDS : Antimicrobial Susceptibility, Broth Microdilution, Rapidly Growing Mycobacteria

INTRODUCTION

A wide variety of infections have been associated with rapidly growing mycobacteria, involving the lungs, skin,bones, central nervous system (CNS), prosthetic heart valves, and also in disseminated infections.[1] It is important to distinguish rapidly growing mycobacteria from others quickly, as conventional antituberculous drugs are ineffective for the treatment of infections caused by them. In order to optimize susceptibility testing and facilitate the interpretation of susceptibility results, the Clinical and Laboratory Standards Institute (CLSI) recommends that isolates be identified, at least to differentiate between *M. chelonae*, *M. fortuitum,* and *M. abscessus*.[2]

MATERIALS AND METHODS

This study included the isolation of RGM from all clinical specimens including lymph node aspirates, pus, tissue, urine, and sterile body fluids of suspected cases of extrapulmonary tuberculosis (EPTB) received between January 2007 and April 2008. All the specimens were collected with aseptic precautions in sterile leak proof containers and transported to the laboratory. The specimens were processed on the same day for microscopy and culture using standard procedures. [1] Smears were stained with the help of the Ziehl Neelsen (ZN) technique. Specimens were inoculated on Lowenstein Jensen (LJ) media, after decontamination and concentration procedures, and incubated at 37°C. The cultures were examined every day for one week and thereafter once a week for eight weeks. Isolates that were obtained within a week were confirmed as acid fast bacilli by the ZN stain. Identification to species level was achieved on the basis of growth on MacConkey's agar, nitrate reduction, and susceptibility to polymixin B 300 units. Minimum inhibitory concentrations (MIC) were determined by broth microdilution using Sensititre CA MHBT (Trek Diagnostic Systems Limited, Imberhorne Lane, East Grindstead, West Sussex RH 19 IQX UK) according to the CLSI guidelines.[3] Inoculum suspensions were prepared by emulsifying three to five colonies in sterile water to a density of 0.5 Mac Farland standard. Fifty microlitres were transferred to a tube of cation adjusted Mueller Hinton broth with TES buffer. One hundred microlitres of this suspension was transferred to each well of the sensititre CA MHBT MIC plate dosed with linezolid, clarithromycin, amikacin, cefoxitin, ceftriaxone, imipenem, minocycline, tobramycin, ciprofloxacin, gatifloxacin, amoxyclav, and trimethoprim sulphamethoxazole in appropriate dilutions.All the wells were covered with adhesive seal and incubated at 30°C. Growth appeared as turbidity or as a deposit of cells at the bottom of the well. The susceptible and resistant breakpoints used were those recommended by the CLSI. [3] The disc diffusion method provides a good screening technique for RGM.[4] As the Sensititre CA MHBT had only 12 antimicrobials, susceptibility testing was also carried out on Mueller Hinton agar, using the Kirby Bauer disc diffusion method to the following antibiotic discs supplied by Hi Media Laboratories Pvt. Ltd. A 406 Bhaveshwar Plaza, Mumbai 400086: erythromycin (15 mg), tetracycline (30 mg),vancomycin (30 mg), piperacillin (100 mg), gentamicin (10 mg), and polymixin B (300 units).

RESULTS

Of the 1062 specimens received for mycobacterial cultures from various clinical specimens from cases ofEPTB during the study period from January 2007 to April 2008, 104 (9.79%) grew mycobacteria. Of the 104 mycobacterial isolates, six (5.76%) were rapid growers, which had been obtained from pus and lymph node aspirates. Of these six isolates, three were of *M. abscessus*, two belonged to *M. fortuitum*, and one was of *M. chelonae*.Table 1 Shows the MIC breakpoints used for categorization of susceptibility of rapidly growing mycobacteria and the antimicrobial susceptibility of rapidly growing mycobacteria by broth microdilution assay.

All three isolates of *M. abscessus* were resistant to all antibiotics by broth microdilution. However, on a disc diffusion test all showed sensitivity to vancomycin,gentamicin, erythromycin, and piperacillin, and in addition, two showed sensitivity to tetracycline. Of the two isolates of *M. fortuitum*, one showed sensitivity to all antimicrobials tested by broth microdilution except Imipenem. This isolate was obtained from the pus aspirated from a scapular abscess in a known case of systemic lupus erythematosus (SLE), and the patient had responded to amoxycillin-clavulanic acid. This isolate was also sensitive to vancomycin and gentamicin when tested by the Kirby Bauer disc diffusion test. The other isolate of *M. fortuitum* was resistant to all antimicrobials by broth microdilution, but was sensitive to tetracycline, vancomycin, and gentamicin. The only isolate of *M. chelonae* obtained from pus collected from a dental abscess showed intermediate resistance to amikacin (MIC 32 mg/ml) and minocycline (MIC 4 mg/ml) and resistance to all other antibiotics by broth microdilution. This isolate was however sensitive to vancomycin and gentamicin.

DISCUSSION

Nontuberculous mycobacteria have been reported with varying frequencies worldwide, while in India isolation rates are between 0.7 and 34%.[5] *M. fortuitum*, *M. abscessus*,and *M. chelonae* have

been commonly reported to cause skin and soft tissue infections, joint and bursae infections,wound infections, and injection abscesses. In our study RGM constituted 6/104 (5.76%) of all mycobacterial isolates,*M. abscessus* being the predominant isolate. In a study on nontuberculous mycobacteria isolated from a tertiary care centre in S. India, MV Jesudasan and P Gladstone reported 100 (2.23%) isolates of *M. chelonae* and *M. fortuitum* out of 4473 mycobacterial isolates. [6] However, the predominant isolate was *M. chelonae*. Unlike our study their study had also included pulmonary specimens. Amoxycillin has modest activity against *M. fortuitum*. Addition of clavulanic acid to amoxycillin enhances the *in vitro* activity against *M. fortuitum*.[5] In our study only one isolate of *M. fortuitum* showed sensitivity to amoxicillin clavulanic acid combination (MIC 4/2 mg/ml). Macrolides and clarithromycin are important agents for treatment of pulmonary and cutaneous infections caused by *M. chelonae*, *M. abscessus*, and 80% of *M. fortuitum*.[2] We observed that only one of the two isolates of *M. fortuitum* was sensitive to clarithromycin (MIC 0.25 mg/ml), but they were all resistant to erythromycin. All the three isolates of *M. abscessus* and the only isolate of *M. chelonae* were resistant to clarithromycin, which has been reported in the past.[6]

In summary, in the present study *M. abscessus* and *M. chelonae* appeared to be resistant organisms, with *M. chelonae* showing intermediate resistance to amikacin and minocycline. However, all the six isolates showed sensitivity to vancomycin and gentamicin on disc diffusion test. Also all three isolates of *M. abscessus* were sensitive to pipercillin and erythromycin. Further studies are required to test their sensitivity to these four antimicrobials by using the microbroth dilution test before they can be prescribed to patients when resistance is seen, even to most commonly used antimicrobials such as clarithromycin, amikacin, and cefoxitin. We wish to emphasize that reporting of rapidly growing mycobacteria from clinical settings along with their sensitivity patterns is an absolute need of the hour.

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