RESEARCH ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY OF RAPID GROWING MYCOBACTERIA INFECTED PATIENTS WITH NTM AT TERTIARY CARE CENTER.

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Introduction:
Nontuberculous mycobacteria (NTM) represented as a large class within the family of Mycobacteriaceae. More than one fifty NTM species are widely distributed in the environment and isolated most frequently from nature (Stout, Gadkowski et al. 2011). NTM infection has been come out as public health problem in humans. Many of these are important human pathogens that cause pulmonary and extrapulmonary infections. Pulmonary NTM is quite prevalent and challenging (Lin, Russell et al. 2018). NTM can be acquired by direct inoculation in the skin, ingestion and inhalation (Griffith, Aksamit et al. 2007). The clinical sign and symptoms of both infections NTM and MTBC are the same but treatment is always different (Singh, Maurya et al. 2013). NTM can be differentiated into slowly growing mycobacteria (SGM) and rapidly growing mycobacteria (RGM) (Griffith, Aksamit et al. 2007). RGM comprise a diverse group of species, including Mycobacterium abscessus (M. abscessus), Mycobacterium fortuitum (M. fortuitum), Mycobacterium chelonae (M. chelonae) and various other rare species moreover they have been reported to cause pulmonary disease in humans (Colombo and Olivier 2008; Yano, Kitada et al. 2013). A report from USA suggested that 10% of pulmonary NTM disease cases were due to RGM (Marras and Daley 2002). A previous study showed that 36% of all NTM infections were RGM in India (Shenai, Rodrigues et al. 2010).

Identification of RGM is very significant for clinical and epidemiological studies because of their spread worldwide (Rahideh, Farnia et al. 2014). NTM has been reported a common causative RGM in respiratory infection from several countries (Prevots, Shaw et al. 2010; Winthrop, McNelley et al. 2010; Alcaide, Peña et al. 2017). M. abscessus, a RGM species have lesser possibility of cure (Jarand, Levin et al. 2011). Pulmonary infection caused by RGM increased in Asia and mainly affect the immune compromised individual (Piersimoni and Scarparo 2009). Irrespective of various studies from India on NTM, the exact susceptibility and burden of disease by RGM still remains unclear. For clinicians and research scholars, treatment and diagnosis for RGM infections are very essential (Colombo and Olivier 2008; Bicmen, Coskun et al. 2010). The variations in sensitivity patterns of species and resistance to 1st line anti TB medication create challenges in the approach to treatment and varied with completely different members of this group of mycobacteria. The drug treatment is the therapy of choice but varies with species, with the distinction being that between SGM and RGM. However, there are many challenges to treatment of RGM diseases i.e. drug therapy is expensive and long, and may causes drug-related toxicities (Bicmen, Coskun et al. 2010; van Ingen, Boeree et al. 2012). Therefore, RGM infections need personalized treatment supported the results of drug
susceptibility testing (DST), which will facilitate to choose the most suitable antimicrobial therapy (Gray, Kong et al. 2014).

In India, data related to DST of NTM isolated from clinical specimens is less. The aim of this study was perform the antibiotic susceptibility testing of RGM isolated from various clinically suspected cases of pulmonary tuberculosis.

Method:-
This study was conducted at the department of microbiology of a tertiary care hospital between 2013 to 2015. Ethical approval from the institutional Ethical Review Board was obtained. Samples were collected in sterile containers and they were then transported to the laboratory and were processed immediately for microscopy (Ziehl–Neelsen [ZN] stain) and culture specimens were decontaminated using NALC NaOH (N-acetyl-L-cysteine-sodium hydroxide) method and further inoculated into the vials of the BacT/Alert 3D system (bioMerieux, France) which containing modified Middlebrook 7H9 with an antibiotic supplement. BacT/Alert 3D vials was monitored continuously by the BacT/Alert 3D system. Positive vials for the presence of acid fast bacilli (AFB) were subjected to smear microscopy. No growth after six weeks of incubation was treated as negative for mycobacteria. Then confirm positive culture was further identified by phenotypic test of NTM by 3-day arylsulfatase test, growth on MacConkey's agar without crystal violet, 5% NaCl tolerance test, nitrate reduction test and MPT64 Antigen test. After that further characterization at the species level was done by Line probe assay. Antimicrobial Susceptibility test of RGM was performed as per the guidelines provided by CLSI using Sensititre® RAPMYCO panel test (TREK Diagnostic Systems Magellan Biosciences, West Sussex, and UK) microdillution method.

Susceptibility testing from broth micro dilution-
Sensititre® RAPMYCO was used per the manufacturer instructions. Inoculum suspension was ready in sterile water to a density of 0.5 MacFarland standards. Fifty microlitres of the suspension were transferred to a tube of cation adjusted Mueller Hinton broth (CAMHBHT) with TES buffer. 100 µl of this suspension was transferred to each well of the Sensititre CAMHBHT plate containing antibiotics in appropriate dilutions (Trimethoprim/sulfamethoxazole (TMP-SMZ), Linezolid, Ciprofloxacin, Imipenem, Moxifloxacin, Cefepime, Cefoxitin, Amoxicillin – clavulanic acid (AMC), Amikacin, Ceftriaxone, Doxycycline, Minocycline, Tigecycline, Clarithromycin, Tobramycin, and positive control). All wells were covered with adhesive seal and incubation was done at 30°C in a non-CO2 incubator for 72 hours. The results of visual reading of growth were read manually. Growth appeared as turbidity or as a deposit of cells at the lowest of the well. If poor, plates were re incubated for up to an additional forty eight hours. Interpretations of minimum inhibitory concentrations (MIC) were done according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). Antimicrobial agents and breakpoints used were those recommended by the CLSI guidelines (Standards. 2004) by Sensititre RAPMYCO are given in Table 1.

Results:-
Of the 125 RGM isolates from pulmonary specimens studied. Majority of RGM were *M. abscessus* consisting of 65 (52%) strains, followed by 43 (34.4%) strains of *M. fortuitum* and 17 (13.6%) of *M. chelonae*. All the isolates were identified to species level by conventional biochemical tests and further confirmed by LPA based techniques using species specific primers. Most frequently recovered species was *M. abscessus*. The results of antimicrobial susceptibility test of RGM are shown in [Table 2].

The susceptible patterns of 65 *M. abscessus* isolates were amikacin (90%), linezolid (74%), clarithromycin (60%), minocycin (35%), imipenem (18%), cefoxitin (15%), moxifloxacin (10%), tobramycin (6%), ciprofloxacin (5%), and TMP-SMZ (3%). Amoxiclave and cefepime were reported to be (100%) resistant.

*M. fortuitum*, a total 43 clinical isolates sensitive to the antimicrobial agents such as amikacin (100%), TMP-SMZ (100%), tigecycline (100%), clarithromycin (76%), moxifloxacin (76%), linezolid (72%), doxycycline (72%), imipenem (69%), ciprofloxacin (35%), minocyclin (33%) and tobramycin (19%). All *M. fortuitum isolates were resistant to cefepime.*

All 17 *M. Cheloneae* (100%) were sensitive to clarithromycin and amikacin. Mostly isolates were sensitive to tigecycline (88%), moxifloxacin (88%), linezolid (76%) doxycycline (71%) tobramycin (59%), imipenem (69%), minocyclin (29%) and cefoxitin (24%). All 17 (100%) *M. cheloneae* isolates were resistant to amoxiclave, TMP-SMZ and cefepime.
Discussion:

NTM has been increasing over the past few decades in many areas of the world in Pulmonary diseases (Thomson 2010). RGM are increasingly being recognized as important human pathogens. Medical treatment for RGM should be based on sensitivity profiles. These types of studies are very limited in India (Set and Shastri 2011). Many species of NTM are being recognized as important human pathogens in both non-TB and TB endemic areas. DST may be even more critical in TB endemic areas in which NTM is more likely to be mistaken for TB. Similarly, DST clinical isolates of NTM is an important decision support tool that clinicians can choose suitable therapy and thus, improve the management and outcomes of some NTM diseases (Brown-Elliott, Nash et al. 2012). DST of RGM is likely to be of increasing importance in selecting an optimal and effective drug therapeutic regimen, as the resistance pattern varies with different species. Some studies have shown that M. abscessus, M. fortuitum, and M. chelonae are important human pathogens among RGM isolates [21–24].

Amongst the 125 isolates studied, M. abscessus was found to be most common comprising 52% of the RGM isolates, 34.4% belong to M. fortuitum, and 13.6% were M. chelonae. In a study by Shenai S et al, 43.75% of the RGM isolated were M. fortuitum and 56.25% were M. abscessus (Shenai, Rodrigues et al. 2010). 34.5% were M. fortuitum, 46% were M. abscessus and 19.5% were M. chelonae reported from Taiwan (Yang, Hsueh et al. 2003).

In patients with M. abscessus, the majority of isolates were susceptible to tigecycline and amikacin followed by linezolid and then clarithromycin. For M. fortuitum the majority of strains were sensitive to amikacin, tigecycline and TMP-SMZ followed by clarithromycin, moxifloxacin, doxycycline and imipenem. M. chelonae was found to be more sensitive for amikacin, clarithromycin, tigecycline, moxifloxacin followed by doxycycline, imipenem and linezolid.

In this study susceptibility of amikacin in M. abscessus 90%, M. fortuitum 100% and M. chelonae 100%. Amikacin was found to have good activity against RGM species (Shen, Wu et al. 2007; Fernández-Roblas, Martin-de-Hijas et al. 2008; Gayathri, Therese et al. 2010). Other amino glycoside tested was tobramycin to which isolates found 81% of M. fortuitum and 94% of M. abscessus to be resistant to tobramycin which is similar to the previous study (Gayathri, Therese et al. 2010) and disagreement with another study (Welch and Kelly 1979). The tetracycline class of antibiotics includes minocyclin, doxycycline and tigecycline belongs to M. chelonae, M. abscessus and M. fortuitum were mostly resistant to minocyclin in the current study, which is concordance with the previous study (Huang, Lee et al. 2008). While M. abscessus, were mostly resistant to doxycycline. Doxycycline susceptibility rates to be 5%, 15% and 56% for M. abscessus, M. chelonae and M. fortuitum isolates, respectively reported by other study (Wallace, Brown-Elliott et al. 2002). Tigecycline potentially used for treating infection by RGM. In contrast, tigecycline displayed 100% activity and successfully inhibited all M. fortuitum strains, similar with previous reports (Fernández-Roblas, Martin-de-Hijas et al. 2008; Huang, Chen et al. 2013). In our study, moxifloxacin as a newer fluoroquinolone demonstrated low activity against M. abscessus, which was consistent with results from previous study in Taiwan (Yang, Hsueh et al. 2003). Most strains of M. chelonae were more susceptible to moxifloxacin than other quinolones which was in agreement with previous study (Sriram and Sarangan 2017). Whereas M. abscessus and M. chelonae isolates were mostly resistant against ciprofloxacin which is another fluoroquinolone while, ciprofloxacin was active against isolates of M. fortuitum similar findings were also reported previously (Wallace, Bedsole et al. 1990; Brown-Elliott, Wallace et al. 2002; Fernández-Roblas, Martin-de-Hijas et al. 2008).

Clarithromycin is related to the macrolide class of antibiotics and this agent displayed good activity against M. abscessus, M. fortuitum and M. chelonae, in our study which was in agreement with previous studies (Nash, Zhang et al. 2005; Park, Kim et al. 2008; Broda, Jebbari et al. 2013; Tang, Lye et al. 2015). Among the cephalosporin group, in our study, 52 out of 65 M. abscessus (80%), 38 M. fortuitum (88%), and 13 M. chelonae (76%) were resistant to cefotixin which is in agreement with the other study (Sriram and Sarangan 2017). We found 97% of M. abscessus and up to 90% M. fortuitum to be resistant to ceftriaxone similarly reported by Gayathri et al (Gayathri, Therese et al. 2010). Cefepime was not active against either M. abscessus, M. fortuitum or M. chelonae in our study.

TMP-SMZ has the highest activity against M. fortuitum. In Taiwan previously reported, moderate resistance to TMP-SMZ against M. fortuitum (51%) (Yang, Hsueh et al. 2003), and high susceptibility to TMP-SMZ has been reported from the United States, which is consistent with our finding (Brown-Elliott and Wallace 2002; Brown-Elliott, Nash et al. 2012). They also found high resistance to TMP-SMZ in their study which was on isolates of M. abscessus and M. chelonae similarly reported in our study (Heidarieh, Mirsaedi et al. 2016). Imipenem was active against 29 (69%) of M. fortuitum isolates in the present study which findings is similar from Taiwan, however the isolates of M. abscessus in our study were 18% sensitive to imipenem which is similarly reported from Korea.
(Lee, Jeong et al. 2007). Imipenem might be useful clinically in treatment regimens for these organisms (Griffith, Aksamit et al. 2007).

**Table 1**: Antimicrobial agents and MIC breakpoints for RGM

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---------------------|-------------|--------------|-----------|
| Amikacin            | ≤16         | 32           | ≥64       |
| Tobramycin          | ≤2          | 4            | ≥8        |
| Tigecycline         | ≤4          | -            | >4        |
| Minocyclin          | ≤1          | 2–4          | ≥8        |
| Ciprofloxacin       | ≤1          | 2            | ≥4        |
| Moxifloxacin        | ≤1          | 2            | ≥4        |
| Clarithromycin      | ≤2          | 4            | ≥8        |
| Linezolid           | ≤8          | 16           | ≥32       |
| Imipenem            | ≤4          | 8–16         | ≥32       |
| Sulfamethoxazole    | ≤2/38       | -            | ≥4/7      |
| Cefepime            | ≤8          | 16           | ≥32       |
| Cefoxitin           | ≤16         | 32–64        | ≥128      |
| Amoxicillin-clavulanic acid | ≤8/4      | 16/8        | ≥32/16    |
| Ceftriaxone         | ≤8          | 16–32        | ≥64       |
| Doxycycline         | ≤1          | 2–4          | ≥8        |

Abbreviation: MIC (minimal inhibitory concentration).

**Table 2**: Antibiotic susceptibility pattern of Non tuberculous Rapid growing mycobacteria

| Antimicrobial agents | Mycobacterium abscessus (n=65) | Mycobacterium fortuitum (n=43) |
|---------------------|---------------------------------|--------------------------------|
|                     | S (I = 65)          | I (R) | S (I = 65) | I (R) | S (I = 65) | I (R) |
| Amikacin            | 58(90%)              | -     | 43(100%)   | -     | 17(100%)   | -    |
| Amoxiclavine        | -                   | -     | 65(100%)   | 03(5%)| 40(95%)    | -    |
| Cefoxitin           | 10(15%)              | 03(5%)| 52(80%)    | 02(5%)| 38(88%)    | 04   |
| Cefepime            | -                   | 65(100%)| -    | -     | 43(100%)   | -    |
| Ceftriaxone         | 02(3%)               | -     | 63(97%)    | 04(99%)| 39(91%)    | -    |
| Ciprofloxacin       | 03(5%)               | -     | 62(95%)    | 15(35%)| 28(65%)    | 02(12%)|
| Clarithromycin      | 39(60%)              | -     | 26(40%)    | 33(76%)| 10(24%)    | 17(100%)|
| Imipenem            | 12(18%)              | 22(34%)| 31(48%)    | 29(69%)| 14(31%)    | 10(59%)| 07(41%)|
| Linezolid           | 48(74%)              | 07(11%)| 10(15%)    | 31(72%)| 09(21%)    | 13(76%)| 04(24%)|
| Minocyclin          | 23(35%)              | -     | 42(65%)    | 14(33%)| 29(67%)    | 05(29%)| 12(71%)|
| Tobramycin          | 04(6%)               | -     | 61(94%)    | 08(19%)| 35(81%)    | 10(59%)| 07(41%)|

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### Conclusions:
Public Health Problems in RGM pulmonary diseases are going to increase. The treatment of pulmonary infections due to RGM is clearly difficult. Due to the lack of facilities and expertise, many laboratories these types of infections are undiagnosed. Accurate and correct identification of isolates to the species level and antibiotic susceptibility of RGM species for proper treatment and management of patients is important.

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|--------|---|---|---|---|---|---|---|
| TMP-SMZ| 02(3%)| - | 63(97%)| - | - | - | 17(100%) |
| Moxifloxacin| 07(10%)| 04(7%)| 54(83%)| 33(76%)| 05(12%)| 05(12%)| 15(8%)|
| Doxycycline| 6(9%)| 05(8%)| 54(83%)| 31(72%)| - | 12(23%)| 12(71%)|
| Tigecycline| 60(93%)| - | 05(7%)| 43(100%)| - | - | 15(88%)|

TMP-SMZ- Trimethoprim/sulfamethoxazole
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