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# DETERMINING THE EFFICACY OF ISOXYL, A MYCOLIC ACID INHIBITOR, IN VITRO AGAINST MYCOBACTERIA OTHER THAN MYCOBACTERIUM TUBERCULOSIS (MOTT) STRAINS

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## Abstract

*Mycobacteria other than Mycobacterium tuberculosis (MOTT) cause infections more commonly in the presence of predisposing factors and underlying diseases. They are also notably resistant to commonly used antituberculosis drugs. Total 11 clinical isolates MOTT were included in the study. Drug susceptibility testing of these isolates was performed by Resistant Ratio method. Minimum inhibitory concentration (MIC) pattern of these isolates of MOTT to mycelia acid synthesis inhibitors namely, Isoxyl(ISO) and Isoniazid (INH) were determined by agar dilution and broth dilution method. Minimum bactericidal concentration (MBC) pattern of these isolates to ISO and INH were also determined. Out of 11 MOTT isolates, 3 isolates were characterized as Mycobacteriumscrofulaceum, 3 isolates as Mycobacteriumfortuitum, 2 isolates as Mycobacteriumflavescens, 1 isolates as Mycobacterium terrae and 2 isolates as Mycobacteriumkansasi depending upon the results of biochemical tests. The MBC range of INH was found to be 0.025 to 6.4 µg/ml and of ISO was found to be 0.6 to 20 µg/ml. Bactericidal activity of ISO was 7.25 times lower than the activity of INH. It is well known that most MOTT species are more resistant to chemotherapeutic agents other than tubercle bacilli.The inhibitory activity of ISO was more to MOTT strains than Mycobacterium tuberculosis strains.There was low bactericidal activity of ISO to MOTT strains, but better than for Mycobacterium tuberculosis strains.*

**Keywords**

MOTT, ISO, MBC, MIC

## 1. Introduction

Although Tuberculosis (TB) is treatable, current treatments have limitations that are contributing to the spread of the disease. Treatment duration of at least six months is required which leads to patient's non-compliance. The most effective strategy for treating TB is Directly observed treatment, short-course (DOTS), which is cumbersome, labour intensive, expensive and with unpleasant side effects; particularly for such a long treatment regimen. Multi Drug Resistant tuberculosis (MDR TB) has become more prevalent in recent years and second line drugs are not as effective as the standard therapy and are more toxic and expensive.

It is important to treat latent TB infections (LTBI) and infections due to Mycobacteria other than Mycobacterium tuberculosis (MOTT) in certain risk patients such as the Human Immunodeficiency Virus (HIV) infected ones. Although Mycobacterium tuberculosis, Mycobacteriumbovis and Mycobacteriumleprae are established pathogens predominating human mycobacterial infections, non-tuberculous Mycobacteria are increasingly being reported as etiological agents of human infections(Wolinsky, 1979). MOTT comprising of over 95 species are naturally seen as saprophytes (Katoch, 2004), but are known to cause four different categories of infections in humans such as pulmonary infections resembling tuberculosis, extra pulmonary infections affecting lymph nodes, skin and soft tissue, multifocal disseminated infections and infections in immunocompromised individuals such as Acquired Immunodeficiency syndrome (AIDS) and transplant patients. The growing population of HIV infected individuals and other immunosuppressed / immunocompromised patients coupled with better diagnostic techniques has led to an increase in the number of MOTT being reported in human infections in recent years(Karak et al., 1996).

MOTT produces infections more commonly in the presence of predisposing factors and underlying diseases; they are also notably resistant to commonly used antituberculosis drugs. These factors augment morbidity and limit therapeutic options in such infections. MOTT has been reported worldwide with varying frequencies, while in India isolation rates are between 0.7% and 34% (Chakrabarti et al., 1990).The standard LTBI treatment regimen lasts from 2 months to 12 months depending on the medicines used. While, MOTT strains are resistant to most of standard ant tuberculosis drugs, the current antituberculosis drugs although effective when properly administered ultimately cannot win the fight against the TB epidemic. There is an urgent need for new ant tuberculosis drugs (Bates, 2012). New drug that Shortens the duration of treatment or significantly reduce the number of doses needed to be taken under DOTS supervision, which would have major impact as compliance and overall cure rate, improve treatment of MDR strains, provide a more effective treatment of LTBI to prevent the progression from infection to diseases, have less side effects and toxicity.

There is an urgent need to develop new effective antituberculosis compounds that can increase the permeability of the Mycobacterium cell wall by inhibiting the synthesis of cell wall components and enhance the activity of conventional drugs as a result of increased penetration of

these latter agents to susceptible internal targets(David et al., 1988).This enhancement of antimicrobial activity theoretically affords the use of lower concentration of antibiotics associated with toxicity(Matlola et al., 2001).As drug development is a long and expensive process, it becomes predominant to re-examine drugs that were formerly deemed effective against TB and increase the permeability of the Mycobacterial cell wall. One such drug is Isoxyl (ISO).

ISO is an old drug, used for the clinical treatment of TB in 1960's (Winder, 1982).Some author's demonstrated modest therapeutic efficacy of ISO monotherapy in cases of untreated pulmonary TB of various degree of difficulty. (Urbancik, 1970; Titscher, 1966) it was concluded that Ionized (INH) and ISO were more effective than monotherapy with either drug.(Schmidt, 1970) The NCDDG group led by Dr. Patrick Brennan recently evaluated this drug and found it to be effective against MDR strains of *M. tuberculosis*(Phetsuksiri et al., 1999).Hence there was a thought to do more work on this compound, as it is an old drug and have proven its efficacy. Present study was conducted with the objective of determining the efficacy of ISO,*in vitro* against MOTT strains.

## 2. Materials and Methods

### 2.1 Mycobacterium Strains

A total 113 clinical isolates of Mycobacteriawere collected from Department of Microbiology of P.D. Hinduja Hospital and Medical Research Centre, Mumbai. All clinical isolates were defined as M. tuberculosis or MOTT according to growth rates, susceptibility to para-nitrobenzoic acid, semi-quantitative catalyse test,pigmentation properties of colonies, nitrate reduction ,niacin accumulation tests etc.(Winn & Koneman, 2006).

### 2.2 Drug susceptibility testing

Drug susceptibility testing of clinical isolates of MOTT was performed by Resistant Ratio method (Cannetti, 1963).Standard *M. tuberculosis* strain H37Rv was also included in the study. Following drug concentrations (µg/ml) were used in the study

- INH; ( Lupin Pharmaceuticals, India): 0.025, 0.05, 0.1 and 0.2 µg/ml;
- Ethambutol (EMB); (Lupin Pharmaceuticals, India): 1.0, 2.0, 4.0, 8.0 and 16 µg/ml.

Resistance ratio was the minimum inhibitory concentration of the test strain divided by

the minimal inhibitory concentration of standard strain of *M. tuberculosis* H37Rv. Strain with resistance ratios of 8 or more were considered resistant to those drugs. A ratio of 4 was considered suggestive of resistance, while strain with resistance ratio of 2 was considered as susceptible strain. While, drug susceptibility testing of clinical MOTT isolates to Rifampicin (RF) (Lupin laboratories, India) was performed by Absolute concentration method. Drug concentrations used were 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml.

### 2.3 Determination of Minimum inhibitory concentration by agar dilution method

Minimum inhibitory concentration (MIC) pattern of clinical isolates of MOTT to mycolic acid synthesis inhibitors namely, ISO (Cayman Chemical Co., U.S. A.) and INH were determined by agar dilution method (Hawkins, Wallace Jr, & Brown, 1991). Middle brook 7H10 Agar (HiMedia Laboratories Pvt ltd., India) was used to determine the MIC pattern of MOTT isolates. Following concentrations (µg/ml) of the drugs were used for the assay

- ISO: 0.07, 0.15, 0.3, 0.6, 1.2, 2.5, 5, 10, 20 and 40 µg/ml.
- INH: 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 µg/ml.

Serial two fold dilutions of individual drug were prepared in sterile water for injection. Test strains were inoculated in sterile Dubos broth with glucose and albumin supplement with 0.05% Tween 80 (HiMediaLaboratories PvtLtd., India) and incubated at $37^0$c for 7 to 10 days to achieve the optical density of 0.1 at 540 nm and then diluted 10 fold in 0.1% Tween 80 containing normal saline. Bacterial suspension in 5-µl quantities was spotted on agar plates containing various drug concentrations. The control plates containing no drug were also inoculated. The plates were incubated at $37^0$c for 14 days. The MIC'S were defined as the minimum concentrations of drugs that completely inhibited the growth of the test organism or allowed growth of not more than five colonies.

### 2.4 Determination of Minimum inhibitory concentration by broth dilution method

MIC pattern of clinical isolates of MOTT to ISO and INH were determined by broth dilution method(Cruikshank, 1968; Tomioka et al., 1993)**.**To determine MIC of MOTT isolates to my colic acid inhibitor ISO and INH, Dubos broth was used. Following concentrations (µg/ml) of the drugs were used for this assay

- ISO 0.035, 0.07, 0.15, 0.3, 0.6, 1.2, 2.5, 5, 10, 20, 40, 80 and 160µg/ml.
- INH: 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4µg/ml.

Serial two-fold dilution of individual drug was prepared in sterile Dubos broth with glucose and albumin supplement after dissolving it in suitable diluents. 4.5 ml of Sterile Dubos broth with glucose and albumin supplements was dispensed in sterile tubes. 0.5 ml of required drug concentration (10X) was then added to obtain desired concentration. Each of the drug containing tube and drug free tube were inoculated with 0.1 ml. of test culture adjusted to McFarland No. 1 turbidity. A tube containing the highest concentration of the drug without any culture was also incubated as a drug control to check if the drug agent contributed to the turbidity of the medium. Drug free tube inoculated with test culture and medium control were also used. Inoculated and un-inoculated tubes were incubated at 37º C for 15 days. Results were obtained by observing the tubes for inhibition of growth, judged by a lack of opacity in tube. The MICs were defined as the minimum concentrations of drugs that completely inhibited the growth of the test organism.

### 2.5 Determination of Minimum bactericidal concentration (MBC) by broth dilution method

MBC pattern of clinical isolates of MOTT to ISO and INH were determined by broth dilution method (Heifets, 1991; Reddy, 1995)**.**Following concentrations (µg/ml) of the drugs were used for this assay.

- ISO: 0.07, 0.15, 0.3, 0.6, 1.2, 2.5, 5, 10, 20, 40, 80 and 160µg/ml;
- INH: 0.003, 0.006, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4µg/ml.

After 15 days of incubation for MIC determination of the drug; drug concentration higher than the MIC was used to determine the MBC of that drug. These concentrations were diluted to $10^{-3}$ to $10^{-4}$ in 10 ml of Dubos broth. 0.1 ml of these diluted drug concentration were inoculated on Lowenstein Jensen medium (LJM) slants. (HiMedia Laboratories Pvt Ltd.,India) The slants were incubated at $37^0$Cfor three weeks. Colony forming units(CFU) was enumerated on LJM slants. Lowest concentration of the drug that killed 99.9% of organisms was considered as the MBC of that drug.

## 3. Results

**Table 1:** *Typical biochemical characteristics of test Mycobacterial strains*

| Sr. no. | Characters studied | M. tuberculosis (n=102) | M. scrofulaceum (n=3) | M. fortuitum (n=3) | M. flavescens (n=2) | M. terrae (n=1) | M. kansasi (n= 2) |
|---|---|---|---|---|---|---|---|
| 1 | Growth within 7 days | - ve | - ve | +ve | - ve | +ve | -ve |
| 2 | Colony texture | Rough | Smooth | smooth | smooth | smooth | Smooth |
| 3 | Growth at 25º C<br>37º C | - ve<br>+ve | +ve<br>+ve | +ve<br>+ve | +ve<br>+ve | +ve<br>+ve | +ve<br>+ve |
| 4 | Pigment production Dark<br>Light | ve<br>ve | -ve<br>+ve | -ve<br>-ve | -ve<br>-ve | -ve<br>-ve | +ve |
| 5 | Niacin | +ve | -ve | -ve | -ve | -ve | - ve |
| 6 | Growth inhibition on PNBA media | +ve | -ve | -ve | -ve | -ve | + ve |
| 7 | Aryl sulphatase 5 days<br>10 days | NT | -ve<br>-ve | +ve<br>+ve | -ve<br>-ve | +ve<br>+ve | -ve<br>-ve |
| 8 | Catalase semi-quantitative | -ve | +ve | +ve | +ve | +ve | + ve |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9 | Catalase 68º C / | -ve | +ve | +ve | +ve | +ve | + ve |
| | 20mins. | | | | | | |
| 10 | Nitrate reduction | +ve | -ve | +ve | -ve | -ve | - ve |
| 11 | 5% NaCl tolerance | NT | -ve | +ve | -ve | +ve | -ve |
| 12 | Bile tolerance | NT | -ve | +ve | -ve | +ve | -ve |
| 13 | Urease | NT | +ve | +ve | -ve | +ve | +ve |
| 14 | Ion uptake | NT | -ve | -ve | -ve | +ve | -ve |
| 15 | Pyrazimidase | NT | +ve | -ve | +ve | -ve | +ve |
| 16 | Tween 80 hydrolysis<br>5 days<br>10days | NT NT | -ve<br>-ve | -ve<br>-ve | -ve<br>-ve | -ve<br>-ve | NT |

**Key:** NT – Not tested -ve – negative +ve – positive.

Out of 113 clinical isolates, 102 isolates were characterized as *M.tuberculosis*, and 11 were characterized as MOTT. Out of 11 MOTT isolates 3 isolates were characterized as *M. scrofulaceum*, 3 isolates as *M. fortuitum*, 2 isolates as *M. flavescent*, 1 isolates as M. *terrae* and 2 isolates as *M. Kansas*. [Table 1]

**Table 2:** *Initial drug resistance of MOTT to individual drug*

| Sr. No. | Drug under study | Total *MOTT* studied | Resistant strains | % Resistance | Susceptible strains | % Susceptible |
|---|---|---|---|---|---|---|
| 1 | INH | 11 | 5 | 45.45 | 6 | 54.54 |
| 2 | RF | 11 | 5 | 45.45 | 6 | 54.54 |
| 3 | EMB | 11 | 7 | 63.63 | 4 | 36.36 |

Out of 11 isolates characterized as MOTT by biochemical tests, 5 (45.45%) isolates were resistant to INH, 5 (45.45%) isolates to RF and 7(63.63%) isolates to EMB. Applying chi-square test, the number of resistant strains between the three drugs was not found significant. (P-value= 0.6155) [Table 2]

**Table 3:** *Initial drug resistance to the combination of drugs:*

| Serial No. | Resistance to drug combination | Number of strains | % Resistance | Highest resistance in drug combination |
|---|---|---|---|---|
| 1 | 1 drug | 4 | 36.36 | EMB |
| 2 | 2 drug | 2 | 18.18 | RF+EMB,INH+EMB |
| 3 | 3 drug | 3 | 27.27 | INH+EMB+RF |

In each drug combination, the resistance to EMB was observed in each drug combination in case of MOTT strains. [Table 3]

**Table 4:** *MIC pattern of MOTT strains in solid media***.**

| Sr. No. | Mycolic acid inhibitors | Geometric mean MIC +/-SD | MIC Range | p-value (Unpaired t-test) |
|---|---|---|---|---|
| 1 | INH | 0.34+/-0.47 | 0.4 to >6.4 µg/ml | 0.087 Difference is not significant |
| 2 | ISO | -0.02+/-0.48 | 0.3 to 5 µg/ml | |

Difference between geometric mean MIC of INH and ISO in solid medium was not found statistically significant. (P-value=0.087) [Table 4]

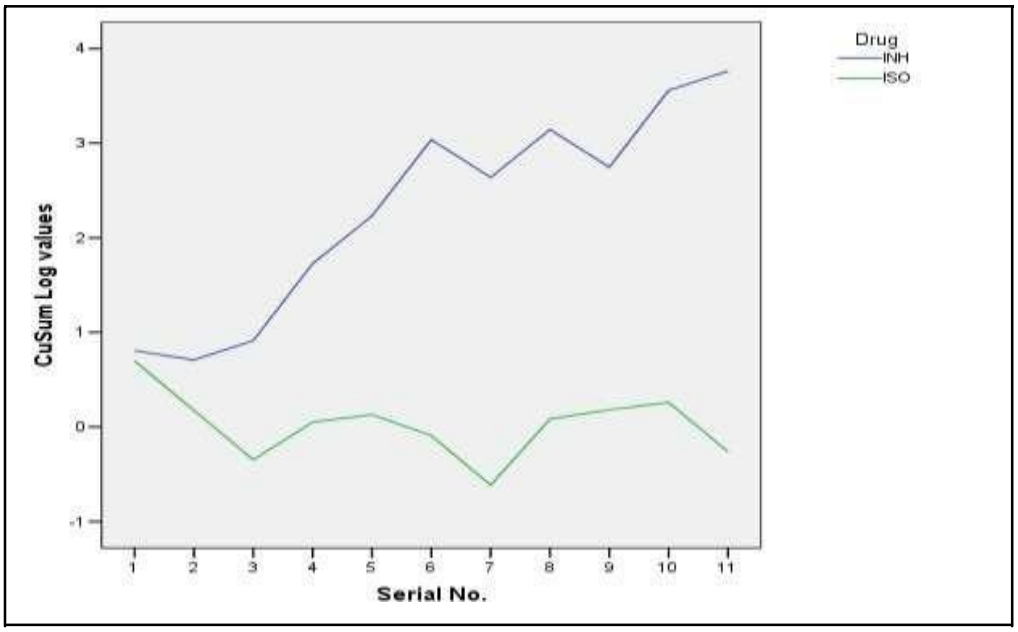


**Figure 1:** *MIC pattern of Mycolic acid inhibitors to MOTT strains in solid media*

**Table 5:** *MIC pattern of MOTT strains in liquid media***.**

| Sr. No. | Mycolic acid inhibitors | Geometric mean MIC +/-SD | MIC Range | p-value (Unpaired t-test) |
|---|---|---|---|---|
| 1 | INH | -1.03+/-0.72 | 0.0125 to 6.4 µg/ml | 0.408 Difference is not significant |
| 2 | ISO | -0.812+/-0.47 | 0.035 to 1.25 µg/ml | |

Difference between geometric mean MIC of INH and ISO in liquid medium was not found statistically significant. (P-value=0.408) [Table 5]

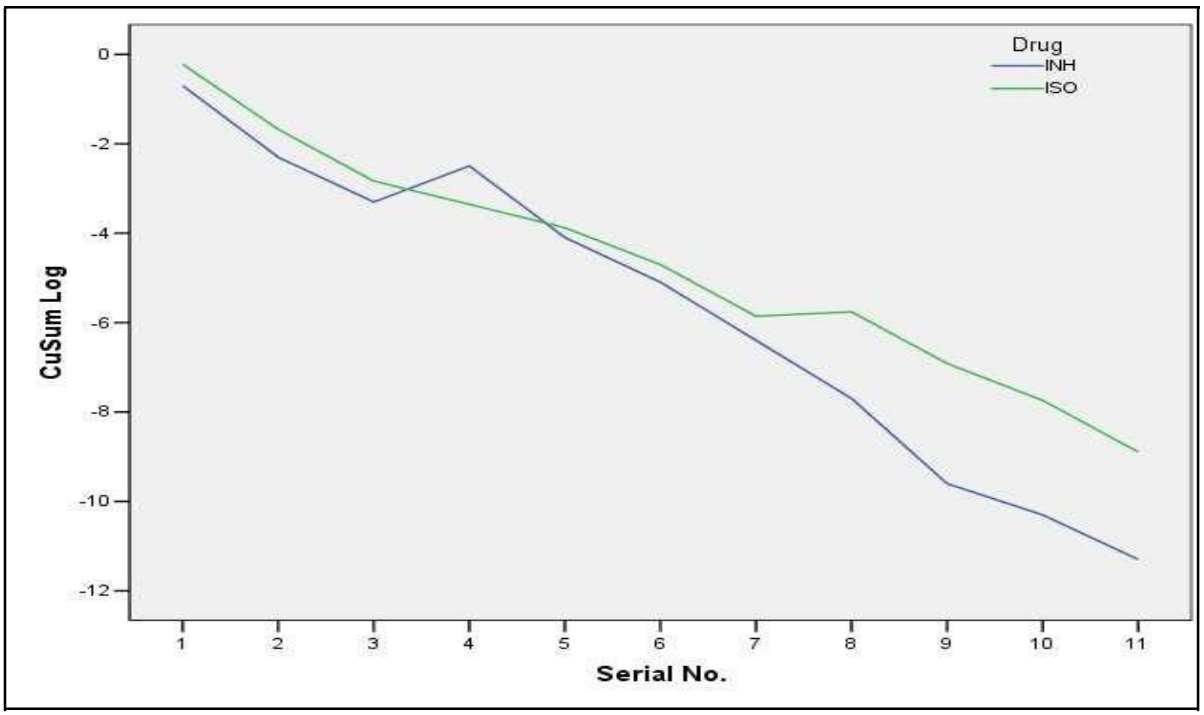


**Figure 2:** *MIC pattern of Mycolic acid inhibitors to MOTT strains in liquid media*

**Table 6:** *MBC pattern of MOTT strains*

| Sr. No. | Mycolic acid inhibitors | Geometric mean MBC +/-SD | MBC Range | p-value (Unpaired t-test) |
|---|---|---|---|---|
| 1 | INH | 0.08+/-0.68 | 0.025 to 6.4 µg/ml | 0.073 Difference is not significant |
| 2 | ISO | 0.58+/-0.46 | 0.6 to 20µg/ml | |

Difference between geometric mean MBC of INH and ISO in liquid medium was not found statistically significant. (P-value=0.073) [Table 6]

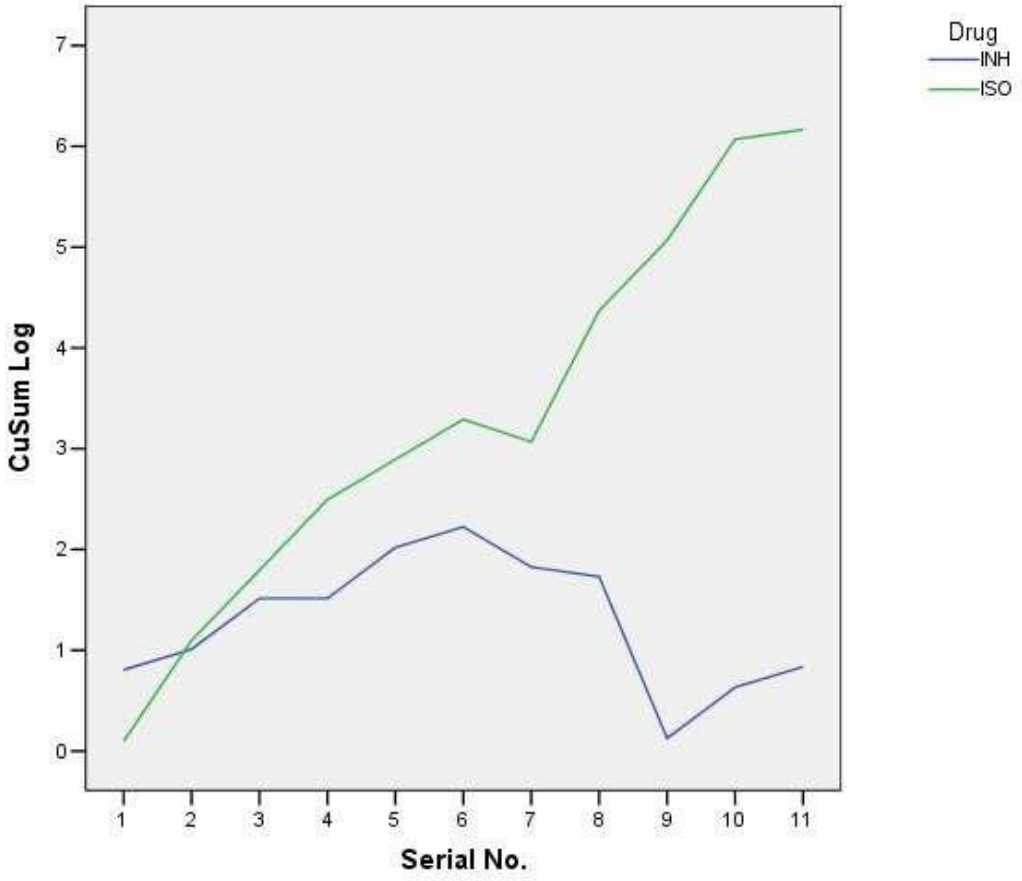


**Figure 3:** *MBC pattern of Mycolic acid inhibitors to MOTT strains*

## 4. Discussion

The incidence of TB has reduced in developed countries but infections due to MOTT are on the rise, while in developing countries like India, TB is still a major health problem. MOTT is also reported frequently as causative agents of human pathogenesis (Wolinsky, 1979). In our study, we came across 11 strains of Mycobacterium, which were characterized further to differentiate MOTT strains.We identified 2 isolates of *M. Kansas*, 3 isolates of *M. scrofualaceum*. 3 isolates of *M. fortuitum*, 2 isolates of *M. flavescent* and 1 isolate *M. terrae*. In our study MOTT bacilli were isolated from sputum specimens.

Some authors documented MOTT isolation rate of 9.3% from various clinical specimens and *M. fortuitum* was the commonest isolate (2.8%), *M. avium* (0.4%) and *M. szulgai* (0.2%)

(Chakrabarti et al., 1990).While some authors reported MOTT prevalence of 17.4 % from sputum specimens from patients with fibrocavitary pulmonary disease. This prevalence is higher than reports of the other workers. (Karak et al., 1996).Authors from South India isolated 173 (3.9%) MOTT from various clinical specimens. Of the 173 MOTT strains, 115 (66.5%) were identified to species level. *M. fortuitum* (41%) and *M. chelonei*(46%) accounted for majority of MOTT. Other MOTT species were *M. szulgai*(3%), *M. terrae* (3%), *M. scrofulaceum* (1%), *M. favascenens* (1%), *M. gordonae* (1%), *M. smegamitis*(2%) and *M. similes* (1%)(Jesudason & Gladstone 2005).

Although there are many reports from India, the exact burden of MOTT infections still remains unclear in India. Regular documentation and reporting of these MOTT strains from clinical settings along with their sensitivity profiles is essential to be aware of the possible spectrum of diseases associated and preferred treatment options. The management of MOTT infections includes medical treatment with various antimicrobial agents based on susceptibility patterns and surgical treatment as in the case of lymphadenitis, skin or soft tissue infections. Since most of these organisms are resistant to commonly used antimicrobial agents, susceptibility testing becomes mandatory before initiation of an effective therapy.

It is well known that most MOTT species are more resistant to chemotherapeutic agents other than tubercle bacilli (Jesudason & Gladstone, 2005).In our study total 11 strains of MOTT species were identified by method described earlier, were tested for sensitivity to INH and SM by Resistance Ratio method and RF by Absolute Concentration method. We noted 1 isolate of *M. Kansas* resistant to INH and RF and another isolate resistant to RF and sensitive to INH and EMB.

It was found that2.03% isolates of *M. Kansas* resistant to RF, while 6.91% isolates resistant to EMB (Yates & Collins, 1981). Some noted sensitivity of most strains of *M. Kansas* to RF and EMB, but among the few resistant strains, most were resistant to EMB than RF (Kolinsky, 1979). While others noted and reported that 2 isolates of *M. Kansas* resistant to INH, while one isolate resistant to EMB, RF and INH in combination. Some also noted 1 isolate of *M. fortuitum* resistant to INH and EMB, susceptible to RF, another isolate resistant to EMB and susceptible to INH and RF and another isolate resistant to all the drugs under study (Rosenzweig, 1979). Some studies noted resistance of *M. fortitum* to EMB and RF (Kolinsky, 1979), while

other studyreported highly resistant strains of *M. fortuitism (*Yates & Collins, 1981). Retrospective analysis showed 1 strain of *M. fourtitum* resistant to Amikacin while another sensitive to it. While susceptibility to oflaxacin, ciprofloxacin and gentamycin were 27%, 21% and 19.2% respectively (Jesudason& Gladstone, 2005).

We noted 1 isolate of *M. scrofulaceum* resistant to INH and susceptible to RF and EMB, another isolate resistantto RF and EMB and susceptible to INH. And another isolate resistant to EMB and susceptible to INH and RF. Others noted *M.avium-intracellualarae-scrofulaceum* group sensitive to EMB and some strains resistant to RF (Rosenzweig, 1979). While some noted 100% strains under study resistant to RF and 98% strains resistant to EMB.(Yates & Collins, 1981).The study in past reported 1 isolate of *M. scrofulaceum* resistant to EMB and sensitive to INH and RF (Rosenzweig, 1979 ). The study found all the strains were resistant to all the 4 drugs under study namely EMB, RF, INH and SM (Jesudason& Gladstone, 2005).We noted 1 isolate of *M. terrae* susceptible to all the drugs under study.While some showed highest susceptibility of *M. terrae*to all drugs tested namely SM, EMB, INH and RF (Jesudason& Gladstone, 2005).The study in past found 2 isolates of *M. terrace* sensitive to EMB, INH and RF, (Rosenzweig, 1979). We noted 1 isolate of *M. flavescent* resistant to all the drugs under study while another isolate susceptible to all the drugs.

Some authors reported sensitive strains of *M. flavescent* EMB and RF (Tsukamura et al., 1988). While others reported, 49% strains resistant to RF and 3.8% strains resistant to EMB.(Yates & Collins, 1981).The study in 1979 reported 1 isolate of *M. flavescent* resistant to INH and RF and sensitive to EMB, while another one sensitive to EMB, INH and RF (Rosenzweig, 1979).Recently reported 78.26 % MOTT to be resistant to INH and RF combination with or without one or more drug (Dharmshala, 2005) Though treatments of patients with Mycobacteriosis need not necessarily depend upon the results of drug susceptibility tests. Nevertheless such tests are done and they are usually included in the investigations necessary for the identification of these Mycobacterium.

These 11 strains of MOTT were further tested for MIC against various concentrations of ISO in solid and liquid media. We observed that all strains were susceptible in the range of 0.3 to 5µg/ml in solid media while in liquid media, they were susceptible in the range of 0.035 to 1.25 µg/ml. We observed that all strains were susceptible to ISO in the range of 0.3 to 5 µg/ml (G.M.

MIC:-0. 02 µg/ml) and 0.035 to1.25 µg/ml (G.M. MIC: -0.81 µg/ml) in solid and liquid medium respectively. It seems that ISO is more active in the liquid medium against MOTT strains. There is scarce literature available of sensitivity of ISO against MOTT strains. Bactericidal activity of ISO was low, in the range of 0.6 to 20 µg/ml We observed that ISO was bactericidal to MOTT strains in the range the range of 0.6 to 20 µg/ml(G.M. MBC :0.58).It was reported in 1970 that *M. Kansas* was much more sensitive i*n vitro* to the ISO than other Mycobacterium species. These properties provide a supplementary test for the characterization of the species (Tacquet et al., 1970).

We reported high inhibitory activity of ISO on *M. Kansas* strains. It is reported that *in vivo*, in the pneumoconiosis guinea pig infected with *M. Kansas*, the therapeutic activity of this substance is excellent, greater than that of ETH, EMB and INH used separately in animals equally sensitive to Mycobacterium attacks through previous pulmonary dust covering. These experimental findings make it possible to recommend the use of ISO in humans, in the treatment of *M. Kansas* chronic pulmonary infections (Tacquet et al., 1970)**.**It was reported in 1970 that in one out of 3 patients with lung disease due to infection with atypical Mycobacterium treatment with ISO and Cycloserine has been found effective (Tacquet et al., 1970). Marked inhibitory activity of ISO against MOTT strains, make us think the suitability of it for treatment of these organisms.

## 5. Conclusion

Following conclusion can be drawn from these studies. The incidence of TB has reduced in developed countries but infections due to MOTT is on the rise, while in developing countries like India, TB is still a major health problem. MOTT is also reported frequently as causative agents of human pathogenesis. Regular documentation and reporting of these MOTT strains from clinical settings along their sensitivity profiles is essential to be aware of the possible spectrum of diseases associated and preferred treatment options. It is well known that most MOTT species are more resistant to chemotherapeutic agents other than tubercle bacilli. The inhibitory activity of ISO was more to MOTT strains than *M. tuberculosis* strains. There was low bactericidal activity of ISO to MOTT strains, but better than for *M. tuberculosis* strains. ISO can be further studied, so that ISO or its derivatives in different forms can be included as one the

drug for treating MOTT.

## 6. Acknowledgement

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