



Mycobacterium tuberculosis prevalence in Suburbs of Mumbai – A Case Study

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ABSTRACT

Tuberculosis is one of the life threatening communicable disease. Detection of the disease at the earliest is necessary, if not it could result in lethal effect. Various tests are available for diagnosis but each has its own limitations. Conventional microscopy has low sensitivity while nonconventional culture has prolonged incubation for reporting positivity. While nucleic acid amplification techniques are very rapid, sensitive and less complicated to perform makes its more preferred to use in early diagnosis. The current case study is aimed at understanding the easy, accurate assessment of the tuberculosis suspected cases and their identification based on the recent scientific analytical approaches. Rifampicin susceptibility analysis isolates the mdr cases and the future possible alternate treatments that can be applied.

Key words: gene-x pert, NAAT, MDR, rifampicin

INTRODUCTION

Tuberculosis is one of the life threatening communicable diseases which is caused by bacterium *Mycobacterium tuberculosis* (*M. tuberculosis*) is an aerobic, acid fast, non-spore forming, non-motile bacillus that belongs to the family *Tuberculosisceae*. Tuberculosis (TB) is caused by members of *M. tuberculosis* complex that include: *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microti*, *M. pinipedi*, *M. caprae*, and *M. mungi*. Active TB disease develops in 10% of patients, while 90% individuals enter into latency phase that can reactivate at a later time especially if the immunity of the individual declines after, once infected. (Khalid *et al.*, 2014). The disease usually affects lungs, known as “pulmonary tuberculosis” and spreads through air and transmit disease from these patients to other nearby people (Caminero *et al.*, 2010). It is estimated that nearly 10 million new cases and 1.7 million deaths occur annually due to these deadly disease (Collantes).

et al., 2016). Thus, it makes very essential to identify the disease with drug susceptibility. A major obstacle in controlling tuberculosis is the time required by the testes to confirm bacterial presence in specimens and confirm diagnosis (Amita et al., 2016). India accounts for a quarter of global burden of TB and Operations research (OR) has been established in India to identify and control totally resistant TB (Nerges et al., 2012) the major challenges to TB control in Mumbai is to identify the pockets of effected or concentrated areas, drug resistance, diagnosis of TB and defining cure, detecting drug resistant TB, multiple sources of health care. There are various methods used for detection, each having its own limitations. Conventional microscopic method is less sensitive than culture method (Evo et al., 2015). While culture methods take prolonged time of incubation for bacteria to grow and then to detect its susceptibility. So, there is a need for some kind of rapid, easy to perform and sensitive method to detect it and in this aspect nucleic acid amplification test (NAAT) proves to be better in its usage (Kamaram et al., 2016). With help of NAAT early diagnosis could be made, preventing disease spread by prescribing adequate and accurate treatment. NAAT can be performed on pulmonary and extra-pulmonary specimens making its wide usage for diagnosis (Chang et al., 2012).

World health organization (WHO) endorsed NAAT test which can be performed by using xpert mtb/rif. It detects mycobacterium tuberculosis and rifampicin resistance by polymerized chain reaction amplifying 5 overlapping probes complementary to the rifampicin resistance determining region (RRDR) of the m.tb rpoB gene. The test could be performed within 2 hours without cross contamination (Chang et al., 2012).

The PCR amplification process is terminated to minimize cross amplification of non-tuberculosis mycobacteria (NTM) species and to maximize mutation detection (Collantes. et al., 2016). Rifampicin is surrogate marker for the mdr-tb therefore assays fulfills the requirement of diagnosing tb & mdr-tb (Collantes. et al., 2016). Gen expert has high sensitivity in detecting disease in smear negative tb also. Thus, it helps in detecting tb in low and middle-income countries. (Evo et al., 2015).

The over and under use of improper antibiotics, often leads to the raise of "superbugs" with resistance to all drugs. In this context, drug resistance index (Acid fast Bacillus) was developed as a composite measure that

combines the ability of antibiotics to treat infections with extend of their use in clinical practice. It is an epidemiological and communications tool to convey trends in the drug resistance to non-experts. (Aditi et al., 2014).

Studies indicated that Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis are current threats due to high mortality rates (12). However, right combination and rational use of available antituberculosis drugs can be helpful to treat these two. A susceptibility study of the isolate will be helpful to decide the line of medication and the resistance index always gives us an idea of the level usage of a particular drug. During the current study survey, an attempt was made to identify the prevalence of the isolate, gender wise analysis of susceptibility, resistance and negative while using different modes of assessments.

MATERIAL AND METHODS

A wide range of samples covering pulmonary and extra pulmonary specimens (around 2,805samples) of both sexes were collected from different parts of Mumbai suburbs and studied. Pulmonary specimens such as sputum and bronchial lavage; extra pulmonary specimens viz. Urine, pus mainly from cervical lymphadenopathy, gastric lavage mainly from pediatric group, endometrium from infertile patients, first morning urine specimens and various body fluids such as cerebro spinal fluid, pleural fluid, ascetic fluid etc. were considered for analyses. The samples were analysed with gene-xpert and also rifampicin drug susceptibility along with normal testing procedures.

Various tests were advised for detection such as Acid-fast Bacillus smear study, Acid fast Bacillus culture, gene-xpert and cytology study. The current study considered all the data analyzed using gene xpert to assess the presence of disease and rifampicin drug insusceptibility pattern.

Acid fast Bacillus testing is prescribed when pulmonary tb symptoms such as viz., lingering, chronic cough produces phlegm or sputum, sometimes with bloody streaks, fever, chills, night sweats, loss of appetite , unexplained weight loss ,weakness, fatigue and chest pain or extra pulmonary tb symptoms such as back pain and paralysis (spinal tb), weakness due

to anaemia (tb in the bone marrow), altered mental state, headache, and coma (tb meningitis), joint pain or abdominal pain are observed. Which vary depending on the area of the body that is affected. It can detect several different types of acid-fast bacilli especially *Mycobacterium tuberculosis*.

A few different tests may be used to help identify Acid fast Bacillus as the cause of an infection:

- An Acid-fast Bacillus smear is used as a rapid test to detect mycobacteria. *M. Tuberculosis*. The smears provide presumptive results within a few hours and help to make early decisions. however the test is less sensitive than culture to diagnosis a mycobacterial infection.

- NAAT : Guidelines from the centers for disease control and prevention suggest this test in special cases to identify potential tuberculosis patients when they show signs and symptoms of tb . Nucleic acid amplification test (NAAT) is a molecular test for tb performed along with an Acid-fast Bacillus smear test. The principle of NAAT detection is to identify the microorganisms in the sample by identifying the genetic components by amplifying / replicating using pcr techniques. It helps in reducing the amount of time necessary for a presumptive diagnosis of tuberculosis to less than 24 hours. However, it narrows the identification to a complex of mycobacteria. This test is found to be fairly sensitive and specific provided, with specimens where acid-fast bacteria were seen on the smear rather than those on on Acid fast Bacillus-negative by smear. The test methods are approved for respiratory samples confirmed with an Acid-fast Bacillus culture. These tests enable health practitioner with a quick answer and gives scope to isolate potentially infectious people and minimize the spread of the disease.

- Acid fast Bacillus cultures are used to diagnose active *M. tuberculosis* infections from similar other infections that may be due to nontuberculous mycobacteria, or any other cause. These cultures are used to help determine the pulmonary confined conditions of tb / spread to extra-pulmonary conditions as well as to monitor the effectiveness of treatment and its efficacy. This test results take long time but more sensitive than an Acid-fast Bacillus smear due to slow growth of mycobacteria.

- Susceptibility testing is another important test performed along with Acid fast Bacillus culture and determines efficacy of antibiotic that treats the mycobacterial infection. *M. Tuberculosis* may be resistant to one or more drugs commonly used to treat tb. Routine susceptibility testing of *M. tuberculosis* are now replaced with molecular tests which identify the genes in bacteria that confer resistance to the most commonly prescribed drugs.

The Drug Resistance Index (DRI) is composite measure with a combination of ability of antibiotics to treat infections with the extent of their use in clinical practice. The DRI helps quantify and communicate overall changes in the effectiveness of the antibiotic arsenal within a given setting (hospital, region, country) in a more intuitive way (Aditi *et al.*, 2014). This index is indicated as resistance drug and use relationship in a scale from 0-1. A value of "1" means infections are untreatable with any of the antibiotics used. A value of "0" means all isolates included in the calculations were susceptible. However, the values in between express overall susceptibility of infections, adjusted for local prescribing practices. Antibiotic Resistance is defined as proportion of non-susceptible isolates over the total number of isolates. Isolates showed varying degree of resistance to antibiotics those were used in the treatment. MDR is calculated ratio of number of antibiotics isolate is resistance to / total number of antibiotics tested.

RESULTS

Around 2805 samples (pulmonary and extrapulmonary) were collected and tested to detect presence of disease and predict the drug resistance. Of these, 57.30% and 42.70% were represented by male and female subjects. Samples of sputum were more (65%) followed by fluid, pus samples and others. (fig.1).

Positive indication to the prevalence in >70% in pus samples followed by bronchial lavage, sputum, tissue, gastric lavage and fluids. However, urine and endometrium samples showed negative results to the tests (fig.2).

Overall study indicated that the mtb of sputum samples showed susceptibility to the Rifampicin in the both the genders (780 out of 1800 cases).

Gender wise the prevalence indicated that mtb -ve cases were more in men, however they are more susceptible

to the disease than women. Overall, the women found to more resistant to this health hazard than men (Fig.3).

Table 1:

	Male				Female			
	mtb detected		mtb -ve	Total	mtb detected		mtb -ve	Total
	Resistant	Susceptible			Resistant	Susceptible		
Sputum	126	456	474	1056	132	324	288	744
Tissue	6	30	24	60	0	12	11	23
Branchial lavage	1	23	12	36	0	6	6	12
Fluids	0	144	114	258	Na	Na	126	126
Pus	6	78	42	126	102	12	30	144
Gastric lavage	0	3	12	15	0	6	0	6
Urine	0	0	18	18	0	0	30	30
Endometrium	0	0	0	0	0	0	84	84
Grand total	139	734	696	1,569	234	360	575	1,169

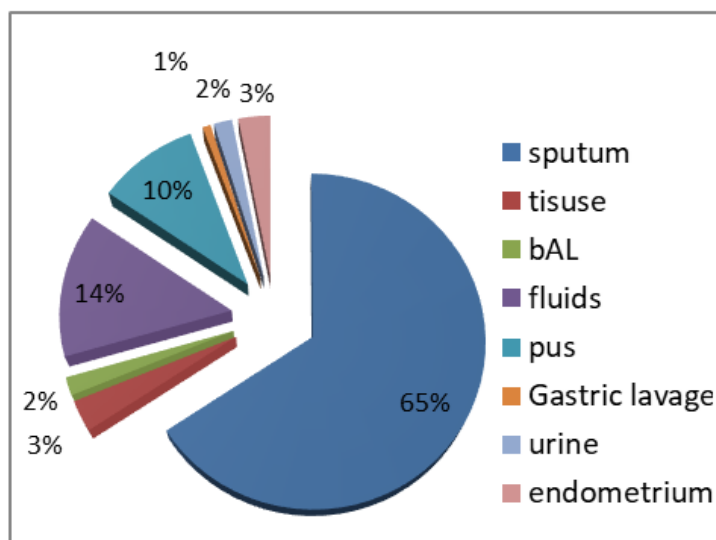


Fig.1 Composition of samples

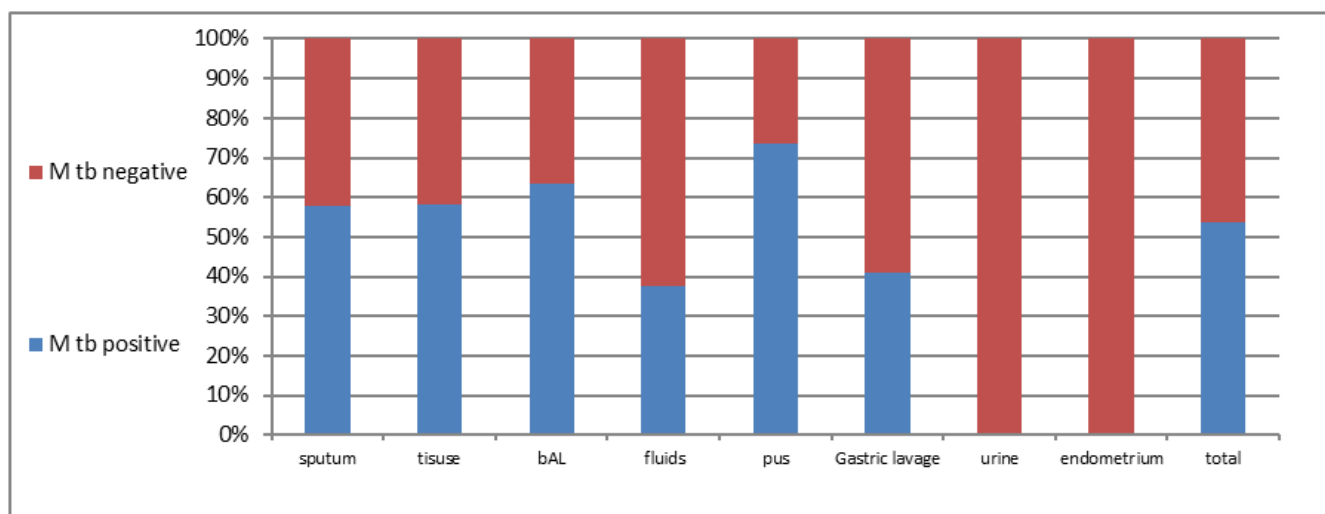


Fig.2 : Prevalence of m.tb in different specimens

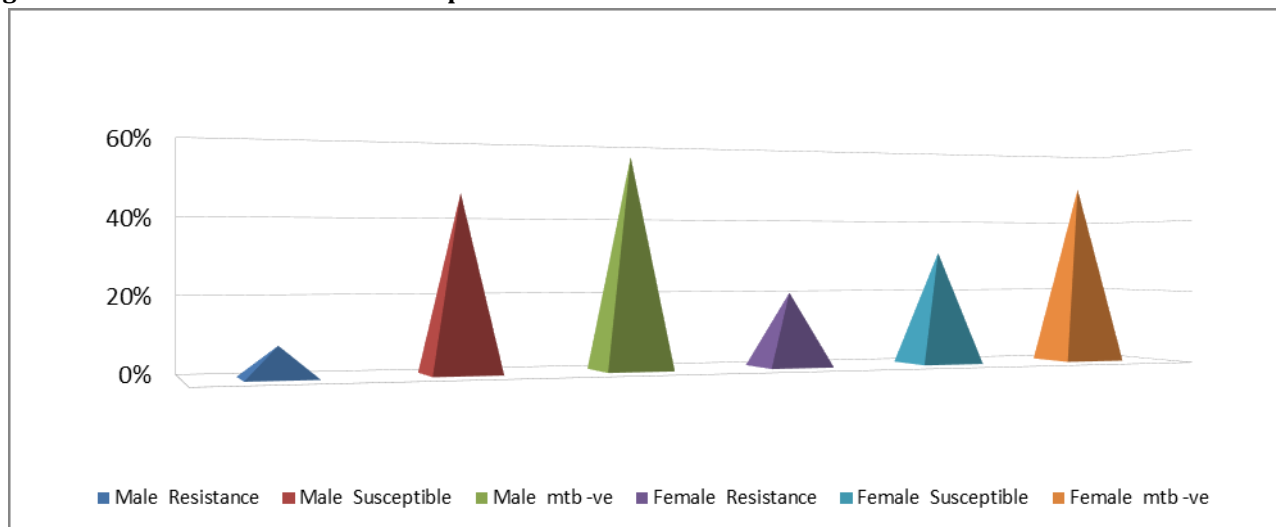


Fig.3 Gender wise Mtb prevalence

Drug resistance of 202 M. Tb isolates indicated the 72.77% were susceptible, 24.75% were resistance and 2.47% were indeterminate. Of the 72.77% susceptible isolates indicate a drug resistance index of 0.2 to Streptomycin and Pyrazinamide; 0.4 for a combination of drugs (Isoniazid and Pyrazinamide) and 0.6 DR index value for triple combination of drugs (Streptomycin + Isoniazid + Rifampicin). Out of 24.75 % resistance isolates, drug resistance Index for all 5 drugs was found to be 1.0 followed by 0.8 drug resistance index for triple combination of drugs (Pyrazinamide + Ethambutol + Rifampicin).

DISCUSSION

The current study indicated the presence of m.tb in different specimens of pulmonary and extra-pulmonary specimens both in male and female subjects. Pulmonary specimens mainly sputum has shown only 53.21 % detection in males when compared to 61.90% in females. It means presence of m.tb infection is more in female. It could be due to weak immune system due to various hormone imbalance conditions seen during life span. Same results are also seen in specimens of pus mainly collected from cervical lymph adenopathy aspiration. In male % of detection is 66.66% while in females it is more that is 79.16%. In gastric lavage specimen detection in male is only 20 % while in female it is 100 %. However, the endometrium and urine samples have not shown any positive results. An error was observed in results of mainly 6 specimens which could be due to bubbles while delivering specimens in

cartilage or due to improper mixing of buffer and specimens.

Overall, eight specimens mainly sputum (Caminero *et al.*, 2010) and pus (Evo *et al.*, 2015) indicated the presence of m.tb clearly while Rifampicin results shows intermediate results. In these case Acid fast Bacillus culture is needed to perform. Once Acid fast Bacillus grows then first line akt drugs testing was carried out to check Rifampicin susceptibility. 27% of the variants were found to be multi drug resistant while around 73% were susceptible as compared to a less number of indeterminants

CONCLUSION

Current study indicated that Genexpert is an very useful, extremely quick NAAT test for detection of *M. tuberculosis* with drug rifampicin susceptibility and resistance. Thus it helps in preventing spreading of multi drug resistance bugs by providing adequate proper timely treatment for curing tuberculosis. The subjects of suburbs tested with the above techniques for Tuberculosis prevalence and drug resistance indicated more resistance in women to the health problem. On the contrary, men are more susceptible with high values of mtb + ve responses. The current study may help the overall understanding of the emergence of multi- and totally drug resistant TB in Mumbai which can be vital for health workers and policy makers to address it in a more stringent way.

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