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Mycobacterium Tuberculosis: Pattern of First Line Drug Resistance

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ABSTRACT

Background: Tuberculosis (TB) is an airborne infectious bacterial disease that is curable but needs continuous treatment by multiple drugs for a prolonged duration of time. This is further compounded by the emerging drug resistance which is gradually increasing to the extent that totally drug resistant Mycobacterium tuberculosis are being identified in the tuberculosis high burden regions. **Objectives:** This study is intended to explore the in-vitro resistance pattern of Mycobacterium tuberculosis isolates to first line anti-tuberculosis drugs. **Study design:** Retrospective/Observational study. **Settings:** Charsada Teaching Hospital affiliated with Jinnah Medical College Peshawar. **Duration:** January 2015 to December 2016. **Methodology:** Record of 873 samples received for Mycobacterium tuberculosis culture and sensitivity were retrieved. The sensitivity results were analyzed and resistance to first-line tuberculosis drugs was noted. **Results:** A total of 873 samples for TB culture and sensitivity were processed, from January2015 to December 2016 (two years). Positive growth was achieved in 31 samples. The sensitivity results showed that 8 isolates (25.8%) of Mycobacterium tuberculosis were resistant to at least one first-line anti-tuberculosis drugs. The resistance pattern to first-line drugs was as follows: Streptomycin 6.5%, Isoniazid (INH) 9.7%, Rifampicin 3.2% and Ethambutol 6.5%. The MDR-TB isolates encountered (resistant to both INH and Rifampicin) were 3.2%. **Conclusions:** One quarter of Mycobacterium tuberculosis isolates were resistance to one or more first-line tuberculosis drugs. Maximum and minimum resistance was encountered to INH and Rifampicin, respectively. MDR-TB (resistant to both INH and Rifampicin) was also encountered. All efforts should be put together for public awareness, early diagnosis, adequate treatment and surveillance.

Keywords: Mycobacterium tuberculosis, MDR-TB, First-line tuberculosis drugs, Resistance pattern.

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INTRODUCTION

Tuberculosis (TB) is an airborne chronic bacterial infection that is curable but is difficult to treat. It has been known in the history by various names like consumption, phthisis, scrofula, Pott's disease and white plague. According to experts the genus Mycobacterium originated more than 150 million years ago but Mycobacterium tuberculosis originated about 15,000–20,000 years ago. It has been known to cause disease and death in ancient civilizations of Greece, Chile, Peru and Egypt.[1]

It is still infecting nearly one third of world population with a daily addition of 5000 new cases and loss of two lives every third minute.[2]

It grows slowly with doubling time of about 18 hours, this accompanied by genetic mutations and selective evolution of resistant strains, mandates treatment by multiple drugs for a prolonged duration of time. However; the downside of prolonged multiple drug therapy is poor patient compliance, giving the pathogens an opportunity to develop antibiotic resistance.[3]

According to the Global tuberculosis report 2017, tuberculosis is the ninth leading cause of death worldwide.[4] According to WHO HQ Report for 2016, Pakistan had an incidence of 518000 TB cases for that year, ranked fifth among TB high-burden countries and had the fourth highest prevalence of multidrug-resistant TB (MDR-TB) globally.[5]

The purpose of this study is to explore the in vitro resistance pattern for Mycobacterium tuberculosis isolates to first line anti-tuberculosis drugs.

METHODOLOGY

Study Design: Retrospective/Observational study.

Settings: Charsada Teaching Hospital affiliated with Jinnah Medical College Peshawar-Pakistan.

Duration: January 2015 to December 2016.

Methods: A total of 873 different samples (Table 1) were sent for Mycobacterium tuberculosis culture and sensitivity. Data was retrieved irrespective of patient's age or gender, from the archives of Charsada Teaching Hospital affiliated with Jinnah Medical College Peshawar. It was ensured that all samples pertaining to one patient were included in the study as one case. As a departmental policy, smears had been prepared from these specimens and stained with Ziehl–Neelsen stain. Contaminated specimens like sputa were liquefied with N-acetyl-L-cysteine, and decontaminated using sodium hydroxide, then neutralized with buffer, and concentrated by centrifugation before being inoculated on Lowenstein–Jensen slopes and incubated at 37^0 C for 6–8 weeks. Mycobacterium tuberculosis isolates from Lowenstein-Jensen (LJ) medium were stained with Ziehl-Neelsen stain and examined under a microscope. Drug

susceptibility testing was performed by sub-culturing the pure isolates on drug containing LJ medium by using standard concentrations against first-line TB drugs (Table II) i.e rifampicin (40 µg/ml), isoniazid (0.2 µg/ml), streptomycin (4 µg/ml), and ethambutol (2 µg/ml), using proportion method according to standard guidelines. Sensitivity material for pyrazinamide testing was not available at that time, so sensitivity to it could not be tested. Mycobacterium tuberculosis ATCC H37RV isolate that is characteristically susceptible to all anti-TB drugs, was used as a reference negative control strain.

The definitions used were as follows: Multidrug-resistant tuberculosis (MDR-TB): Strains resistant to both INH and Rifampicin. Extensively drug resistant tuberculosis (XDR-TB): Strains resistant to both INH and Rifampicin, a fluoroquinolone and at least one of second-line injectable drugs (Amikacin, Kenamycin, Capreomycin, Viomycin). Totally drug resistant tuberculosis (TDR-TB): Strains of Mycobacterium tuberculosis that are resistant to all first and second-line TB drugs.[2,6,7]

RESULTS

A total of 873 samples for TB culture and sensitivity were processed, from January 2015 to December 2016 (two years). These samples were collected from different sources (Table 1), mostly from sputum, followed by freshly removed lymph nodes, pus from abscesses and pleural fluid. From a total of 873 samples that were cultured, positive growth for Mycobacterium tuberculosis was achieved in 31 samples, depicting a positive yield of 3.6%. The sensitivity results showed that the organisms were mostly sensitive to the first line antibiotics, except for 8 cases (25.8%) which were resistant. The resistance pattern of those 8 resistant cases was as follows: Streptomycin 6.5%, Isoniazid (INH) 9.7%, Rifampicin 3.2% and Ethambutol 6.5% (Table 2). Only one isolate (3.2%) was resistant to both INH and Rifampicin (MDR-TB).

Table 1: Source of specimen and mycobacterium tuberculosis positivity

Sample Source	Sample Number	Number of Positives
Sputum	693	26
Lymph Node	108	03
Soft Tissue Abscess	41	01
Pleural Fluid	31	01
Total	**873**	**31**

Table 2: Drug resistance result

Drug	Resistant
Streptomycin	02 (6.5%)
Isoniazid (INH)	03 (9.7%)
Rifampicin	01 (3.2%)
Ethambutol	02 (6.5%)
Total	8 (25.9%)

DISCUSSION

Tuberculosis (TB) is a chronic infection caused by Mycobacterium tuberculosis. Mycobacterium has seven species,[7] most common being Mycobacterium tuberculosis that causes pulmonary and extra-pulmonary TB.[8]

Mycobacteria are rod-shaped; non-spore forming obligate aerobic bacteria encased in a lipid rich waxy envelope. They are slow growing, having doubling time of about 18 hours.[9] Mycobacteria are transmitted through airborne particles; after being inhaled they reach the lung alveoli.[7] Once inside the alveoli, these are ingested by macrophages, where they can multiply. Some macrophages are able to kill the bacteria while some die in the process. Within about 2 months after exposure to Mycobacteria, lesions typical of tuberculosis appear in the lung.[9] Successful transmission of Mycobacterium tuberculosis depends on the immune status (susceptibility) of the exposed individual, load of tubercle bacilli in the droplet nuclei (infectiousness), frequency and duration of exposure (Proximity).[7]

One in 10^6 to 10^8 tubercle bacilli develops spontaneous mutations that render it resistant to commonly used anti-tuberculosis drugs.[9] Resistant tubercle bacilli emerge when anti-tuberculosis drugs are used singly. The first-line drugs used to treat tuberculosis include isoniazid, rifampicin (the two commonly used drugs), pyrazinamide, ethambutol, and streptomycin. Resistance to isoniazid develops due to deletions or mutations in the catalase-peroxidase gene (katG) and in the inhA gene that encodes an enzyme in mycolic acid synthesis. Resistance to streptomycin develops due to mutations in ribosomal S12 protein genes. Resistance to rifampicin develops due to mutations in the rpoB gene.[3]

Multidrug-resistant Mycobacterium tuberculosis strains (MDR-TB, resistant to isoniazid and rifampicin) are presently the main obstacle in the treatment and therefore in the control of tuberculosis. These strains are prevalent in certain geographic locations and they usually emerge due to an irregular drug supply, inappropriate regimens, or poor patient compliance.[10]

The incidence of tuberculosis and the number of resultant deaths has declined over the years due to advances made in TB prevention and treatment. As a result, about 49 million lives were saved all over the world between the year 2000 and 2015.[11]

Pakistan, according to WHO HQ Report for 2016, had an incidence of 518,000 TB cases for that year. Among TB high-burden countries Pakistan ranked fifth and it accounted for 61% of TB burden in the WHO Eastern Mediterranean Region. According to the same report, the incidence of multidrug-resistant TB (MDR-TB) for 2016 was 27,000 cases.[5,12]

Rumina et al conducted a study spanning over 17.5-year (1990 to 2007), MDR-TB showed increasing resistance in the provinces of Sindh, Punjab, and NWFP.[13]

Agha Khan University Hospital in collaboration with Swedish Institute for Infectious Diseases Control, found that Central Asian Strain (CAS) was predominant in the province of Punjab, whereas East African-Indian strain was prevalent in the province

of Sindh. Also, Beijing strains were found to be associated with younger age.[14]

In this retrospective study conducted at Charsada Teaching Hospital affiliated with Jinnah Medical College Peshawar. From a total of 873 different types of samples (Table I) cultured, Mycobacterium tuberculosis were grown in 31 samples. Most of the positive cultures were from the specimens of sputa (total: 693, positive: 26, i.e. 3.8%) from patients of pulmonary tuberculosis. This was followed in descending order by samples from extra-pulmonary sites like freshly removed lymph nodes (sent without any fixative, total: 108, positive: 03, i.e. 2.8%), pus from cold abscesses (total: 41, positive: 01, i.e. 2.4%), and pleural fluid (total: 31, positive: 01, i.e. 3.2%).Therefore, the positive yield varied from 2.4% for pus to 3.8% for sputa, with an average of 3.6%. In a large study conducted at institute of public health Lahore, Pakistan from January 2012 to December 2013, out of 6006 suspected specimens cultured, 2445 (40.7%) were positive.[15] This shows a high yield of 40.7% in comparison to our yield (3.6%). This could result from the prevalence of tuberculosis in the catchment area of those hospitals (tuberculosis is common in the province of Punjab as compared to Khyber Pakhtunkhwa),[14] size of studied samples, clinical threshold of suspicion, and better diagnostic facilities.

In the present study, out of 31 isolates of mycobacterium tuberculosis, 8 (25.9%) isolates showed resistance to one or more first line tuberculosis drugs. The resistance of Mycobacterium tuberculosis isolates (Table II) was maximum to INH (n=3, 9.7%). This was followed in descending order by Streptomycin and Ethambutol (each n=2, 6.5%). Rifampicin faced least resistance (n=1, 3.2%). The Mycobacterium tuberculosis isolate that showed resistance to Rifampicin was also resistant to INH (MDR-TB, 3.2%). This is in corroboration with a study conducted by Kamal et al on 122 isolates of Mycobacterium tuberculosis collected from centers in Peshawar and Abbottabad in the Province of Khyber Pakhtunkhwa (KPK) Pakistan. They found that resistance was observed to Isoniazid in 8.4%, to Streptomycin in 5.9%, to Rifampicin in 2.5%, and to Ethambutol in 1.6%.Moreover, the Primary Multidrug resistance in their study was 2.5%.[6] In the study (6006 cases) in Institute of public health Lahore, Pakistan from January 2012 to December 2013, among 2367 cases, the drug resistance was as follows: Resistance to isoniazid in 233 (9.9%) cases, resistance to rifampicin in 241 (10.2%) cases, resistance to streptomycin in 70 (2.9%) cases, resistance to ethambutol in 65 (2.7%) cases, and 221 (9.3%) cases were found to have MDR-TB[15]. All these values are high as compared to our study and that of Kamal et al (both conducted in KPK province) thereby implying higher prevalence of tuberculosis disease and MDR-TB in the Punjab province.[13] A study conducted in Indonesia, spanning over four years from January 2011 to December 2014, out of 127 isolates, 20.5% were resistant to at least one of the first line anti-TB drugs and 0.8% of them were multidrug resistance (MDR). Resistance to Ethambutol, INH, Rifampicin, and Streptomycin was seen in 6.3%, 6.3%, 4.7%, and 1.6% of isolates, respectively.[8] The figures depicted in this study also correlate well with our study except for the MDR-TB which in this study is quite low, probably for the reason that the study was conducted in the rural settings of Indonesia in newly diagnosed cases, whereas our study consisted of mixed patient population of newly diagnosed cases and relapse treatment cases.

In conclusion, a quarter of Mycobacterium tuberculosis isolates were resistant to one or more first-line anti-tuberculosis drugs. Maximum resistance was encountered to INH and minimum resistance to Rifampicin. MDR-TB (resistant to both INH and Rifampicin) was also encountered in the study. All efforts should be put together to adequately treat the patients, reduce the emergence of drug resistance, and prevent its transmission through public awareness. Moreover; it is recommended that the patients should be treated according to drug sensitivity test results. If such susceptibility results are unavailable locally, then the drugs should be selected according to the known community pattern of drug resistance and later modified once the susceptibility test results become available.

CONCLUSION

One quarter of Mycobacterium tuberculosis isolates were resistance to one or more first-line tuberculosis drugs. Maximum and minimum resistance was encountered to INH and Rifampicin, respectively. MDR-TB (resistant to both INH and Rifampicin) was also encountered. All efforts should be put together for public awareness, early diagnosis, adequate treatment and surveillance.

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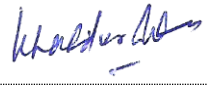
Dr. Khalid Ur Rehman Hashmi Associate Professor, Pathology Faisalabad Medical University, Faisalabad	Methodology & Recording data	
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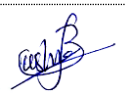
Dr. Bushra Shaheen Associate Professor, Biochemistry Independent Medical College, Faisalabad	Data Collection	
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