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Pyrazinamide Resistance among Multi Drug Resistant Tuberculosis Patients in Karnataka: Cross Sectional Study from a Referral Centre

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ABSTRACT

This study aimed to investigate the rate of occurrence of pyrazinamide (PZA) resistance among patients with MDR-TB from the clinical specimens submitted to a referral centre in Bengaluru, Karnataka. PZA has been used for almost 50 years as first line drug for short course chemotherapy against MTB and its inclusion has significantly shortened the treatment duration to 6 months. However, resistance to PZA is associated with poor treatment outcomes and its drug susceptibility testing (DST) is not routinely performed in public health laboratories in India due to technical difficulties. This study followed a structured approach to investigate PZA resistance among MDRTB patients in Karnataka. Relevant demographic and clinical information was collected from Laboratory registers. The specimens yielding the growth of Mycobacterium tuberculosis were subjected to DST for PZA using validated techniques and the rate of PZA resistance was determined. Our results showed 4% of PZA resistance among MDRTB specimens. These results can contribute to the understanding of local epidemiology of drug resistant TB and update public health strategies for TB control in a particular geographical region. The findings may highlight the need for routine DST for PZA in public health laboratories in India and further research to better understand the associated factors with PZA resistance. This study also emphasizes the importance of continued surveillance of drug resistance patterns to guide evidence-based interventions for TB control and management.

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Introduction

In India Multi drug resistant tuberculosis (MDR-TB) continues to be a major public health problem [1-2]. As per 2020 statistics of global TB report, approximately 10 million people fell ill with TB in 2019 and half a million among them developed rifampicin resistance-TB and 78% were diagnosed with MDRTB. Thus, an important contributor to morbidity and mortality from TB is resistance to first or second line anti-TB drugs [3]. According to World Health Organization (WHO) 2018 statistics, globally about 0.48 million new cases of rifampicin-resistant TB were reported, of which 78% were multi drug resistant TB [4].

According to the new WHO drug classification (2016), patients with rifampicin-resistant or MDR-TB require a regimen with at least five effective TB medicines during the intensive phase: pyrazinamide and four core second-line TB drugs. Agents from group D1 are added if they are considered to add benefit (e.g., high-dose isoniazid in patients without high-level isoniazid resistance) [5]. Group D1 includes pyrazinamide, ethambutol, and high-dose isoniazid. Group D2 includes bedaquiline and delamanid. These may have the efficacy needed to be part of a future hypothetical alloral group B, given that evidence on their safety is growing, although it is still incomplete. Data regarding their safe combination and whole treatment duration over the recommended 6 months are gradually emerging. Pyrazinamide can always be used, although its drug susceptibility test is unreliable, not being counted as well among the four active drugs [6].

Pyrazinamide (PZA) is an important anti-TB drug, with effective bactericidal activity against both drug- sensitive and MDR strains. As a prodrug, PZA is converted by pncA-encoded pyrazinamidase (PZase) into pyrazinoic acid (POA) to exhibit bactericidal activities [7]. The accumulation of POA in cells results in cytoplasmic acidification, which depletes cellular membrane potentials, inhibits various intracellular targets, and eventually leads to cell death [8]. PZA is effective against persistent bacteria

in macrophages, which cannot be killed by other anti-TB drugs. Due to its unique antibacterial activity, PZA reduces the treatment time from 12 months to 6months when it is combined with isoniazid and rifampicin. Studies have shown that 72-98% of PZA resistance is due to pncA mutations which are highly diverse and scattered in open reading frames and upstream regulatory regions [9-12]. PZA has antibacterial activity only at a low pH; thus, an acidic environment is needed for PZA susceptibility tests. Due to the complex procedures and the failure probability of PZA susceptibility tests, only a few laboratories perform these experiments [13]. However, pooled studies have shown that approximately 16.2% of TB patients are PZA resistant [14]. Specifically, the PZA resistance rate is 2–7.5% in non- MDR-TB patients and 36–85% in MDR-TB patients [15-16]. Therefore, reliable prediction of PZA resistance before treatment facilitates the development of more effective treatments. Till date, very few studies have been documented regarding the rate of PZA resistance among Mycobacterium tuberculosis and particularly among MDR-TB. Hence, this current study was aimed to investigate the rate of occurrence of PZA resistance among MDRTB patients in Karnataka, South India.

Materials and Methods

Study Design

This study was conducted at a referral centre situated in Bengaluru, Karnataka and in a tertiary care teaching hospital in Mysuru. The study duration was from November 2019 to March 2022. All the clinical specimens received to the centre either for follow-up or newly diagnosed cases that requires programmatic management of DRTB were included in the study. Salivary/ insufficient samples and those samples that satisfies the rejection criteria according to the SOP of the centre were excluded from the study. Total of 1023 suspected MDR-TB clinical specimens that were received in the referral centre were used in the study.

The current study was approved by Institutional Ethics Committee. (Ref No: JSSMC/IEC/18.02.2022/14NCT/2021-22 Dated 03.03.2022)

Study Site

The study site is a TB laboratory well equipped with negative pressure. Standard safety precautions were followed for specimen processing, smear preparation and DST. All specimens were processed following the standard NALC-NaOH method for digestion, decontamination, and concentration (14, 26). The concentrated sediment was resuspended in about 2 to 3 ml phosphate buffer (pH 6.8) and mixed thoroughly. A smear was prepared for acid-fast staining, and irrespective of smear microscopy results, the specimens were subjected to Liquid culture and those specimens that were positive were subjected to DST using MGIT.

Direct Susceptibility Testing Using Mgit 960

At the referral centre the PZA M960 assay is performed according to the manufacturer's instructions, the remaining pellet from the MTB growth positive tube was suspended in phosphate buffer (pH 6.8) up to a final volume of 2 ml and was used as the inoculum for PZA susceptibility testing [17]. The resuspended pellet was diluted 1/10, and 0.5 ml was inoculated into the control tube (also containing polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [PANTA] and the PZA enrichment supplement), while 0.5 ml of the undiluted resuspended pellet was inoculated into the tube containing 100 g/ml PZA (and also containing PANTA and the PZA enrichment supplement). Tubes were incubated in the Bactec 960 MGIT instrument, according to the 21-day protocol for PZA susceptibility testing. Direct DST results from the MGIT instrument were recorded as susceptible (S) or resistant (R).

Interpretation of Direct Dst Results

When the Growth control (GC) reached the growth unit (GU) value of 400 or more, the instrument indicated that the test was complete, the susceptibility set was removed after scanning, and an inventory report was printed. At the time the GU value of the GC was 400 or more and if the GU value of the drug tube was less than 100, the test result was reported as "susceptible," while if the GU value of the drug tube was 100 or more the result was interpreted as "resistant." In case the GU value of the control did not reach 400 within 21 days, the instrument indicated an X200 error, indicating insufficient growth. On the other hand, if the GU reached 400 earlier than day 4, the instrument gave an X400 error, indicating contamination or over-inoculation. For calculation of time to obtain direct DST results, the time it took for the instrument to complete the DST test was recorded. Since the instrument gives time in hours, any time equal to half a day or more was taken as a full day.

M. tuberculosis H37Rv strain was tested with each lot of MGIT 960 PZA medium and drug set for quality control. All positive MGIT tubes were checked for contamination by subculture on blood agar. Smear was prepared from positive tubes and stained by Ziehl Neelson stain to confirm the presence of acid-fast bacilli and Gram stain was done to rule out contamination.

Results

A total of 1023 clinical samples were received at the referral centre during the study period. Of these, 623 were from males and remaining 400 were from females. 583 samples were received for the diagnosis of DRTB 79 were for the diagnosis of DSTB, 124 for follow up of DRTB and 237 were for follow up of DSTB. 286 patients had history of intake of ATT, 316 did not have any history of intake of ATT and history of ATT intake for 421 was not known. On Liquid culture, PZA resistance was seen in 41 (4%) patients.

Among 41 PZA resistant patients, 21 (50%) were known cases of PTB and were on ATT whose samples were sent for diagnosis of DRTB as per guidelines and 11 (26.8%) of patients were known cases of DRTB and were put of DRTB treatment as per guidelines.

Both these categories of patients had a history of anti-tubercular treatment (ATT), remaining 9 (21.95%) patients of 41 did not have any history of ATT intake and are newly diagnosed cases whose samples were sent for diagnosis of DSTB. Of total 41, 23 (56.09%) patients of PZA resistance were males and 18 (43.90%) females. Over all PZA resistance observed in this study during this study period in Karnataka is 4%.

Discussion

The global control and management of tuberculosis (TB) is faced with the formidable challenge of worsening scenarios of drug-resistant disease. Pyrazinamide (PZA) is an indispensable first line drug used for the treatment of TB. It plays a key role in reducing TB relapse rates, shortening the course of the disease treatment from 9-12 months to 6 months, and the treatment of patients infected with bacillary strains that are resistant to at least isoniazid and rifampicin. Additionally, it is the only first-line anti-TB drug most likely to be kept with all new regimens, which are aimed at reducing the treatment period of susceptible,

multi-drug resistant and extensively drug-resistant TB. It has a preferential sterilizing activity against nonreplicating persister bacilli with low metabolism at acid pH in vitro or in vivo during active inflammation where other drugs may not act so well. PZA seem to have a non-specific cellular target and instead, exerts its anti-mycobacterial effect by disrupting the membrane energetics, the trans-translation process, acidification of the cytoplasm and perhaps coenzyme A synthesis, which is required for survival of Mycobacterium tuberculosis (MTB) persisters. Indeed, the emergence of MTB strains resistant to PZA represents an important clinical and public health problem. The essential role of PZA in TB treatment underlines the need for accurate and rapid detection of its resistance. The need to test isolates of M. tuberculosis for PZA susceptibility has become more apparent as it is being used not only as front-line drug but also in treatment of MDR-TB in DOTS plus programme along with other drugs. Use of PZA in treatment regimen has reduced the duration of therapy to 6 months. It has good sterilizing activity and kills the semi-dormant tubercle bacilli residing in acid environment. However, testing for susceptibility to PZA is a laborious procedure and required extreme specialization. PZA as an important first-line anti-tuberculosis drug plays a crucial role in the therapeutic treatment of MDR-TB [18-19]. Considering the unique effect of PZA, the detection of PZA among MDR-TB is a significant factor for initiation of PZA in the therapy regimens for these refractory patients. Our study showed 4% PZA resistance. When compared to the global burden, this is a lesser rate. PZA resistance in Ningbo was 59.1%, which is higher than those in Zhejiang Province (43.1%), Shanghai (38.5%), Thailand (49.0%), United states (38.0%), and Beijing (57.7%) [20-24]. We further tried to analyse the correlation between PZA resistance and the gender of the patients. Our study showed 56.09% patients of PZA resistance were males and 43.90% in females. These results from our study showed PZA resistance was higher in males than compared to females (Odds ratio:0.0418, 95% Confidence interval (CI): 0.0302 to 0.0577, P< 0.0001) and is statistically significant. This is in concordance by a study conducted by Jie shi et.al, from Henance province that stated that the PZA resistance rate among their population was significantly higher among men than women [25]. When compared to the burden in India, PZA resistance is between 6-8%. Not many studies have documented about PZA resistance in India and in the year 2020 Liquid culture DST was introduced to linezolid and pyrazinamide by NTEP and the actual statistics are yet to be represented. So, this study can form a base line data with some limitations. First, the sample size was small, and all of the strains were derived from mixed geographical areas in Karnataka. As a result, the results might not be representative and might not apply to any other specific area; therefore, more samples size and multicentric studies will be needed to determine the exact prevalence of PZA resistance.

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Conflicts of Interest: The authors declare no conflicts of interest

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