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# Pyrazinamide monoresistance in clinical isolates

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**Aim:** To determine pyrazinamide (PZA) monoresistance in clinical isolates of *Mycobacterium tuberculosis* complex (MTBC) isolated in hospitals in and around İzmir.

**Materials and methods:** The study was performed between 2004 and 2009 with 150 streptomycin, isoniazid, rifampicin, and ethambutol susceptible MTBC clinical strains isolated in the university hospitals of İzmir and Manisa. The PZA susceptibility of the isolates was determined by the BACTEC 460 TB test.

**Results:** The results indicated that resistance to PZA was present in 5 (3.3%) of the 150 MTBC isolates susceptible to all primary drugs except PZA.

**Conclusion:** It is not essential to perform PZA susceptibility tests routinely along with other primary drugs due to the low PZA monoresistance level in our region.

**Key words:** *Mycobacterium tuberculosis*, antituberculosis susceptibility test, pyrazinamide resistance, BACTEC 460TB

## 1. Introduction

In spite of the advances in methods for the diagnosis and treatment of tuberculosis (TB), the disease remains a public health problem (1).

Susceptibility testing of first-line (primary) anti-TB drugs on *Mycobacterium tuberculosis* is essential for rapid detection of strains resistant to the drugs, providing the patient with effective treatment, and preventing the spread of drug-resistant TB strains by taking urgent and adequate public health precautions. Taking the therapy regimes and resistance rates in the USA into consideration, the Clinical and Laboratory Standards Institute (CLSI) recommended the use of isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA) as primary drugs and included streptomycin (SM) in the category of secondary drugs. Centers for Disease Control and Prevention (CDC) recommend that susceptibility tests of *M. tuberculosis* strains isolated from each patient be carried out for the primary anti-TB drugs and the results of the tests be reported within 30 days of the arrival of the sample at the laboratory. Different populations should determine primary drug panels to be used according to their respective conditions (2,3).

PZA is a synthetic pyrazine analogue of nicotinamide and exhibits bactericidal activity only at a slightly acidic pH (pH 5.5). When added to anti-TB drug combinations, it shortens the length of treatment from 9 months to 6 months. This, in turn, facilitates patient compliance and reduces the risk of MDR-TB development (4). Unlike conventional antibiotics that are effective against commonly encountered bacilli, PZA demonstrates sterilizing activity by killing 95% of the semidormant tuberculosis bacteria in the acidic pH environment inside macrophages (5).

Since PZA is active at low pH and the growth of mycobacteria is difficult in low pH environments, using the agar proportion method, which is the reference method for susceptibility testing of other primary drugs, is not adequate for determining PZA susceptibility (6). Thus, the CLSI recommends the use of the BACTEC 460 TB (Becton Dickinson, Sparks, MD, USA) susceptibility test, a liquid-based commercial test system, as the reference test for determining the susceptibility of *M. tuberculosis* complex (MTBC) isolates to PZA. The pH of the broth is adjusted to 6 in the BACTEC 460 TB system, which is a fast and reliable method that has been used for many years to

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evaluate PZA susceptibility. However, since PZA is costly and, unlike the other drugs, requires different equipment and working methods, its clinical use is limited (2). Consequently, the PZA susceptibility test is not performed in most of the TB laboratories in this country.

When the data of our mycobacteriology laboratory were retrospectively analyzed for the period between 2004 and 2008, it was found that nearly 2300 clinical samples were received annually. The growth rate of MTBC in the culture of these samples was determined as 4%. For 85% of isolates, susceptibility testing was performed against 4 antituberculous drugs. Eighty-six percent of isolates tested were found to be totally susceptible to all 4 drugs. The susceptibility patterns of these isolates for PZA, which were susceptible to each of the 4 drugs, could not been determined, since susceptibility testing for PZA was not being used during this period on a routine basis.

The present study aimed to determine PZA monoresistance in MTBC clinical isolates and to find out whether it is essential to include PZA in the primary susceptibility test panel.

## 2. Materials and methods

One hundred MTBC strains isolated from different patients and sent to Dokuz Eylül University Mycobacteriology Laboratory between 2004 and 2008 and 50 MTBC strains isolated in hospitals in and around Manisa (all of which were found to be susceptible to INH, RIF, EMB, and SM) were included in the study. All isolates had previously been isolated by BACTEC 460 TB and conventional methods and were stock strains characterized as MTBC by the BACTEC 460 TB NAP test. The susceptibility of MTBC isolates to the other primary antituberculosis drugs had also been determined by the BACTEC 460 TB susceptibility test.

Stocked MTBC isolates were revived and their susceptibility to PZA was studied using the BACTEC 460 TB indirect proportion susceptibility test. A critical concentration of 100 μg of PZA/mL was used in the susceptibility test. *M. tuberculosis* H37Rv (ATCC 27294) was used as the control strain.

For the differentiation of *M. bovis* and *M. tuberculosis*, a commercial test based on opposite hybridization (Genotype MTBC, Hain Lifescience, Germany) was applied.

## 3. Results

In our study, resistance to PZA was determined in 5 of the 150 *M. tuberculosis* complex isolates, which were susceptible to all primary anti-TB drugs (INH, RIF, EMB, and SM) except PZA. The PZA monoresistance rate of MTBC strains isolated in the 2 neighboring cities İzmir and Manisa was determined as 3.3%.

It was determined that PZA susceptibility test results were obtained in an average duration of 6 days (min: 4 days, max: 14 days) using the BACTEC 460 TB system.

The investigation of the 5 isolates demonstrating PZA monoresistance with the Genotype MTBC test (Hain Lifescience, Germany) showed that 3 of them were *M. bovis* and the remaining 2 were *M. tuberculosis*.

## 4. Discussion

*M. tuberculosis* is one of the leading causes of deaths due to a single infectious agent. In recent years, the increase in multidrug resistant strains in the general population has reached a striking level (7,8). Global research shows that drug-resistant TB has become widespread around the world and a threat to TB control programs in many countries. Drug resistance complicates the treatment and frequently causes unsuccessful treatment results (9). As the patients with drug resistance receive unsuitable drugs, they keep on spreading bacilli and act as sources of infection. Additionally, the use of drugs that are not useful to the patients and the prolongation of hospital stay result in substantial socioeconomic losses. By determining anti-TB drug resistance as soon as possible and starting the appropriate treatment, the treatment cost is reduced and the contamination chain is broken.

In anti-TB susceptibility tests, tests using agar media last about 3 weeks while primary isolation doubles this duration. However, the CDC mentions that test results should be obtained within 30 days of the arrival of the sample at the laboratory. In order to achieve this, the agency recommends the use of commercial systems (BACTEC 460 TB, MGIT 960, VersaTrek) approved by the Food and Drug Administration (FDA), which yield fast results, as the reference methods in the susceptibility test (2). In our study, anti-TB testing was carried out using the BACTEC 460 TB system, which is recommended as a reference.

PZA, RIF, and INH are the most important primary anti-TB drugs, which, when used in combination, shorten the duration of the treatment up to 6 months. They facilitate patient compliance, reduce the risk of MDR-TB development, and help to a great extent in preventing relapses. PZA is included in both primary and secondary treatment schemes. Thus, resistance to PZA constitutes an important public health problem (10).

An important restriction in the anti-TB susceptibility testing of PZA is that the drug is active at low pH (pH 5.5), whereas it is difficult to grow mycobacteria under such pH conditions. Before the BACTEC 460 TB PZA susceptibility testing method was developed, the proportion method on Löwenstein–Jensen (LJ) medium or the enzymatic pyrazinamidase assay had been used as susceptibility

tests. The test results were obtained with 4 to 5 weeks of delay using the proportion method on LJ medium and with 7 days of delay with the pyrazinamidase assay (10). Moreover, due to the acidic medium used in the LJ method, *M. tuberculosis* growth may be limited, which, in turn, results in unreliable test results. In a study conducted by Stottmeier et al., it was shown that 20% of the *M. tuberculosis* clinical isolates could not grow in acidic 7H10 agar (pH 5.5) (11). BACTEC 460 TB is considered the reference method for PZA susceptibility testing (2,12). On the other hand, most laboratories have now replaced the 460TB system with the nonradiometric BACTEC MGIT 960 (Becton Dickinson, Sparks, MD, USA) system. Due to the potential for false resistant results during PZA testing with the BACTEC 960, laboratories should consider retesting all PZA-resistant isolates with the BACTEC 460 TB reference method before reporting results (13). In one study, the new PCR-based in vitro synthesized PZase assay was recommended as a safer alternative to BACTEC 460 TB for PZA testing. This method showed some significant advantages, such as its fast speed, simplicity, and potential of being a direct test and giving results easy to read by the naked eye. However, more clinical testing is needed to further evaluate the method and more studies are needed to confirm its potential (14).

The pH of BACTEC 460 TB PZA test medium is set at 6. This medium is able to identify the differences between PZA-susceptible and PZA-resistant strains and the test results can be obtained in about 5 days (15). In our study, PZA susceptibility test results were obtained in an average of 6 days (min: 4 days, max: 14 days). This duration is reasonable enough for an anti-TB susceptibility test.

The CLSI recommends that, together with INH, RIF, and EMB, PZA be included in the primary drug panel and the drug's susceptibility test be carried out. However, the agency also recommends that populations should determine their respective primary drug panels according to their respective conditions (2,12). Although BACTEC 460 TB is a rapid method, it brings additional costs. Thus, the CLSI mentions that PZA can be excluded from the primary test panel in populations where resistance to the drug is rarely seen (2,3).

PZA monoresistance has been reported by many studies conducted in different countries: Furtado and Brum (16) reported a PZA monoresistance rate of 1.7% in Portugal, Alrajhi et al. (17) reported a PZA monoresistance rate of 3.6% in Saudi Arabia, Javaid et al. (18) reported a monoresistance rate of 4.6% in Pakistan, Louw et al. (19) reported a monoresistance rate of 2.1% in South Africa, and Cheng et al. (20) reported a monoresistance rate of 2.8% in Canada. On the other hand, the study by Ruddy et al. (21) conducted in Russia reported a considerably high PZA resistance compared to other countries. The authors investigated strains isolated from civilians and prison inmates and determined that, among the civilian patients, PZA resistance was present in 6.1% of new cases and 14.6% of previously treated cases. As for the inmates, PZA resistance was determined in 13% of the new and 5.3% of the previously treated cases. In that study, it was emphasized that PZA resistance may be present even in new cases in populations where primary drug resistance is high. Additionally, the study reported that the crowded prison setting and the impossibility of isolating inmates with resistance caused an increase in the contamination of resistant strains. However, the PZA monoresistance rate was not reported in that study.

Although PZA is used as a first-choice drug in this country, it is not tested on a routine basis. Thus, the data related to PZA monoresistance are not sufficient. In a meta-analysis by Yolsal et al. (22), it was reported that the PZA resistance rate was 1.6% between 1984 and 1989 and 6.5% between 1990 and 1995. However, the gradually increasing resistance rates do not represent PZA monoresistance but the PZA resistance rates in all isolates. In the study by Karadağ et al. (23) conducted on 50 MTBC strains isolated from hospitals in and around the city of Samsun, the authors detected PZA resistance in 1 (2%) isolate and reported that this particular isolate was also resistant to INH. The data introduced in these studies are the general resistance rates in all isolates. As for the studies on PZA monoresistance conducted in Turkey, PZA monoresistance was detected in 2 (1.8%) of 109 MTBC isolates in the study by Özkütük et al. (24) conducted between 2004 and 2007 in and around the city of Manisa, and in 2 (3.1%) of 65 MTBC isolates in the study by Şenol et al. (25) conducted between 2005 and 2007 in İzmir. In our study, PZA monoresistance was detected in 5 (3.3%) of the 150 MTBC isolates. This rate is close to others reported in this country as well as in many other countries. It is gratifying that PZA monoresistance is low in our region.

Strains of *M. bovis*, a member of MTBC, display an intrinsic resistance to pyrazinamide. Although *M. bovis* rarely causes TB among humans, preventing its contamination to humans and the use of unnecessary treatment by differentiating *M. bovis* from *M. tuberculosis* are crucial for public health (26). Although some PZA-susceptible *M. bovis* species are defined, they are very rare. Thus, it has been hypothesized that *M. bovis* can be used in identifying PZA monoresistance (27). In a study by Hannan et al. (28), which was based on molecular methods, of the 5 clinical isolates, 2 were identified as *M. bovis* and the remaining 3 were identified as *M. tuberculosis*. As for the study by Özkütük et al. (24), of the 2 clinical isolates with PZA monoresistance, 1 was identified as *M. bovis* and the other as *M. tuberculosis*, using molecular methods. In

our study, the investigation of the 5 clinical isolates based on molecular methods revealed that 3 isolates were *M. bovis* and the remaining 2 were *M. tuberculosis*. Our data and those acquired by other authors show that, although detection of PZA monoresistance can provide aid in identifying *M. bovis* species, it is not convenient for use alone as an indicator.

In conclusion, it was determined that the level of PZA monoresistance is low in our region. Thus, taking the economic factors into consideration, we think that it is not essential to include PZA in the primary drug panel for susceptibility testing and a PZA susceptibility test can be more beneficial when resistance to RIF or to at least 2 primary anti-TB drugs is seen.

## References


1. Scorpio A, Lindholm-Levy P, Heifets L, Gilman R, Siddiqi S, Cynamon M et al. Characterization of pncA mutations in pyrazinamide-resistant *Mycobacterium tuberculosis.* Antimicrob Agents Chemother 1997; 41: 540–3.
2. National Committee for Clinical Laboratory Standards. Susceptibility Testing of Mycobacteria, Nocardia, and other aerobic Actinomycetes; Approved Standard, NCCLS document M24-A, 2003, Pennsylvania, USA.
3. Woods GL. Susceptibility testing for mycobacteria. Clin Infect Dis 2000; 31: 209–11.
4. Davies AP, Billington OJ, McHugh TD, Mitchison DA, Gillespie SH. Comparison of phenotypic and genotypic methods for pyrazinamide susceptibility testing with *Mycobacterium tuberculosis*. J Clin Microbiol 2000; 38: 3686–8.
5. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis 2003; 7: 6–21.
6. Salfinger M, Heifets LB. Determination of pyrazinamide MICs for *Mycobacterium tuberculosis* at different pHs by the radiometric method. Antimicrob Agents Chemother 1988; 32: 100–4.
7. World Health Organization. Global Tuberculosis Control, Surveillance, Planning, Financing. Geneva: WHO Report; 2008.
8. Bilgiç H. Dünyada tüberkülozun durumu. VI. Ulusal Mikobakteri Sempozyumu Kitabı. Kızılcahamam. Türk Mikrobiyoloji Cemiyeti 2006: 17–21.
9. Mphahlele M, Syre H, Valvatne H, Stavrum R, Mannsaker T, Muthivhi T et al. Pyrazinamide resistance among South African multidrug-resistant *Mycobacterium tuberculosis* isolates. J Clin Microbiol 2008; 10: 3459–64.
10. Krishnamurthy A, Almeida D, Rodrigues C, Mehta A. Comparison of pyrazinamide drug susceptibility of *M. tuberculosıs* by radiometric BACTEC and enzymatic pyrazinamidase assay. Indian J Med Microbiol 2004; 22: 166–85.
11. Stottmeier KD, Beam RE, Kubica GP. Determination of drug susceptibility of mycobacteria to pyrazinamide in 7H10 agar. Am Rev Respir Dis 1967; 96: 1072–5.
12. Della-Latta P. Mycobacteriology and antimycobacterial susceptibility testing. In: Isenberg HD (Editor in Chief), Clinical Microbiology Procedures Handbook. 2nd ed. Washington, DC: ASM Publishers; 2004.
13. Chedore P, Bertucci L, Wolfe J, Sharma M, Jamieson F. Potential for erroneous results indicating resistance when using the Bactec MGIT 960 System for testing susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. J Clin Microbiol 2010; 48: 300–1.
14. Zhou M, Geng X, Chen J, Wang X, Wang D, Deng J et al. Rapid colorimetric testing for pyrazinamide susceptibility of *M. tuberculosis* by a PCR-based in-vitro synthesized pyrazinamidase method. PLoS One 2011; 6: e27654. Epub 2011 Nov 10.
15. Miller MA, Thibert L, Desjardins F, Siddiqi SH, Dascal A. Growth inhibition of *Mycobacterium tuberculosis* by polyoxyethylene stearate present in the BACTEC pyrazinamide susceptibility test. J Clin Microbiol 1996; 34: 84–6.
16. Furtado C, Brum L. Laboratory surveillance of drug resistance tuberculosis in Portugal in 2000–2001. Rev Port Pneumol 2003; 9: 279–91.
17. Alrajhi AA, Abdulwahab S, Almodovar E. Risk factors for drug-resistant *Mycobacterium tuberculosis* in Saudi Arabia. Saudi Med J 2002; 23: 305–10.
18. Javaid A, Rizvi N, Angari M. Primary drug resistance to anti-tuberculous drugs in Karachi. J Coll Physician Surg Pak 2008; 18: 699–702.
19. Louw GE, Warren RM, Donald PR, Murray MB, Bosman M, Van Helden PD et al. Frequency and implications of pyrazinamide resistance in managing previously treated tuberculosis patients. Int J Tuberc Lung Dis 2006; 10: 802–7.
20. Cheng S Ji, Thibert L, Sanchez T, Heifets L, Zhang Y. pncA mutations as a major mechanism of pyrazinamide resistance in *Mycobacterium tuberculosis:* spread of a monoresistant strain in Quebec, Canada. Antimicrob Agents Chemother 2000; 44: 528–32.
21. Ruddy M, Balabanova Y, Graham C, Fedorin I, Malomanova N, Elisarova E et al. Rates of drug resistance and risk factor analysis in civilian and prison patients with tuberculosis in Samara Region, Russia. Thorax 2005; 60: 130–5.
22. Yolsal N, Malat G, Dişçi R, Orkun M, Kılıçaslan Z. Türkiye'de tüberküloz ilaçlarına direnç sorununun 1984–1989 ve 1990–1995 yılları için karşılaştırılması: Meta-analiz. Klimik Derg 1998; 11: 6–9.

23. Karadağ A, Tokaç M, Güvenli A, Sünbül M, Günaydın M, Saniç A. Klinik örneklerden izole edilen tüberküloz basili kompleksinin majör antitüberküloz ilaçlara direnç oranları. Ankem Derg 2004; 18: 189–92.

24. Özkütük N, Ecemiş T, Sürücüoğlu S. Pyrazinamide monoresistant *Mycobacterium tuberculosis* in Manisa region, Turkey. Mikrobiyol Bul 2008; 42: 585–90.

25. Şenol G, Coşkun M, Gündüz A, Biçmen C, Gayaf M, Özsöz A. Investigation of pyrazinamide resistance in multidrug-resistant tuberculosis cases in Hospital of Pulmonary Diseases, Izmir, Turkey. Mikrobiyol Bul 2008; 42: 591–7.

26. Dankner WM, Waecker NJ, Essey KM, Thompson M, Davis CE. *Mycobacterium bovis* infection in San Diego: a clinicoepidemiologic study of 73 patients and a historical review of a forgotten pathogen. Medicine 1993; 72: 11–37.

27. Nolte FS, Metchock B. Mycobacterium, In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds.), Manual of Clinical Microbiology, 7th ed. Washington, DC: ASM Press; 1999. p. 399–437.

28. Hannan M, Desmond PE, Morlock GP, Mazurek GH, Crawford JT. Pyrazinamide-monoresistant *Mycobacterium tuberculosis* in the United States. J Clin Microbiol 2001; 12: 647–50.