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Susceptibility of *Mycobacterium tuberculosis* to Pyrazinamide and Its Relationship to Pyrazinamidase Activity

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Pyrazinamidase activity has been associated with pyrazinamide-susceptible *Mycobacterium tuberculosis* strains. The detection of pyrazinamidase activity by the Wayne method was found to be of limited value when compared with the results of standard pyrazinamide susceptibility tests, especially when a high level of pyrazinamide resistance was found. When resistance to pyrazinamide reached a level of 150 to 200 μg/ml, there was too much variability in Wayne test results to accurately define pyrazinamide susceptibility.

The difficulty in performing pyrazinamide (PZA) susceptibility tests derives from the fact that an acid pH of the medium is required to demonstrate activity of the drug (5), and *Mycobacterium tuberculosis* strains do not grow well at pH 5.5 (6). Growth can be improved by pretesting the albumin enrichments before they are incorporated into 7H10 medium (2); however, some *M. tuberculosis* strains still do not grow in acidified medium (2, 4, 6).

It has been reported that PZA-susceptible *M. tuberculosis* strains have a pyrazinamidase (PZase) that hydrolyzes PZA to pyrazinoic acid and that PZA-resistant strains lack this enzyme (1, 3, 4, 7). PZase activity is easily detected by the Wayne method by using freshly prepared ferrous ammonium sulfate solution (9). To overcome the problem of an acid medium, detection of PZase activity has been recommended as a useful indicator for screening *M. tuberculosis* for PZA susceptibility (4). The difficulty in obtaining PZA susceptibilities for some *M. tuberculosis* strains makes the detection of PZase activity an interesting alternative to the present procedure.

We examined the PZase activity of PZA-resistant mutants of *M. tuberculosis* strain H37Rv (TMC 102, lot 771; Trudeau Mycobacterial Culture Collection) as well as fresh clinical isolates of *M. tuberculosis* submitted from state health laboratories to the Centers for Disease Control, Atlanta, Ga., for standard mycobacterial drug susceptibility testing (8). PZA susceptibility tests were performed in Middlebrook 7H10 agar (low pH) (GIBCO Diagnostics, Madison, Wis.) with a final pH of 5.5 (2). Cultures that appeared to be resistant to 25 μg of PZA per ml were selected for additional testing. H37Rv PZA-resistant mutants were picked from colonies appearing on 7H10 agar containing 50 μg of PZA per ml. The level of resistance to PZA was further defined for all isolates by testing for growth at PZA concentrations ranging from 25 to 300 μg/ml.

The standard Wayne test (9) was performed with a heavy inoculum of actively growing cells. After adding the developing reagent, we recorded any color reaction (pink to red) as positive. Both PZase-positive and -negative strains were included as controls in all tests.

The PZase test results from 24 H37Rv mutants were compared with the levels of PZA resistance for each isolate (Table 1). Of the mutants, 14 gave a PZase-positive reaction; the remaining 10 isolates were negative. Of the 14 isolates that were PZase positive, 11 were resistant to 40 to 100 μg of PZA per ml. The other three PZase-positive isolates were resistant to 150 μg of PZA per ml, and one of these was resistant to 250 μg of PZA per ml. In addition, 36 clinical isolates of

TABLE 1. PZase activity and PZA resistance of *M. tuberculosis* H37Rv mutants

No. of mutants tested	Level of PZA resistance (μg/ml)	PZase test	
		+	−
8	40	8	0
2	60	2	0
1	100	1	0
3	150	2	1
2	200	0	2
6	250	1	5
2	300	0	2

TABLE 2. PZase activity and PZA resistance of clinical isolates of *M. tuberculosis*

No. of strains tested	Level of PZA resistance (μg/ml)	PZase test	
		+	−
3	25	3	0
12	50	12	0
8	100	8	0
3	150	3	0
2	200	1	1
1	250	0	1
7	300	2	5

M. tuberculosis that were resistant to PZA were tested for PZase activity. Of these clinical isolates, 29 were PZase positive. Of these 29, 26 were resistant to ≤150 μg of PZA per ml. Further, three strains which were resistant to 200 to 300 μg of PZA per ml still retained PZase activity (Table 2).

These data indicate that, when the level of PZA resistance is ≤100 to 150 μg/ml, the organisms retained PZase activity, whereas at a resistance level of ≥150 μg/ml, the majority of the mutants lost enzyme activity. There was, however, no direct correlation between loss of PZase activity and the level of PZA resistance. Since highly PZA-resistant strains are not always pyrazinamidase negative, care should be taken in defining susceptibility solely on the basis of the Wayne test.

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