Original Article

Minimal inhibitory concentrations of first-line drugs of multidrug-resistant tuberculosis isolates

Nicolas Schönfeld, Thorsten Bergmann[1], Silvan Vesenbeckh, Harald Mauch[1], Gudrun Bettermann[1], Torsten T. Bauer, Holger Rüssmann[1]

Department of Pneumology, Lungenklinik Heckeshorn, [1]Institute of Microbiology, HELIOS Klinikum Emil von Behring, Walterhöferstr. 11, Berlin, Germany

ABSTRACT

Context: The treatment of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) is consistently difficult. Besides resistances, drug availability can be problematic and costs for therapy are high. **Aims:** Our aim was to evaluate alternatives in treatment of MDR and XDR TB other than using second-line drugs. **Materials and Methods:** We analyzed retrospectively the minimal inhibitory concentrations (MICs) of first-line drugs for 44 multidrug–resistant *Mycobacterium tuberculosis* isolates determined in our institute over a period of 20 years (1990 - 2010, *n* = 44). Drug susceptibility testing (DST) was performed using the proportion method on Lowenstein–Jensen Medium or Middlebrook 7H10 agar. MICs were defined as the lowest drug concentration after two-fold serially diluted concentration of the drugs that inhibits growth of more than 99.0% of a bacterial proportion of the tested *M. tuberculosis* within 14 to 21 days of incubation at 37°C. **Statistical Analysis Used:** Summation. **Results:** The MICs of isoniazid and ethambutol were equal or slightly above the critical concentration in most of the strains (92% and 84%, respectively), defined as "low-level resistance". Rifampicin and streptomycin exhibited very high MICs in most of the strains (100% and 77%, respectively), indicating a "high-level resistance". **Conclusion:** Our results indicate that isoniazid and ethambutol could still play a role in treating MDR and XDR TB patients if low-level resistance is detected. Quantitative DST seems to be promising for the recognition of residual drug activity, but has to be confirmed by clinical studies.

KEY WORDS: Ethambutol, isoniazid, low-level resistance, minimal inhibitory concentrations, multidrug-resistance, tuberculosis

Address for correspondence: Dr. Nicolas Schönfeld, Department of Pneumology, Lungenklinik Heckeshorn, HELIOS Klinikum Emil von Behring, Walterhöferstr. 11, Berlin, Germany. E-mail: Schoenfeld.Berlin@t-online.de

INTRODUCTION

In multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB), second-line drugs have to be used for treatment but with less effectiveness, more toxicity, and higher costs. The availability of second-line drugs is another problem, especially in resource-limited, low income countries. Drug susceptibility testing (DST), which determine the minimal inhibitory concentration (MIC) instead of the critical concentration, show that some first-line drugs act inhibitory in slightly higher concentrations *in vitro* describe as "low-level resistance," especially for INH indicating a possible effectiveness *in vivo*.[1-4] Furthermore, the critical concentrations, analogues also called "breakpoint" - concentrations for some drugs do not correspond exactly to the serum or tissue concentration achieved by conventional treatment dosages.[4-10] Therefore, a detected resistance *in vitro* does not necessarily mean a phenotypic resistance *in vivo*. In this context, drugs could be effectively used in the treatment of MDR and XDR TB although they were found to be resistant *in vitro* using conventional critical concentrations.[11,12] In this study, we report about the MICs of first-line drugs in 44 MDR-TB strains.

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MATERIALS AND METHODS

This is a retrospective analysis performed from all clinical isolates, which were sent between 1990 and 2010 to

the Institute of Microbiology, which is affiliated to the Lungenklinik Heckeshorn (HELIOS Klinikum Emil von Behring) in Berlin, Germany. Forty four patients were detected to be infected with a MDR-TB strain; these strains were included in the present study.

During the study period, the standard method to determine DST of isolates changed from Lowenstein-Jensen (LJ) medium to Middlebrook 7H10 medium. Therefore, our data contain isolates cultivated and tested with both media. DST was performed by using the proportion method on Lowenstein–Jensen Medium for 26 strains, and on Middlebrook 7H10 agar for 18 strains, the latter as agar dilution method.[4-6,8,13] In this study, the susceptibility of the first-line drugs isoniazid (INH), ethambutol (EMB), rifampicin (RMP), and streptomycin (SM) were analyzed. Critical concentrations of the drugs, that are used for the decision whether a strain is susceptible or resistant to first-line drugs, are shown in Table 1. MICs were defined as the lowest drug concentration after two-fold serially diluted concentration of the drugs [Table 2] that inhibits growth of more than 99.0% of a bacterial proportion of the tested *M. tuberculosis* strains, either on Lowenstein–Jensen medium or solid Middlebrook medium, within 21 (7H10) to 28 (LJ) days of incubation at 37°C.

Table 1: Critical drug concentrations for LJ and 7H10 medium*

Lowenstein-Jensen medium		Middlebrook 7H10 medium	
Drug	Critical concentration* "breakpoint" (µg/ml)	Drug	Critical concentration* "breakpoint" (µg/ml)
INH	0.25	INH	0.25
RMP	32.0	RMP	2.0
EMB	2.0	EMB	5.0
SM	4.0	SM	2.5

*Strains are judged as resistant, if MICs are ≥ one step (two-fold) dilution above the critical concentration. INH: Isoniazid, RMP: Rifampicin, EMB: Ethambutol, SM: Streptomycin

Table 2: Tested concentration for drug susceptibility

Medium	Drug	Tested concentrations (µg/ml)
LJ	INH	0.12/0.25/0.5/1.0/2.0/4.0/8.0
	RMP	4.0/8.0/16.0/32.0/64.0/128.0/250.
	EMB	0.25/0.5/1.0/2.0/4.0/8.0/
	SM	2.0/4.0/8.0/16.0/32.0/64.0/128.0
7H10	INH	0.12/0.25/0.5/1.0/2.0/4.0/8.0
	RMP	1.0/2.0/4.0/8.0/16.0
	EMB	2.5/5.0/10.0/20.0/40.0/80.0
	SM	1.25/2.5/5.0/10.0/20.0

INH: Isoniazid, RMP: Rifampicin, EMB: Ethambutol, SM: Streptomycin

Low-level resistance was defined as follows: Except for isoniazid, the drugs are described as low-level resistant if the MIC is two-fold higher than the critical concentration. For EMB, was defined as MIC at maximum of 8.0 µg/ml in LJ, 20.0 µg/ml on 7H10-medium. Low-level resistance for SM was defined as MIC 16.0 µg/ml in LJ and 10.0 µg/ml on Middlebrook medium. For isoniazid, low-level resistance was defined as MIC being between 0.5 and 8.0 µg/ml (critical concentration between 0.25 and 4.0 µg/ml) because higher serum and tissue concentrations can regularly be achieved by conventional treatment dosages of 3.0-5.0 mg/kg body weight/day and especially with high–dose INH therapy (15.0-20.0 mg/kg/body weight/day).[1,3,9,10]

RESULTS

INH

For the 26 isolates grown on LJ-medium, 24 (92.3%) strains showed low-level resistance: Two with a MIC at a level of 1.0 µg/ml. 11 strains were inhibited at levels over 1.0 up to 2.0 µg/ml, while 10 isolates showed a MIC up to 4.0 µg/ml and 1 strain a MIC of 8 µg/ml. Only 2 isolates grew at a concentration above 8.0 µg/ml [Table 3], and therefore, were categorized as high-level-resistant. On 7H10 medium (18 strains, Table 3), 15 (83.3%) strains exhibited low-level resistance: 4 strains showed MICs at levels up to 4.0 µg/ml, while 12 isolates were found to be

Table 3: Minimal inhibitory concentrations (MICs) of MDR-*M. tuberculosis*-isolates on different media (*n* = 44)

Medium	INH			RMP			EMB			SM		
	MIC (µg/ml)	No. of isolates/Total no. of isolates	%	MIC (µg/ml)	No. of isolates/Total no. of isolates	%	MIC (µg/ml)	No. of isolates/Total no. of isolates	%	MIC (µg/ml)	No. of isolates/Total no. of isolates	%
LJ	1.0	1/26	7.7	32.0	1/26	3.8	<0.5	2/26	7.7	<4.0	4/26	15.4
	>1.0-2.0	11/26	42.3	>32.0-64.0	11/26	42.3	0.5	1/26	3.8	4.0	1/26	3.8
	4.0	10/26	38.5	>64.0-128.0	7/26	26.9	1.0	5/26	19.2	8.0	1/26	3.8
	8.0	1/26	3.8	>128.0-256.0	7/26	26.9	>1.0-2.0	14/26	53.8	> 8.0-16.0	3/26	11.5
	>8.0	2/26	7.7				4.0	4/26	15.4	32.0	2/26	7.7
										>32.0-64.0	2/26	7.7
										>64.0	13/26	50.0
7H10	4.0	3/18	16.7	4.0	1/18	5.6	<0.5	1/18	5.6	5.0	1/18	5.6
	8.0	12/18	66.7	>16.0	17/18	94.4	5.0	2/18	11.1	10.0	1/18	5.6
	>8.0	3/18	16.7				10.0	12/18	66.7	>20.0	16/18	88.9
							20.0	3/18	16.7			

INH: Isoniazid, RMP: Rifampicin, EMB: Ethambutol, SM: Streptomycin, MIC: Minimal inhibitory concentration

inhibited at a concentration of 8.0 μg/ml INH [Table 3]. Only 3 isolates revealed high-level resistance with a MIC above 8.0 μg/ml. Altogether, 39 (88.6%) MDR strains showed low-level resistance.

EMB

On LJ medium, MICs of 84.6% of the isolates were below or equal to the critical concentration of 2.0 μg/ ml (23 isolates: MIC ≤2.0 μg/ml), and 4 (15.4%) strains showed MICs of 4.0 μg/ml. On 7H10 medium, we found only 3 isolates, which showed a MIC below or equal to 5.0 μg/ ml. 11 samples were detected with a MIC up to 10 μg/ml, which were regarded as "low-level resistance" according to the definition. Only 3 isolates were detected with a MIC of 20.0 μg/ml [Table 3]. In summary, for EMB, all but 1 strain showed sensitivity (3 strains, 16.7%) or low-level resistance (12 strains, 66.7%).

SM

On LJ medium, just 5 (19.2%) isolates showed sensitivity with MICs below or equal to 4.0 μg/ml. One sample reached a concentration up to 8.0 μg/ml (3.8%), and therefore, was regarded as low-level-resistant, while most of the samples showed a MIC above 8.0 μg/ml (76.9%). Similarly, only in 2 (11.1%) isolates, the bacterial growth was inhibited at concentrations above 10 μg/ml on 7H10 medium, while most isolates showed MICs above 20 μg/ml (88.9 %, Table 3). In summary, although most of the strains were highly resistant, 8 (18.1%) strains showed either sensitivity (5 strains) or low-level resistance (3 strains).

RMP

All MDR-strains were judged as highly resistant strains; see also the known one-step-mutation of RMP-resistance.[2,3]

DISCUSSION

The results for treatment of MDR-TB and XDR-TB patients with second-line drugs are still disappointing. There are only few data, showing that isoniazid could be part of effective treatment of MDR-TB. On molecular level, the INH-resistance is divided into 2 sections. The high-resistance is based on a mutation of the *katG* gene and results in an inactivation of catalase-peroxidase.[1] Low-level-resistance of INH is mostly caused by mutations of the *inhA* promoter region and could lead to phenotypic susceptibility *in vivo*.[1,2,14]

92.3% of our INH-resistant isolates showed a MIC of ≤8.0 μg/ml on LJ medium, and 83.3% on 7H10 medium, and therefore, exhibit a "low-level resistance" according to our definition and as described by other authors.[1-3] With a normal dosage of 3-5 mg/kg/day, serum concentrations of almost up to 4 μg/ml can be achieved.[15] Yet, even high-dose INH (16-20 mg/kg/day) is classified into the group of agents with an unclear role in the treatment of drug-resistant TB by the World Health Organization.[16] In our eyes, INH should be discussed stronger than before as an effective alternative in treatment of MDR-TBs based upon individual "low-level resistance" assessed by MIC testing. This was also proposed by others working groups, suggesting that high–dose INH therapy might be effective in these cases.[1,2]

For EMB, only 3 isolates (16.6%) showed high-level resistance on 7H10 medium, whereas no MICs above 8 μg/ml were detected on LJ medium. Similar result were reported by Springer *et al*.[2] Therefore, EMB can also be discussed as another alternative option in the treatment of MDR-TB patients with normal or higher daily doses (up to 25 mg/kg body weight), depending on the results of DST and additionally MIC-testing for the detection of low-level resistance.

For SM, 75.0% of strains (17 on LJ, 16 on 7H10) exhibited high-level resistance [Table 3]. Only 5 isolates were detected to be susceptible, 6 strains showed low-level resistance. Low-level resistance for SM was also described by other authors.[2] In absence of studies, which could show the effectiveness of higher doses for treatment, it is not possible to conclude from DST and MICs on the possible efficiency of SM therapy in MDR-TB patients. For patients with high-level resistance for SM and RMP of the MDR-TB strains *in vitro*, the exclusion of these drugs from treatment seems to be justified.[2,3]

CONCLUSION

The determination of MICs is proposed to be performed in case of multidrug-resistant and/or extensively-resistant *M. tuberculosis* isolates revealed by conventional DST. Thus, especially INH, but also EMB could be identified as resistant at a low level, and therefore, could be considered for the treatment of MDR-TB patients within a treatment regimen of otherwise shown active drugs. However, prospective clinical studies have to be performed on safety and efficiency, especially of INH treatment in selected patients suffering from MDR-TB and potentially INH susceptible strains according to MIC results.

REFERENCES

1. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis 2010;10:621-9.
2. Springer B, Calligaris-Maibach RC, Ritter C, Böttger EC. Tuberculosis drug resistance in an area of low endemicity in 2004 to 2006: Semiquantitative drug susceptibility testing and genotyping. J Clin Infect Microbiol 2008;46:4064-7.
3. Schaaf HS, Victor TC, Engelke E, Brittle W, Marais BJ, Hesseling AC, *et al*. Minimal inhibitory concentration of isoniazid in isoniazid-resistant *Mycobacterium tuberculosis* isolates from children. Eur J Microbiol Infect Dis 2006;26:203-5.
4. NCCLS. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes, vol. 23: Approved standard M24-A. Wayne, PA: National Committee for Clinical Laboratory Standards; 2003.
5. DIN Deutsches Institut für Normung e. V. Medical microbiology - Diagnosis of tuberculosis. Part 8: Methods for the determination of susceptibility of tubercle bacilli to chemotherapeutic agents. Berlin, Germany: Beuth Verlag; 2009.
6. Kent PT, Kubica GP. Public health mycobacteriology. A guide for the level III laboratory. Atlanta, GA: Center for Disease Control and Prevention; 1985.

7. Geerligs WA, Van Altena R, De Lange WCM, Van Soolingen D, van Der Werf TS. Multidrug-resistant tuberculosis: Long-term treatment outcome in the Netherlands. Int J Tuberc Lung Dis 2000;4:758-64.
8. Schaberg T, Reichert B, Schülin T, Lode H, Mauch H. Rapid drug susceptibility testing of *Mycobacterium tuberculosis* using conventional solid media. Eur Respir J 1995;8:1688-93.
9. Kim SJ. Drug-susceptibility testing in tuberculosis: Methods and reliability of results. Eur Respir J 2005;25:564-9.
10. Nuernberger E, Grosset J. Pharmacokinetic and pharmacodynamics issues in the treatment of mycobacterial infections. Eur J Clin Microbiol Infect Dis 2004;23:243-55.
11. Böttger E. Drug-resistant tuberculosis. Lancet 2001;357:1288-9.
12. Espinal MA, Kim SI, Suarez G, Kam KM, Kohmenko AG, Migliori GB, *et al*. Standard short-course chemotherapy for drug-resistant tuberculosis: Treatment outcomes in 6 countries. JAMA 2000;283:2537-45.
13. Richter E, Mauch H, Beer R, Diel R, Hillemann D, Hofmann H, *et al*. MIQ 5: Tuberkulose/Mykobakteriose. In: Podbielski A, Herrmann M, Kniehl E, Mauch H, Rüssmann H, editors. Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik. 2nd ed. München: Elsevier GmbH Urban and Fischer; 2010. p. 1-75.
14. Gagneux S, Burgis MV, DeRiemer K, Encisco A, Muños S, Hopewell PC, *et al*. Impact of bacterial genetics on the transmission of isoniazid-resistant *Mycobacterium tuberculosis*. PloS Pathog 2006;2:e61.
15. Peloquin CA, Namdar R, Dodge AA, Nix DE. Pharmacokinetics of isoniazid under fasting conditions, with food, and with antacids. Int J Tuberc Lung Dis 1999;3:703-10.
16. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis: Emergency update 2008. Geneva: World Health Organization; 2008.

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