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Is second-line anti-tuberculosis drug susceptibility testing reliable?

IN VITRO second-line anti-tuberculosis drug susceptibility testing (DST) often shows poor reproducibility and lack of correlation with clinical response. Nevertheless, many laboratories perform DST of second-line drugs needed to treat multidrug-resistant tuberculosis (MDR-TB). To ascertain current DST practices for second-line drugs and plan further activities, a questionnaire was sent to 21 Supranational Reference Laboratories (SRLs). Results suggest that there are considerable differences in the critical concentrations of drugs and critical proportions of resistance used by the SRLs, and that there is an urgent need for unanimously agreed standards to secure reproducibility of DST of second-line drugs.

Increases in MDR-TB (defined as resistance to at least isoniazid and rifampicin) have led to pressing demands for appropriate treatment with second-line anti-tuberculosis drugs, and accurate and reliable DST not only for individual case management but also for drug resistance surveillance.[1,2] Clinicians, however, are often disappointed with the lack of correlation between second-line DST results and clinical response. This is probably because the testing methods currently used for some second-line drugs have not yet been calibrated with representative samples of clinical isolates of *Mycobacterium tuberculosis*. Isolates should be collected from both tuberculosis cases who fail treatment with second-line drugs and patients who have never been treated with these drugs.[3] DST may also show poor reproducibility because of the lack of standardisation of testing methods within the fragile physicochemical test environment, such as pH, medium inspissation, incubation temperatures and periods and presence of antagonistic substances in the medium.

The 21 SRLs contacted in the survey are part of the WHO/IUATLD Laboratory Network for drug resistance surveillance established in 1994, and include prestigious laboratories across the six continents. Annual proficiency testing for first-line drugs has been successfully conducted in the network,[4,5] but no information is available on second-line drugs. The SRLs were asked about second-line DST, testing methods (including media), critical resistance proportions and/or critical concentrations for each drug tested and any proficiency testing exercise performed.

Ten SRLs responded (see Table). On the subject of DST systems, eight SRLs used conventional methods and four used the BACTEC rapid growth method (two used both conventional and BACTEC methods). Of the conventional methods, the proportion method was the preferred choice. Culture was performed mainly by conventional colony count or growth in drug-free and drug-containing egg-based media (Löwenstein-Jensen [LJ] medium) or agar-based 7H10/7H11 media. All respondents conducted DST without external proficiency assessment.

Table Susceptibility testing for second-line anti-tuberculosis drugs

	DTS systems			Culture media			Critical concentrations of drugs, μg/ml*					
	Conv. methods		Rapid growth methods									
SRL No.	PM	AC		Egg-based	Agar-based	Broth	KM	CPM	ETH	CS	PAS	OFX
1	X			Ogawa			20.0 (1)		20.0 (1)	30.0 (1)	0.5 (1)	
2	X			LJ			20.0 (10)		20.0 (10)	30.0 (10)		2.0 (1)
3	X				7H11		6.0 (1)	10.0 (1)	10.0 (1)	30.0 (1)	8.0 (1)	2.0 (1)
4		X		LJ			40.0	40.0	40.0	30.0	1.0	2.0
5	X				7H11		6.0 (1)	10.0 (1)	10.0 (1)			4.0 (1)
	X			LJ							0.5 (1)	
6	X			LJ				20.0 (10)		30.0 (10)	0.5 (1)	
	X				7H10		4.0 (1)		10.0 (1)			2.0 (1)
7		X		LJ			16.0	32.0	56.0	28.0		2.4
			BACTEC			X			2.5			2.0
8	X				7H10		5.0 (1)	10.0 (1)	10.0 (1)	30.0 (1)		2.0 (1)
			BACTEC			X	5.0	5.0	5.0	50.0		2.0
9			BACTEC			X		1.25	1.25		4.0	2.0
10			BACTEC			X		10.0	5.0	50.0	8.0	2.0

*Numbers in parentheses are critical proportions (%) of resistance.
DST = drug susceptibility testing; SRL = Suprenational Reference Laboratory; Conv. = conventional; PM = proportion method; AC = absolute concentration method; KM = kanamycin; CPM = capreomycin; ETH = ethionamide; CS = cycloserine; PAS = para-aminosalicylic acid; OFX = ofloxacin (laboratory 8 tested ciprofloxacin).

The critical concentrations of drugs and critical proportions of resistance were different for the six drugs (kanamycin [KN], capreomycin [CPM], ethionamide [ETH], cycloserine [CS], para-aminosalicylic acid [PAS], and ofloxacin [OFX]) tested in the two SRLs using the absolute concentration method in LJ medium, while only CS and PAS were tested with similar criteria in two of the three SRLs using the proportion method in LJ medium. The remaining drugs were either not tested or were limited to one SRL. For agar media (7H10 and 7H11), the criteria for CPM, ETH and CS were similar, but they were different for KM and OFX. Finally, using BACTEC, only CS and OFX showed similar criteria for all SRLs. It is important to note that DST for CS in BACTEC may be unreliable because of the difficulty in determining the critical concentration in 7H12 broth. Furthermore, regardless of the method and medium, similar criteria were used for OFX in seven of eight SRLs, suggesting that OFX is stable in the various test environments for which results may be reproducible.

The wide variations in the testing systems and methods used by the SRLs reflect difficulties in securing reproducibility and optimising the clinical relevance of the results obtained. These findings cast doubts on the reliability of DST results and underline the need to standardise the methods and criteria for second-line DST. A proficiency testing exercise among SRLs to understand the reproducibility of DST and a study to optimise the clinical relevance of DST results are currently underway.

A limitation of this study is that it is not known whether the 11 SRLs that did not reply to our request do not test second-line drugs or whether they simply decided not to participate in the study. Although some of the 11 may test second-line drugs, there is no reason to believe our results would be different with the addition of their information.

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