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SHORT COMMUNICATION

Evaluation of the accuracy of the microplate Alamar Blue assay for rapid detection of MDR-TB in Peru

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SUMMARY

Tuberculosis control is hampered by the widespread increase in multidrug resistance. Rapid drug susceptibility testing would greatly aid in the adequate treatment of the disease. This study evaluates the usefulness of the colorimetric method using Alamar Blue for the rapid detection of resistance to rifampicin and isoniazid in 63 clinical isolates of *Mycobacterium tuberculosis* in Peru. Results obtained by receiver operating characteristic curve analysis and measures of gain in certainty showed greater diagnostic accuracy than with the gold standard, the proportion method on Löwenstein-Jensen medium.

KEY WORDS: tuberculosis; MABA; drug resistance; accuracy

MULTIDRUG-RESISTANT *Mycobacterium tuberculosis* (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampicin (RMP), constitutes an important problem for tuberculosis (TB) control because patients have a lower probability of cure.[1,2] Prompt detection of drug resistance allows early alternative treatment with second-line drugs.[2] Conventional methods for drug susceptibility testing (DST) require long periods of incubation to give results.[2] Tests using colorimetric reagents such as redox indicators measuring bacterial viability may provide a low-cost alternative, with the potential for easy implementation in developing countries with a high burden of TB and drug resistance.[1,3,4]

The aim of this study was to evaluate the accuracy of the colorimetric microplate Alamar Blue assay (MABA) for rapid detection of resistance to RMP and INH in clinical isolates of *M. tuberculosis* in Peru.

MATERIALS AND METHODS

M. tuberculosis isolates from 63 pulmonary TB patients at the Institute of Tropical Medicine Alexander von Humboldt—Universidad Peruana Cayetano Heredia in Lima, Peru, were used. Growth on Löwenstein-Jensen (LJ) medium was suspended in sterile Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleate-albumin-dextrose-catalase) enrichment (7H9-S), adjusted to a McFarland nº 1 standard with 7H9-S, and a 1:20 dilution used as the inoculum for MABA. All manipulations were performed with appropriate safety hoods.

Stock solutions of the drugs were prepared according to standard procedures.[4,5] DST was performed using the proportion method on LJ medium for each isolate, according to standard procedures.[6]

DST with MABA was performed in 96-well flat-bottom plates (Nunc International, Rochester, NY, USA) as described by Franzblau et al., with some modifications.[1] The final concentrations tested were 0.031–1.0 μg/ml for INH and 0.062–2.0 μg/ml for RMP. After 5 days' incubation at 37°C, the indicator (20 μl of Alamar Blue [Trek, OH, USA] and 12 μl of sterile 10% Tween 80) was added to a drug-free growth control well. Plates were re-incubated for 24 h. If the control well turned pink, all other wells received the indicator. After a further 24 h of incubation, the colours of all wells were recorded. Wells remaining blue were scored as no growth. The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented a change in colour. If on day 6 there had been no change in the drug-free control, the plate was re-incubated for 3 more days; if still negative, the second control well was used (day 9), repeating the procedure.

Data analysis

The accuracy of MABA was based on the area under the receiver operating characteristic (ROC) curves, with values of 1–0.9 considered as excellent, 0.9–0.8 as good, 0.8–0.7 as fair, 0.7–0.6 as poor and 0.6–0.5 as a failure.[7] Each strain was defined as resistant or susceptible based on the cut-off value that yielded fewer false resistance and false susceptibility values.[7]

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Using Bayesian analysis, the sensitivity and specificity pre-test (based on previous studies) were updated with our results.[8] Predicted values were calculated by the Bayes theorem using the prevalence of resistance for RMP and INH in Peru as an a priori probability.[9,10] The net gain in certainty (ΔC) value that describes the increment in the probability that a patient has or does not have a resistant strain, given the MABA result, was calculated as: $\Delta C = PV - P$, where PV is the predictive value and P is the prevalence.[10]

The proportion method on LJ medium was used as the gold standard. All calculations were carried out using SPSS version 13.0 (SPSS, Chicago, IL, USA) and EPIDAT version 3.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

RESULTS

Rifampicin susceptibility testing

The ROC curve for RMP gave an area under the curve (AUC) of 0.978 (standard error [SE] 0.019). Given a cut-off at 0.125 μg/ml, the false resistance and false susceptibility results for the MABA test are shown in Table 1. The sensitivity and specificity based on Bayesian analysis is shown in Table 1. The positive and negative predictive values for the prevalence of resistance corresponding to new and previously treated patients in Peru are shown in Table 2. The greater net gain in certainty ($\Delta C+$) was observed when a positive result was obtained, in comparison with a negative result.

Isoniazid susceptibility testing

The ROC curve for INH gave an AUC of 0.957 (SE 0.028). Given a cut-off of 0.0625 μg/ml, the number of false-resistant and false-susceptible isolates by the MABA test is shown in Table 1. The sensitivity and specificity based on Bayesian analysis is shown in Table 1. The positive and negative predictive values for a prevalence corresponding to new and previously treated patients are shown in Table 2. Again, the net gain in certainty ($\Delta C+$) was greater when a positive result was obtained.

Table 1 Sensitivity and specificity of MABA in the susceptibility testing of 63 isolates of *Mycobacterium tuberculosis* to RMP and INH

	Proportion method		Sensitivity	Specificity
	Resistant	Susceptible	% (95%CI)	% (95%CI)
MABA-RMP				
Resistant	34	1	98 (95.7–99.0)	99 (97.1–99.6)
Susceptible	1	27		
MABA-INH				
Resistant	33	0	97 (94.8–98.6)	97 (90.0–99.8)
Susceptible	3	27		

MABA = microplate Alamar Blue assay; RMP = rifampicin; INH = isoniazid; CI = confidence interval.

Table 2 NPV and PPV of MABA in the susceptibility testing of 63 isolates of *Mycobacterium tuberculosis* against RMP and INH

	MABA, RMP		MABA, INH	
	New patient	Previous treatment	New patient	Previous treatment
Prevalence (%)	4	14.6	9	16.2
Result: susceptible				
NPV	99.9	99.7	99.7	99.4
ΔC^*-	3.9	14.3	8.7	15.6
Result: resistant				
PPV	80.3	94.4	76.2	86.2
ΔC^*+	76.3	79.8	67.2	70.0

*ΔC is the value that describes the increment in the probability that a patient has or does not have a resistant strain, given the MABA result. Prevalence in Peru.[9]
NPV = negative predictive value; PPV = positive predictive value; MABA = microplate Alamar Blue assay; RMP = rifampicin; INH = isoniazid.

DISCUSSION

Considering the advantages of phenotypic DST, such as microdilution assays, e.g., cost-effectiveness, speed and quantitative MIC results, the MABA test represents an excellent alternative to antimycobacterial DST. The high accuracy observed between MABA and the proportion method agrees with previous studies.[1,3,4] In this study, the MABA has been evaluated keeping in mind not only sensitivity and specificity but also its hypothetical behaviour under different prevalence values (the situation found under field conditions) through its predictive positive and negative values.[8,10] The values shown appear to be smaller than those published in similar studies, but this seems to be due to a methodological bias usually found in these studies. The predictive values of a new DST method for TB are often calculated using the proportion of resistant and susceptible strains of the study sample (frequently near 50%), therefore overestimating these values. The greater net gain in certainty can be a useful measurement of the impact of a new test under different prevalence values.[10]

It should be noted that colorimetric methods such as the MABA in the microplate format are best suited for reference laboratories that follow strict biosafety standards. The test described here can be made more appropriate for mycobacteriology diagnostic laboratories by using screwcap tubes.

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RÉSUMÉ

La lutte contre la tuberculose est menacé par l'augmentation étendue de la multirésistance aux médicaments. Les tests rapides de sensibilité aux médicaments favoriseraient considérablement un traitement adéquat de la maladie. Cette étude évalue l'utilité d'une méthode colorimétrique utilisant le Bleu Alamar pour la détection rapide de la résistance à la rifampicine et à l'isoniazide dans 63 isolats cliniques de *Mycobacterium tuberculosis* au Pérou. Les résultats obtenus par l'analyse de la courbe «receiving operating characteristic» et les mesures d'accroissement de certitude démontrent une très bonne précision du diagnostic par comparaison avec le gold standard, en l'occurrence la méthode des proportions sur milieu de Löwenstein-Jensen.

RESUMEN

El aumento generalizado de las cepas multidrogorresistentes obstaculiza el control de la tuberculosis. Las pruebas rápidas de sensibilidad a los medicamentos podrían favorecer en gran medida el tratamiento adecuado de la enfermedad. En el presente estudio, se evalúa la utilidad del método colorimétrico usando Alamar Blue en la detección rápida de resistencia a rifampicina e isoniacida en 63 aislados clínicos de *Mycobacterium tuberculosis* en el Perú. Los resultados obtenidos, analizados mediante curvas de eficacia diagnóstica y medidas del incremento de certeza ganada, pusieron en evidencia una alta precisión diagnóstica, comparada con la del método de referencia de las proporciones en medio de Löwenstein-Jensen.